

Saline Lakes Around the World: Unique Systems with Unique Values



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Saline Lakes around the World: Unique Systems with Unique Values

Preface

Aharon Oren¹, David L. Naftz² & Wayne A. Wurtsbaugh³

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“All these were joined together in the vale of Siddim, which is the salt sea.”
(Genesis 14: 3)

“From two of our trappers who Came in inform me they had Seen a large lake equal in Size Winipeg & that Bear River & New River discharge their Waters in the Lake ...”
(Peter Skene Ogden, May 1825)

Inland salt lakes are found on all continents. Some have been documented for thousands of years; others have been discovered and explored more recently as shown by the above examples of the Dead Sea and Great Salt Lake, the two largest hypersaline lakes on Earth.

The special properties of inland salt lakes have attracted the attention of explorers and scientists throughout the ages. Charles Darwin visited coastal salt ponds in Patagonia during his travels on the H.M.S. Beagle, and his well known description clearly shows his fascination with this special environment:

“One day I rode to a large salt lake, or Salina, which is distant fifteen miles from the town. During the winter it consists of a shallow lake of brine, which in summer is converted into a field of snow-white salt. ... The lake was two and a half miles long, and one broad. Others occur in the neighbourhood many times larger, and with a floor of salt, two and three feet in thickness, even when under water during the winter. One of these brilliantly-white and level expanses, in the midst of the brown and desolate plain, offers and extraordinary spectacle. A large quantity of salt is annually drawn from the salina; and great piles, some hundred tons in weight, were lying ready for exportation. ... The border of the lake is formed of mud: and in this numerous large crystals of gypsum, some of which are three inches long, lie embedded; whilst on the surface, others of sulphate of magnesia lie scattered about. ... The mud is black, and has a fetid odour. I could not, at first, imagine the cause of this, but I afterwards perceived that the froth, which the wind drifted on shore was coloured green, as if by confervæ: I attempted to carry home some of this green matter, but from an accident failed. Parts of the lake seen from a short distance appeared of a reddish colour, and this, perhaps, was owing to some infusorial animalcula. The mud in many places was thrown up by numbers of some kind of annelidous animal. How surprising it is that any creatures should be able to exist in a fluid,

saturated with brine, and that they should be crawling among crystals of sulphate of soda and lime! And what becomes of these worms when, during a long summer, the surface at least is hardened into a solid layer of salt? Flamingoes in considerable numbers inhabit this lake; they breed here, and their bodies are sometimes found by the workmen, preserved in the salt. I saw several wading about in search of food, - probably for the worms which burrow in the mud; and these latter, perhaps, feed on infusoria or confervæ. Thus we have a little world within itself, adapted to these little inland seas of brine.”

(Darwin 1839)

The International Conference on Salt Lake Research, held at the University of Utah, Salt Lake City, Utah, USA, May 11–16, 2008 in conjunction with the 2008 FRIENDS of Great Salt Lake Issues Forum, brought together 204 scientists, managers and students from 18 countries, who discussed all possible aspects of salt lake research, including physics, chemistry, biology of plants, animals and microorganisms, biogeochemistry, history, economical utilization, conservation, and others. This symposium was the tenth in a series of salt lake symposia, earlier meetings having been held in Adelaide, South Australia (1979), Saskatoon, Saskatchewan, Canada (1982), Nairobi, Kenya (1985), Banyoles, Spain (1988), Lake Titicaca, Bolivia (1991), Beijing, China (1994), Death Valley, California, USA (1999), Khakassia, Russia (2002), and Perth, Western Australia (2005). The Salt Lake City 2008 conference was made possible thanks to the generous financial support from the Center for Integrated Biosystems, Utah State University, CH2MHill College of Natural Resources, Chevron, Utah State University, Dr. Kenneth Sasson, Ducks Unlimited, FRIENDS of Great Salt Lake, The Great Salt Lake Brine Shrimp Cooperative, Inc., Great Salt Lake Minerals Corporation, Kennecott Utah Copper, the Mono Lake Committee, the National Science Foundation, SWCA Environmental Consultants, The Nature Conservancy Tides Foundation, the United States Geological Survey, the Utah Department of Environmental Quality, the Utah Wetlands Foundation, and Westminster College.

Since the Beijing 1994 meeting, these salt lake research symposia are held under the auspices of the International Society for Salt Lake Research (<http://isslr.org>). This society, which grew from the earlier established informal International Consortium for Salt Lake Research (Conte &

Williams 1989). The late W.D. Williams (1936–2002), professor at the University of Adelaide (Walker 2002; De Dekker 2005), was the driving force for the establishment of the society, the newsletter *Salinet* (1989–1993), and the journal *International Journal of Salt Lake Research* (1992–1999, since 2000 merged with the journal *Hydrobiologia*). The thriving society and the series of triennial symposia are part of Bill Williams' rich legacy. The original goal of the society was to bring researchers interested in salt lakes together, but it has grown to encompass not only research but management and education as well.

This book clearly shows the diversity of interest in salt lakes, with 51 chapters (28 full-length papers and 23 extended abstracts, based on presentations made during the May 2008 symposium in Salt Lake City. Saline lakes from every continent except for Antarctica feature in these chapters (Figure 1). Each chapter has been reviewed by one or more members of the editorial team and by at least one outside reviewer. We thank all chapter authors and reviewers for the rapid handling of the manuscripts, enabling the timely publication of this book both as a printed volume and in an online open-access version.

It is not surprising that the Great Salt Lake features in a large number of chapters in this book. The fact that the symposium was held near the shore of the lake attracted many scientists involved in different aspects of Great Salt

Lake studies. Moreover, the FRIENDS of Great Salt Lake (<http://www.fogsl.org>) organized their 2008 Issues Forum within the framework of the conference. We are grateful to the FRIENDS of Great Salt Lake for their contributions to the symposium and to this proceedings volume.

Finally we would like to thank Patsy Palacios, the S.J. and Jessie E. Quinney Natural Resources Research Library, and the Utah State University College of Natural Resources for agreeing to publish these proceedings in the framework of their series and for the excellent cooperation.

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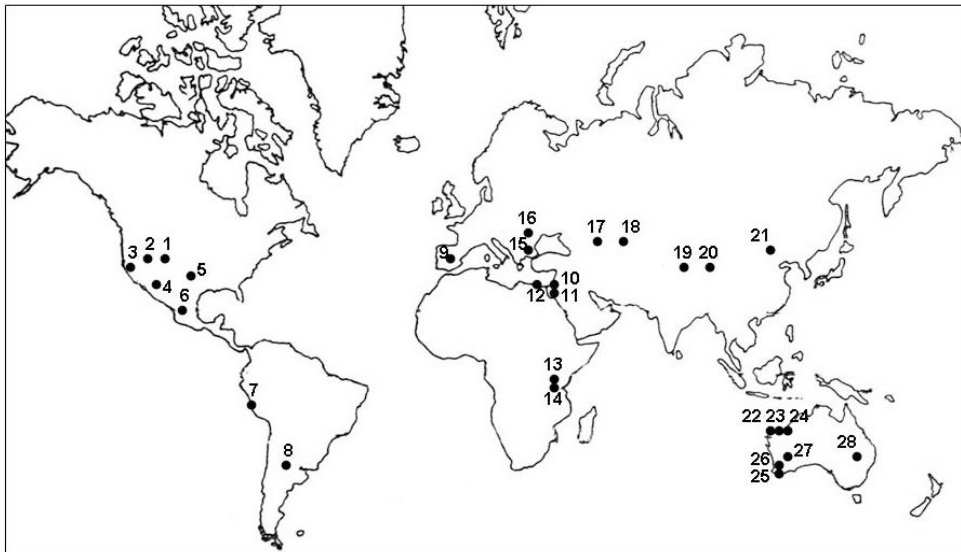


Figure 1—Salt lakes and other saline environments discussed in the chapters in this book: (1) Great Salt Lake, Utah, USA; (2) Walker Lake, California, USA; (3) San Francisco Bay, California, USA; (4) Mojave Desert, California, USA; (5) Guadalupe Mountains, Texas, USA; (6) Lake Alchichica, Mexico; (7) Lima, Peru; (8) Mar Chiquita, Argentina; (9) Inland salterns, Spain; (10) The Dead Sea, Israel/Jordan; (11) Eilat, Israel; (12) Burg-el-Arab, Egypt; (13) Lake Elmenteita, Kenya; (14) Lake Sonachi, Kenya; (15) Braila Salt Lake, Fundata Lake, Techirghiol Lake and Amara Lake, Romania; (16) Solotvine, Ukraine; (17) Caspian Sea, Azerbaijan/ Iran/ Kazakhstan/

Russia/ Turkmenistan; (18) Aral Sea, Kazakhstan/Uzbekistan; (19) Nanyishan oil field, Tibet, China; (20) Zabuye Lake, Tibet, China; (21) Dagenoer soda lake, Inner Mongolia, China; (22) Oslow, North-West Australia; (23) Karratha, North-West Australia; (24) Port Hedland, North-West Australia; (25) Esperance, Western Australia; (26) Eastern Goldfields, Western Australia; (27) Lake Carey, Western Australia; (28) Paroo Area, New South Wales/Queensland, Australia.

Preface - Lynn de Freitas¹

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“To travelers so long shut among the mountain ranges a sudden view over the expanse of silent waters had in it something sublime. Several large islands raised their rocky heads out of the waves. . . . Then, a storm burst down with sudden fury upon the lake, and entirely hid the islands from our view.”

(John C. Fremont, Report of the Exploring Expedition to the Rocky Mountains, 1845)

FRIENDS of Great Salt Lake (<http://www.fogsl.org>), a nonprofit organization founded in 1994 with a mission to preserve and protect the Great Salt Lake Ecosystem through education, research, and advocacy, organized their 2008 Great Salt Lake Issues Forum within the framework of the conference. Although FRIENDS works primarily with local communities with a focus on the lake, they recognize that the system is tied to a much greater “extended” ecosystem, and with other groups in many countries. The millions of migratory birds that utilize the lake for feeding and resting

have migration routes that extend across countries and continents. FRIENDS has begun building a network of contacts in Canada and Mexico to advance efforts in range wide migratory bird conservation, and to help preserve ecosystems there. The purpose of the Great Salt Lake Issues Forum is to bring together Great Salt Lake stakeholders to encourage constructive dialogue about the complexity of the lake and its long term sustainability. Folding the Forum into the 10th Triennial Conference of the International Society for Salt Lake Research allowed local scientists, managers and the public to interact and establish contacts with some of the leading world experts, and prominent conservation groups in other states and countries that work with saline systems. This level of interaction was beneficial to everyone who attended the conference. It will certainly help FRIENDS achieve its goal of ensuring proper management of the Great Salt Lake and other ecosystems in the world.



Biostromes, Brine Flies, Birds and the Bioaccumulation of Selenium in Great Salt Lake, Utah

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ABSTRACT

Benthic organisms and substrates in Great Salt Lake, Utah, were sampled to measure selenium concentrations of prey organisms of the birds that utilize the lake for nesting and during migrations. The sampling was focused on stromatolite biostromes, as these solid reef-like structures cover approximately 23% of the oxic benthic area of the lake and are the principal habitat for brine fly (*Ephydra cinerea*) larvae and pupae. Samples were taken at depths of 1-5 m along two transects in Gilbert Bay where salinities ranged from 116–126 g l⁻¹. Periphyton on the biostromes had chlorophyll levels of 700 mg m⁻², and contained approximately 68% of the chlorophyll in the lake's phytoplankton. Consequently, the biostromes represent a significant component of the lake's primary production. A pumped-bucket sampler effectively sampled brine flies on horizontal surfaces of the biostromes, but not on the sides of the mounded ones encountered in the southern part of the bay. Brine fly larvae and pupae were far more abundant on the biostromes than on the soft substrates, with respective mean densities of 9100 m⁻², 530 m⁻² and 240 m⁻², on biostromes, sand and mud. Total brine fly biomass on biostromes averaged 5.9 g m⁻², which is about 30% of the biomass present in brine shrimp (*Artemia franciscana*) in the water column. The mean selenium concentration in the combined organic matter-inorganic substrates of biostromes sampled in 2007 was $0.3 \pm 0.1 \mu\text{g g}^{-1}$ dry weight. However, when the inorganic carbonates were removed with acid, the remaining organic matter had selenium concentrations of $1.0 \pm 0.1 \mu\text{g g}^{-1}$ dry weight. Mean Se concentrations in larvae, pupae and adult brine flies were 1.3, 1.5 and $1.8 \mu\text{g g}^{-1}$ dry weight, respectively, but the differences were not significant. Although there was a 2500X bioconcentration factor between total dissolved Se (mean = $0.40 \mu\text{g l}^{-1}$) in the overlying water and in the periphyton of the biostromes, the limited data suggested that there was little biomagnification between the periphyton and the brine flies. A review of the diets of birds utilizing Great Salt Lake and other saline lakes suggests that brine fly produced on biostromes are an important diet component for goldeneye ducks (*Bucephala clangula*), American avocets (*Recurvirostra americana*), black-necked stilts (*Himantopus mexicanus*) and California gulls (*Larus californicus*) and perhaps other birds utilizing the lake. Consequently the benthic food web may be important route for uptake of metal contaminants in these birds in Great Salt Lake. The high selenium concentrations in goldeneye ducks

that feed on brine flies suggests that proposed increases in the loading of this contaminant should be reviewed carefully by managers. High mercury levels in goldeneyes suggest that the food web on the lake's biostromes may be an important pathway for other metals into birds.

INTRODUCTION

In lakes and oceans, production processes are often divided between those in the water column and those on the benthic substrates. The food webs of phytoplankton → zooplankton → predators in the water column are better studied than are the periphyton → invertebrate grazer → predator food chains in the benthos. However, a recent review (Vadeboncoeur et al. 2002) documented that in many lakes, food chains based on benthic production are frequently more important for the upper trophic levels than are pelagic food chains. This is particularly true in small or shallow lakes where light penetrates to much of the bottom and consequently fuels primary production by periphyton. For example, in clear, shallow lakes, Vadeboncoeur et al. (2003) found that over 80% of the lake's production came from periphyton, and top predators such as fish and birds are often highly dependent on benthic organisms as food sources (Vadeboncoeur et al. 2002).

In saline lakes, pelagic primary production and food webs are also much better understood than are their benthic counterparts. For example, in Great Salt Lake, Utah, there have been multiple studies of the plankton community (Wirick 1972; Stephens & Gillespie 1976; Stephens 1990; Wurtsbaugh 1988, 1992; Wurtsbaugh & Gliwicz 2001; Marcarelli et al. 2006), but only a single study on the benthic invertebrates (Collins 1980) and none on the primary producers there. The mean depth of Great Salt Lake is near 5 m, and hence a considerable portion of the lake's bottom receives sunlight and can thus support primary production.

Primary production in the sediments of Great Salt Lake has led to the formation of expansive beds of stromatolite biostromes (bioherms) that form when photosynthesis drives up the pH and allows carbonates to precipitate, forming rock-like structures. Only a single study has described the biostromes in the lake. Eardley (1938) provided an assessment of all of the lake's sediment structure, and found that stromatolites covered hundreds of square kilometers, and that they were the only solid

substrate in the lake. The lake may have the most extensive coverage of living stromatolites anywhere in the world, but they are far less known than those in Shark Bay, Australia (Golubic 1992), Cuatro Ciénegas, Mexico (Dinger et al. 2006), or in the Bahamas (Paerl et al. 2001).

The stromatolite biostromes in Great Salt Lake are particularly important as a habitat for brine flies. In the only published study on brine flies in the lake, Collins (1980) found that larval and pupal densities were highest on the calcified biostromes in the lake that provide solid substrates. Mud substrates were secondarily important, and few flies were found on sand substrates. Because brine fly pupae must attach to a solid substrate to undergo metamorphosis, the biostromes may be crucial for their survival in the lake.

The brine flies are likely an important food resource for the extensive bird populations that utilize Great Salt Lake during annual migrations, but it is often assumed that brine shrimp are the dominant prey items. However, in hypersaline ecosystems brine flies are often an important component of bird diets. Herbst (2006) studied bird (including black-necked stilts) use of prey in hypersaline ponds in California and concluded that nearly 90% of all feeding was on brine flies, with the remainder on brine shrimp and corixids. Brine flies (*E. hians*) also have been shown to be an important component of the diet of California gull chicks at Mono Lake, CA (Wrege et al. 2001). Brine flies are also important component diets of eared grebes (*Podiceps nigricollis*) in saline lakes (Jehl 1988) and these birds often concentrate over biostrome areas in Great Salt Lake (personal observation). Another abundant species at Great Salt Lake are red-necked phalaropes (*Phalaropus lobatus*) and Rubega & Inouye (1994) found that brine flies from Mono Lake were a better nutritional source for these birds than were *Artemia*. Few studies on bird diets have been done on Great Salt Lake, but Vest et al. (2008) recently found that larval brine flies were the dominant prey eaten by goldeneye ducks (*Bucephala clangula*), and gulls are often seen feeding on adult flies that accumulate in mass along the shoreline. The limited diet analyses from Great Salt Lake and those from other systems suggest that brine flies may be a very important source of food.

Brine flies may also be an important pathway for the accumulation of contaminants in birds that utilize Great Salt Lake. Benthic food webs may be particularly important for transferring metals to birds and fish, because concentrations are often high in the sediments and because reducing conditions there often mobilize or transform metals into oxic forms (e.g. mercury; Mason 2002). Not all metals respond similarly, however. Selenium, in particular, is

frequently made less bioavailable under reducing conditions. Nevertheless, Vest et al. (2008) found high concentrations of both selenium and mercury in the livers of goldeneye ducks that feed heavily on brine flies, and this has led to a consumption advisory to limit mercury intake in humans (Utah DWR 2008).

The objectives of the study were four-fold. The first objective was to test methods for quantitatively sampling the periphyton and brine flies from the biostromes, mud and sand substrates. Most sampling of brine flies has relied on only semi-quantitative kick-net methods (Herbst 1988), or quantitative samples collected by wading in shallow water (Herbst 1990). The second objective was to determine the abundances and biomasses of the periphyton and brine fly community, and to compare them with the primary producers and brine shrimp (*Artemia franciscana*) in the water column. The third objective was to determine the selenium content in the benthic organisms because of concerns that this metal might bioaccumulate in birds at Great Salt Lake and because the Kennecott Utah Copper Corporation had requested to increase its discharge of this metalloid into the lake. The fourth objective was to construct a food web for the birds utilizing recently available data on bird diets from Great Salt Lake, and other literature on bird feeding habits in saline lakes. The benthic study was part of a larger analysis by the Utah Division of Water Quality of selenium in the lake and its potential impacts on birds (CH2MHill 2008).

STUDY AREA

Great Salt Lake is a 5180 km² closed-basin system in Utah, USA (41.04 N, 112.28 W) bordered on its eastern and southeastern shores by the greater metropolitan area of Salt Lake City. The lake has been impacted by industrial and municipal discharges, and by transportation causeways that divide the system into three large bays. Gunnison Bay (2520 km²), located in the northwest of the lake, has salt concentrations between 280 and 300 g l⁻¹ (28-30%). Farmington Bay (260 km²) in the SE, is very shallow with a mean depth < 1 m and highly variable salinities. The benthic food web study described here was focused on Gilbert Bay (2400 km²), in the central portion of the lake. This bay is separated from Gunnison Bay by a railway causeway. Gilbert Bay typically has surface salinities typically ranging between 120 and 180 g l⁻¹ and supports a large brine shrimp population. The lake elevation during the study was 1279.6 m in June, 2006 (USGS 2007). At this elevation, the respective mean and maximum depths of Gilbert Bay are 5.0 and 9.5 m. In the continental climate, water temperatures in the lake reach 27-28°C in summer and decrease to near 0°C by January (Wurtsbaugh & Gliwicz 2001).

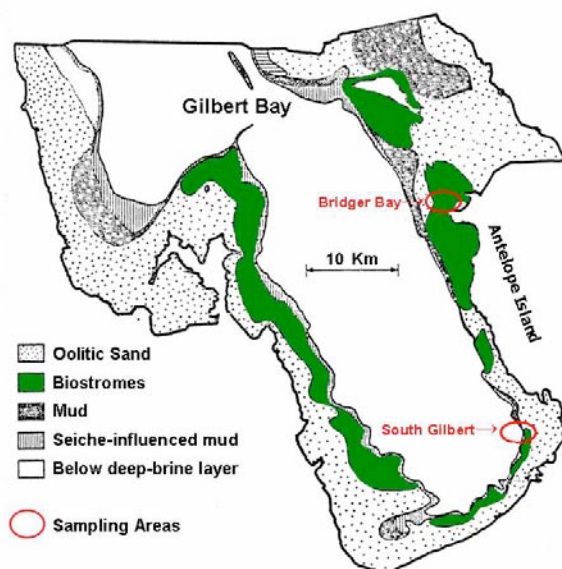


Figure 1—Map of Gilbert Bay showing the two benthic sampling areas. The substrates in the lake are derived from a map in Collins (1980) who used sediment structure data of Eardley (1938).

Gilbert Bay (Figure 1) is meromictic due to infiltration of saturated brines from Gunnison Bay under the railway causeway and into the deeper strata of Gilbert (Loving et al. 2002) creating a deep-brine layer (monimolimnion) below 6.7 m. The upper 6.7 m of Gilbert Bay is well-mixed and oxic. The deep-brine layer is anoxic with substantial hydrogen sulfide, and consequently has no macroinvertebrates. Eardley (1938) provided a detailed map of the benthic structure of Great Salt Lake, although no methods were provided on how this information was collected. The deep brine layer underlies 44% of Gilbert Bay. In the remaining sediments covered by the oxygenated mixed layer, oolitic sand, mud and biostromes represent 62%, 15% and 23% of the substrate, respectively (Table 1).

The biostromes occupy depths of approximately 0.5-3 m in the lake where there is sufficient light for benthic photosynthesis. A single analysis of the microbial composition of the biostromes indicates that the cyanobacterium *Aphanothece* sp. represented over 99% of the cells in the biostromes, but some green algae were present (N. Parker & W.A. Wurtsbaugh, unpublished data). The small 1.4 μm diameter cells are embedded in a mucilaginous matrix that is partially calcified. The growing cyanobacteria change the pH of the water, causing carbonates to precipitate and the biostromes to grow. Treatment with hydrochloric acid dissolves the carbonates, leaving a solid, flexible mucilaginous plate ca. 1 cm thick. Most of the biostromes we have encountered in the lake have a flat plate structure protruding approximately 10-20 cm from surrounding soft sediments. In many places the biostromes have grown together forming a nearly continuous plate. However, during the sampling for this

study we found that biostromes in water about 3 m deep near the SE end of the lake had considerably different structure, with mounds that protruded ca. 0.8-1.5 m from the bottom, and that were ca. 0.5 m in diameter. These were in dense fields with limited space between mounds, although poor visibility precluded assessing them extensively.

Table 1—Morphometric characteristics of Gilbert Bay of Great Salt Lake at a lake elevation of 1280.2 m (4200 ft), which is near the mean historical elevation. The data exclude areas of the southern salt ponds and Farmington Bay. Data derived from Baskin (2005). The thickness of the mixed layer was estimated at 6.7 m (22 ft). The areas of stromatolites, oolitic sand and mud were derived from the proportional areas shown in the map of Collins (1980), with an adjustment to a lake level of 1280.2 m.

Section	Mean Depth (m)	Area of Sediments (km^2)	Volume ($\text{m}^3 \times 10^9$)
Gilbert Bay (total)	5.55	2057	11.42
Deep-Brine Layer		912	1.73
Mixed Layer		1145	9.69
Stromatolites		261	
Oolitic sand		712	
Mud		172	

METHODS

Collection Sites

The primary collections of brine fly pupae and larvae, periphyton, water, and sediment were along two transects in Gilbert Bay (Figure 1) from 14–16 June 2006. The transect for Site 1 began at a depth of 1 m at the SW corner of Bridger Bay on Antelope Island and proceeded westward. Site 2 (Gilbert South) was a N-S transect beginning in the SE end of Gilbert Bay. At each of these sites we sampled at nominal depths of 1, 3 and 5 m. The coordinates and actual depths sampled are shown in Appendix 1. We also collected adult brine flies at three shore locations: rock outcroppings at the SW corner of Bridger Bay, Saltair Beach, and at a beach just north of Kennecott mine tailings on the south shore. Because relatively few sediment samples were collected in 2006, and because some of the analyses were questionable, additional ones were collected at depths of 1-3 m on 28 April 2007. At each site, salinity, oxygen and temperature profiles were measured with an InSitu sonde. Secchi disk measurements were made at each site. On 28 September 2006 we made two additional collections of brine fly larvae and pupae at a depth of 1.9 m at the Bridger Bay sampling site.

Brine Fly & Sediment Collection

Brine fly adults were captured with a fine-meshed butterfly net while running along the beach, or between rocks where brine flies were resting. Netted brine flies were placed in a cooler with dry ice to euthanize and transport them. They were kept frozen at -20°C after return to the laboratory, and then washed with de-ionized water to remove salts, counted, and weighed.



Figure 2—SCUBA diver sampling biostromes in Great Salt Lake with a pump sampler. The inset shows the detail of the bucket and scrub brush used to dislodge brine flies so that they can be pumped to the surface.

Duplicate larval and pupal brine fly samples were collected at each depth. The larvae and pupae were sampled on the biostromes by SCUBA divers using a vacuum pump sampler (Figure 2) similar to that of Voshell et al. (1992). The sampler consisted of an inverted plastic bucket with a port and glove attached to the side of the canister so that a diver could agitate the substrate. A flexible rubber strip on the bottom of the bucket helped seal it against the irregular surfaces of the biostromes. The apparatus sampled an area of 0.075 m^2 . Lead weights (4 kg total) were attached to the lower part of the bucket to increase stability and to keep the unit on the substrate. In order to function effectively, the sampler had to be placed on a relatively level and solid substrate. This precluded sampling on the sides of the dome-shaped biostromes in southern Gilbert Bay. Once the sampler was positioned, the diver jerked the attached pump tube so that the operators in the boat could begin bringing water to the surface with a hand-powered bilge pump (Guzzler Model Vacuum Pump, U.S. Plastics Corp.). The diver then began scouring the substrate with a scrub brush. Pumping continued until three 20 l buckets were filled on the boat. Subsequent analyses indicated that this pumped volume removed an average of 92% of the larvae and pupae from the substrate (W.A. Wurtsbaugh, unpublished data). Samples were sieved through a $500\text{-}\mu\text{m}$ sieve and collected

in an acid-washed 500 ml polyethylene bottle, and stored on ice for transport to the laboratory. A power analysis of the sampling efficacy of the bucket sampler suggested that respective sample sizes of 5 and 36 would be needed to measure selenium concentrations and larval abundances with an allowable error of 30% (Wurtsbaugh 2008).

To sample organic matter and chlorophyll, the diver broke off a portion of the calcified biostromes. Only edge pieces of flat biostromes, or exfoliating pieces of domed biostromes could be collected, and this could have introduced some bias. Sampled pieces were $100\text{--}300\text{ cm}^2$, and usually about 3 cm thick. Pieces of biostromes of known area were frozen and subsequently placed in 95% ethanol to extract chlorophyll overnight at room temperature. The chlorophyll solution was then diluted with ethanol and concentrations measured in a Turner 10-AU fluorometer with the non-acidification method (Welschmeyer 1994). Blanks and standard were analyzed at the beginning of each run. Biostrome and sediment subsamples were dried at 70°C , weighed and then ashed at 450°C for 6-8 h. Ashed samples were re-wetted using deionized water, dried overnight at 70°C , and then weighed to obtain ash-free dry mass (AFDM). Subsamples of biostromes were treated with acid to remove carbonates. These samples were submerged in 1 N HCl until all CO_2 bubbling stopped. This required several hours and necessitated replacing the acid up to three times. By removing the carbonates, this procedure allowed us to determine the selenium concentration of the organic component of the stromatolites.

On sand and mud substrates, brine flies and substrate materials were collected with a 0.050 m^2 , 24 kg, Ponar grab (Wilco, Inc., Buffalo NY). The samples were brought to the surface and discharged into a plastic tub and then sieved through a $500\text{-}\mu\text{m}$ mesh. In all cases, insufficient brine flies were available from the soft sediment samples for selenium analyses. Separate Ponar grab samples were used to collect sediments for selenium analyses. In some cases the upper few millimeters of sediment were sectioned separately with a plastic spatula to determine if there were vertical differences in selenium content.

In the laboratory, larvae and pupae were counted, washed three times with de-ionized water, then weighed and frozen in polyethylene scintillation vials. Composite samples of larvae (mean, 236 individuals; range 47-500) and pupae (mean, 246 individuals; range 21-500) were analyzed for selenium. The brine fly samples were sent to LET Incorporated (Columbia, MO) for selenium analysis by hydride generation-atomic absorption spectrometry on acid-digested samples. The reporting limit for selenium was $0.1\text{ }\mu\text{g Se g}^{-1}$.

Water Samples and Statistical Analyses

Water samples were taken first at all dive sites to avoid disturbed sediments. Collection of water samples occurred 2–5 cm above the sediment surface using 60 ml acid washed syringes. Each syringe was rinsed three times with surface water, and once with water from above the sediments prior to collecting the actual sample. Water samples were taken ca. 5 m apart from each other. On the surface, water in the syringes was filtered through Whatman 47 mm GF/F filters (0.80 μm) and placed in 200 ml acid-washed polyethylene bottle. Two ml of concentrated nitric acid were added to fix samples. Samples were sent to Frontier Geoscience, Seattle, WA for total dissolved selenium analysis by hydride generation and atomic fluorescence spectrometry (HG-AFS). Minimum detection limit for total Se was reported as 0.05 $\mu\text{g l}^{-1}$. The statistical analyses of selenium concentrations and brine fly densities were done using SYSTAT (Chicago, Illinois).

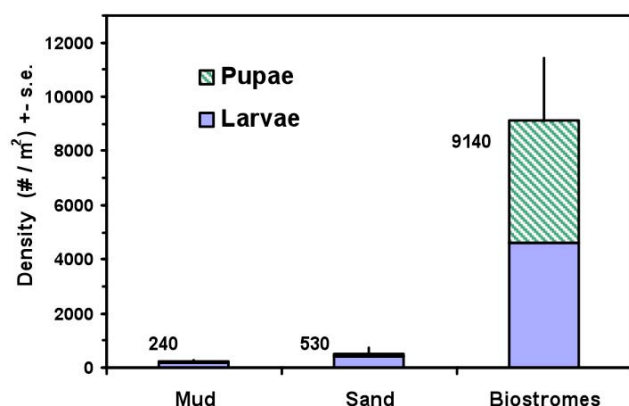


Figure 3—Densities of brine fly (*Ephedra cinerea*) larvae and pupae measured on mud, sand and biostrome substrates in Gilbert Bay of Great Salt Lake during June 2006. Error bars show ± 1 standard error.

RESULTS

Limnological Conditions

During the 14–16 June 2006 sampling, water temperatures ranged from 20.7°C at the surface to 20.4°C near the bottom and oxygen was at saturation. Surface salinities were 116 g l^{-1} at both sites, increasing to 126 g l^{-1} (12.6%) at 5 m. The Secchi depth (0.72 m) at Bridger Bay was influenced by the algal-laden Farmington Bay water reaching the site, as clearer water was observed farther offshore and in other areas of the lake. In the September sampling at Bridger Bay, water temperatures ranged from 20.0°C at the surface to 16.8°C at 1.8 m, and salinities ranged from 136 g l^{-1} at the surface to 146 g l^{-1} at 1.8 m, indicating an overflow of fresher water from Farmington Bay was influencing the site. When benthic substrates were

sampled in April 2007, the Secchi depth was 1.0 m at the Bridger Bay site. Much clearer water was observed in other parts of Gilbert Bay, but transparencies were not measured.

Brine Fly Densities, Biomass, Organic Matter

Brine fly larvae and pupae were very abundant on biostromes but scarce on sand and mud substrates (Figure 3). There was no significant relationship between brine fly densities and depth (linear regression; $p = 0.88$), so data from all depths are pooled for presentation. Total brine fly densities on biostromes averaged 9140 m^{-2} and reached over $1.6 \times 10^4 \text{ m}^{-2}$ in three samples. Larval brine flies on biostromes were significantly (2-way ANOVA; $p = 0.015$) more abundant (7060 m^{-2}) in Bridger Bay than at the Gilbert South site (1470 m^{-2}), but there was no significant difference for pupae ($p = 0.226$; mean = 4600). Combined larval and pupal densities on mud and sand were 240 m^{-2} and 530 m^{-2} , respectively, but pupae were very rare on these substrates. Both larvae and pupae were significantly more abundant on biostromes than on the combined category of sand/mud (t-test; $p = 0.003$). Total brine fly biomass on biostromes sampled in June averaged 5.9 g m^{-2} , but only 0.2 g m^{-2} on sand/mud substrates. Larval and pupal brine fly densities at Bridger Bay in September 2006 were 3160 ± 670 , and $410 \pm 132 \text{ m}^{-2}$, respectively.

Organic matter content was high in the biostromes, but low in the sand and mud substrates (Figure 4). The organic matter content of sand substrates averaged 3% and that in mud was 12%. The organic matter of intact biostrome material was 30%, but when the carbonates were removed by acidification, the remaining material had an average organic matter content of 72% (Figure 4). Chlorophyll *a* concentrations on the biostromes were high, averaging $700 \pm 210 \text{ mg m}^{-2}$. Chlorophyll was not measured on the sand/mud substrates.

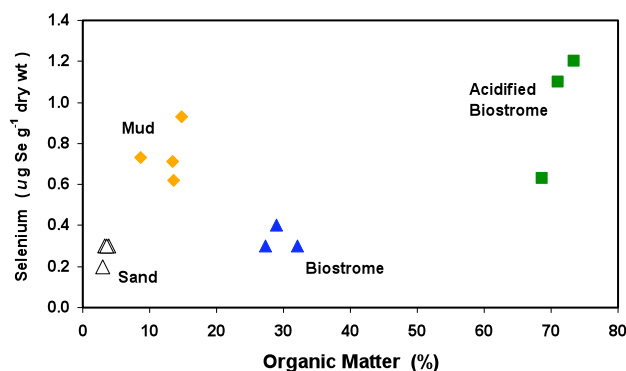


Figure 4—Relationship between organic matter in benthic substrates and their selenium content in replicate samples collected near Bridger Bay in 2007. Biostromes were analyzed either intact (Biostrome), or after acidification to remove carbonates (Acidified Biostrome).

Selenium Concentrations

The concentrations of selenium in water and the benthic organisms were moderate but showed little indication that there is biomagnification (Figure 5). Mean total dissolved selenium concentrations in the water were low ($0.40 \mu\text{g Se l}^{-1}$) and did not differ significantly between the Bridger Bay and the Gilbert South sites ($p = 0.117$). Variability in selenium concentrations in the water was very low with a range of $0.37\text{--}0.43 \mu\text{g Se l}^{-1}$. The mean Se concentration in microbial community on the biostromes was $1.0 \mu\text{g g}^{-1}$ ($980 \mu\text{g kg}^{-1}$). This represents a 2500-fold bioaccumulation factor between the water phase and the microbes. The selenium concentrations in the sand (mean $0.3 \mu\text{g g}^{-1}$), mud ($0.8 \mu\text{g g}^{-1}$) and non-acidified biostrome material ($0.3 \mu\text{g g}^{-1}$) were lower than concentrations measured in the primarily organic material ($1.0 \mu\text{g g}^{-1}$) from the acidified biostromes (Figure 4).

The mean concentrations of Se in brine flies increased from larvae to pupae to the adults, but these differences were not significant ($p > 0.15$). Mean Se content for all three stages of flies average $1.5 \mu\text{g g}^{-1}$. A two-way ANOVA (site x brine fly stage) indicated that the brine flies in Bridger Bay had significantly higher concentrations of selenium than did those in Gilbert South ($p < 0.000$) with a mean difference of 1.6 vs. $1.3 \mu\text{g Se g}^{-1}$.

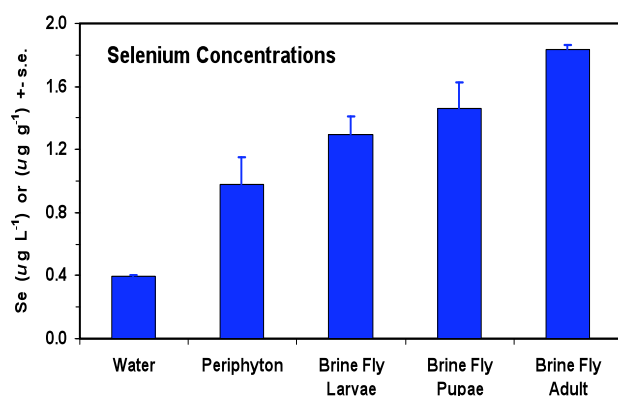


Figure 5—Selenium concentrations in water, periphyton (cyanobacteria + other microbes) and three stages of brine flies in Great Salt Lake. The value for periphyton was from biostrome samples collected in 2007 that were acidified to remove carbonates, leaving primarily organic matter. The selenium concentration for water is in units of $\mu\text{g l}^{-1}$ while that for the periphyton and brine flies is in units of $\mu\text{g g}^{-1}$ dry weight. A Bonferroni-adjusted multiple comparison of the periphyton and brine fly stages indicated that only periphyton and adult brine flies had significantly different selenium concentrations ($p = 0.022$). Error bars show +1 standard error.

DISCUSSION

Food Web Dynamics and Selenium Bioaccumulation

The data collected on the biostromes indicates that they are an important component of the food web in Gilbert Bay, and they may consequently have an important influence of the bioaccumulation of metals such as selenium and mercury. A relative comparison of periphyton on biostromes and the phytoplankton can be done as an approximation of how much production may come from these two sources. Primary production data are not available for the biostromes, so chlorophyll levels in the two can be compared. Biostromes are estimated to underlie an area of 261 km^2 in Gilbert Bay, which is only about 18% of the area where phytoplankton occurs (Figure 6A). However, areal chlorophyll levels are about 380% higher on the biostromes than in the integrated phytoplankton from the 6.7 m thick mixed layer (Figure 6B). Multiplying the areal coverage of the two habitat types by the chlorophyll concentrations indicates the total amount of chlorophyll in the two habitats. This calculation suggests that the cyanobacteria and algae on the biostromes is about 70% of that in the water column (Figure 6C). Note that this calculation does not include the contribution of chlorophyll on the expansive mud and sand substrates in the littoral zone of the lake. Although the cyanobacteria on (and in) the biostromes may not be as accessible to the brine flies as phytoplankton cells are to grazing *Artemia*, this preliminary analysis indicates that the abundant biostromes are an important component of the food web in the lake. This analysis is consistent with recent views on the importance of benthic areas for primary production and the production of invertebrates in lakes (Vadeboncoeur et al. 2002).

The brine flies on the biostromes also represent a significant component of the invertebrates in the lake and contain a large amount of the bioavailable selenium for birds (Figure 7). The biomass of brine flies we measured in June was about 30% of that in *Artemia* (Figure 7A). The seasonality of brine flies is not well known, but Collins (1980) found comparable densities of pupae on biostromes from June through August, suggesting that our measurements in June are at least indicative of the summer period. Selenium concentrations in brine flies were somewhat higher than in *Artemia* (1.5 vs. $1.2 \mu\text{g g}^{-1}$; Figure 7B), contrary to what others have found Great Salt Lake (Adams 2005 unpublished) or elsewhere (Herbst 2006). The resulting estimate of total selenium in the benthic invertebrates suggests that brine flies contain about 38% of the total selenium that is contained in *Artemia*. These comparisons, although based on relatively few samples, indicate that brine flies could be a significant source of selenium for birds in the Great Salt Lake.

In contrast to the biostromes, the sand and mud substrates we sampled had relatively few brine flies associated with them. This is consistent with the findings of Collins (1980), although he did estimate that perhaps 18% of brine fly production could occur on the expansive mud and sand sediments. The soft sediments in much of the lake may produce little periphyton for the brine flies. It is likely that the sands in shallow waters shift so much that algae cannot become well-established. Conversely, in deeper water, periphyton may have insufficient light for photosynthesis. The photic zone at the time of our survey was estimated to be only about 2 m deep (two Secchi depths), so photosynthesis would be restricted below this depth. Our survey sites, however, were located relatively close to discharges of nutrient-laden water (Farmington Bay and the Goggin Drain) from metropolitan Salt Lake City, and the resulting phytoplankton growth in these areas may shade-out periphyton. Secchi depths in the lake often increase to > 3.5 m (i.e. 7 m photic zone) when intense grazing by *Artemia* removes phytoplankton (Wurtsbaugh & Gliwicz 2002), so benthic photosynthesis may often occur throughout the littoral zone sediments. The limitation of stromatolites to depths < 3.5 m (Eardley 1938) suggests, however, that intense benthic photosynthesis may be limited to this zone. A thorough study of benthic primary production will be necessary before we fully understand this process in Great Salt Lake.

The benthic food web is a likely route for selenium transport into birds, because a large portion of the estimated 20 metric tons of selenium in the lake (water + upper 2 cm of sediments) is in the bioactive benthic zone (Table 2). A comparison of data collected by Johnson et al. (2008) and that reported here yields an approximate estimate that 67% of the selenium is in the top 2 cm of the sediments and biostromes in the lake while only 8% is in suspending particulate matter (seston) available for *Artemia* to graze on, and 25% is dissolved in the water (Table 2). Biostromes contain an estimated 4% (upper 2 cm) of the selenium. A large portion of the selenium (46%) is estimated to be in the anoxic sediments below the deep brine layer. It is important to consider that the selenium in the organic material of the biostromes is potentially available to the invertebrates, whereas that beneath the deep brine layer will cycle very slowly to the oxic zone of the lake (Johnson et al. 2008) where invertebrates could take it up. The oxic sand and mud substrates, with an estimated selenium content of 17% of the lake total, could potentially be important for the transfer of selenium to invertebrates. However, the very low numbers of brine flies found on the sand and mud suggests that even the organic selenium in soft sediments would not be utilized to any significant degree. This comparison between the different lake zones is based on a small number of benthic samples from the oxic zone, and several assumptions, so it is clear that more research is needed in order to construct a true estimate of selenium in the different compartments.

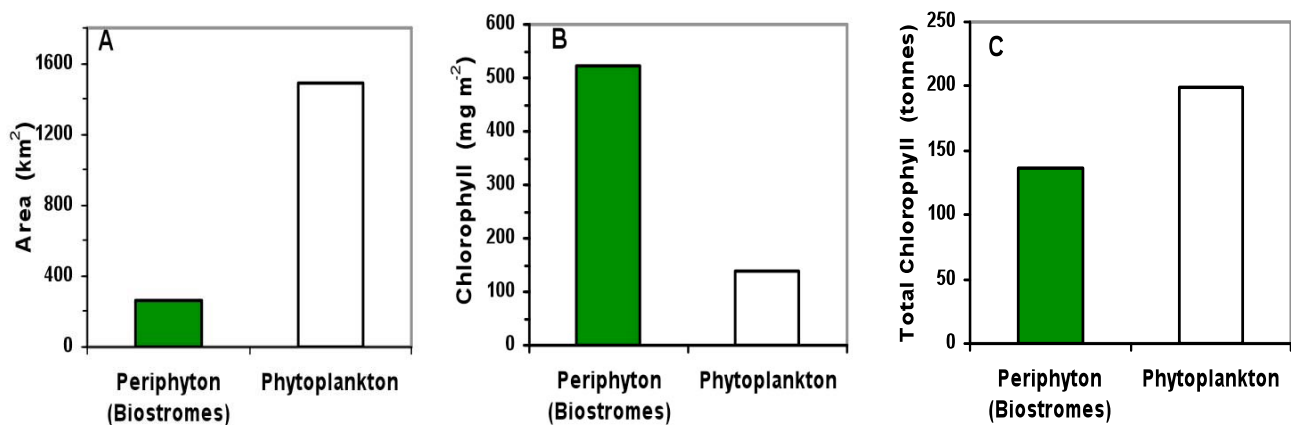


Figure 6—A. Comparison of the area covered by periphyton on biostromes (solid fill) and that of the epilimnion of Gilbert Bay where phytoplankton can grow (open). **B.** Concentrations of chlorophyll on biostromes (solid fill) and that in phytoplankton in the water column (open). **C.** Total chlorophyll estimates (metric tonnes) for Gilbert Bay in periphyton attached to biostromes, and that in the phytoplankton. The latter was based on chlorophyll in Gilbert Bay calculated from data from 2002–2005 of W.A. Wurtsbaugh, and an estimated epilimnetic volume of $9 \times 10^9 \text{ m}^3$.

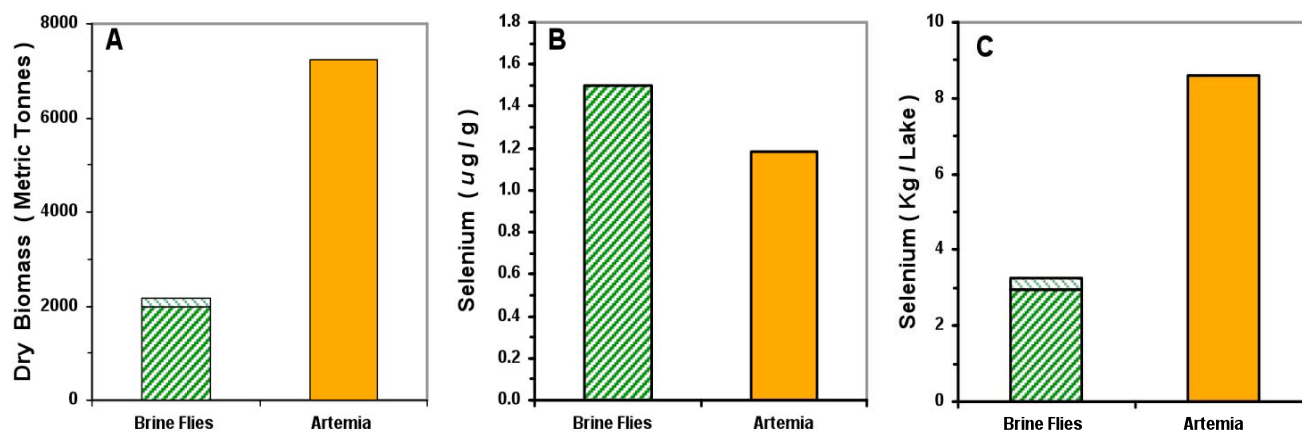


Figure 7A—Comparison of the amount of biomass in brine flies and that in *Artemia*. The brine fly data are from June 2006, and represents the sum of larvae and pupae. The dark green diagonal shading shows brine flies on biostrome substrates. The light green shading represents brine flies on soft sediments. B. Mean selenium concentrations in brine fly larvae and pupae and that in adult *Artemia* (Marden 2008). Selenium concentrations were 2–4X lower in juvenile *Artemia*, but the data from adults was used because: (1) they are likely the main prey of birds, and; (2) under most circumstance they would dominate the biomass. C. Estimated total amount of selenium in brine fly larvae and pupae on biostromes (dark green shading), on soft sediments (light green shading), and in *Artemia*. The *Artemia* data are based on an April–December mean dry biomass of 0.75 mg l^{-1} (data of Brad Marden). Estimates of brine fly biomass and selenium concentrations are based on limited samples.

If the mean estimated selenium content in the biostrome organic material collected in 2007 is correct ($1.0 \mu\text{g Se g}^{-1}$) there appears to be little or no biomagnification up the benthic food web (Figure 5). The selenium content of organic material from biostromes measured in 2006 (Appendix 1) was higher (mean, $1.7 \mu\text{g Se g}^{-1}$), so if this value were used it would suggest a negative biomagnification factor. There was, however, a 2500-fold bioconcentration from the dissolved phase ($0.4 \mu\text{g Se l}^{-1} = 0.4 \text{ ng Se g}^{-1}$ of water) into the periphyton. This bioconcentration factor is likely an overestimate, because much of the selenium reaching the periphyton biofilm does so by sedimentation of particles (Johnson et al. 2008). The mineralization of these particles likely would yield much higher dissolved selenium concentrations in the interstitial water surrounding the microbes than that in the water centimeters above the biostromes. Nevertheless, most of the accumulation of selenium into organic matter must occur between the water and microbes, because the brine fly larvae do not increase concentrations further. This is similar to the results of Brix et al. (2004), who also did not find significant biomagnification of selenium by *Artemia*, and by the relatively low concentration factors reported for selenium by Chapman et al. (1968). Grosell (2008) also found relatively low uptake of selenium by brine shrimp at high salinities, and suggested that this might be due to competitive exclusion of Se uptake by the high sulfate levels in Great Salt Lake. The slight increase in selenium concentrations from larvae to pupae to adults that we found

may be the result of modifications in fat content or other constituents, since feeding does not occur after the brine flies pupate. It is also possible that exoskeletons of pupae are low in selenium, so that molting into the adult stage would increase the selenium concentrations in the flies.

With the limited sampling reported here it is difficult to assess the spatial variations in selenium that may be present in the benthic zone of the lake. We did not anticipate finding higher selenium concentrations at the brine flies from the Bridger Bay site than at the Gilbert South site. The latter is near the discharge points of Kennecott Utah Copper Corporation and the Goggin drain where 55% of the selenium load enters the lake (Naftz et al. 2008), and where Cavitt (2008) found high selenium in shoreline organisms. In contrast, the Farmington Bay discharge near the Bridger Bay site contributes only 13% of the selenium load, and concentrations of the effluent from Farmington Bay are only 56% of those from the Goggin drain (Naftz et al. 2008). The higher concentrations of selenium in the brine flies at Bridger Bay may be attributable to the very high organic content of the effluent from Farmington Bay, which is highly eutrophic (Wurtsbaugh & Marcarelli 2006). Rosetta & Knight (1995) found that brine flies bioaccumulated selenium much faster from a dissolved organic compound than they did from selenate or selenite. Indeed, most uptake of selenium by benthic invertebrates is thought to be via incorporation of organic selenium (Presser & Luoma 2006).

Table 2—Estimates of selenium in different areas of the lake. That in the water column was based on the selenium concentrations reported by Johnson et al. (2008). Sediment estimates assume only a 2 cm thick bioactive layer, and a solids component of 33% (W. Johnson unpublished data) and a density of dry sediment and stromatolites of 1.5 g cm⁻³. Selenium concentrations in the anoxic sediments are from Johnson et al. (2008), whereas the oxic sediment concentrations are from the 2007 data reported here. The Se concentration for the biostromes is for non-acidified samples.

	Se Concentration		Area/Volume	Se (Metric Tons)	Percent
Water column (total)	0.56	μg l ⁻¹	11.4 x 10 ⁹ m ³	6.4	33%
Water column (dissolved)	0.42	μg l ⁻¹	11.4 x 10 ⁹ m ³	4.8	25%
Water column (particulate)	0.14	μg l ⁻¹	11.4 x 10 ⁹ m ³	1.6	8%
Sediments (total)			2057 km ²	13.1	67%
Sediments (anoxic)	1.0	μg g ⁻¹	912 km ²	9.0	46%
Sands (oxic)	0.3	μg g ⁻¹	712 km ²	2.0	10%
Muds (oxic)	0.8	μg g ⁻¹	172 km ²	1.3	7%
Biostromes	0.3	μg g ⁻¹	261 km ²	0.9	4%
Total				19.5	

The selenium content of brine flies is important because birds in Great Salt Lake feed on them extensively (Figure 8). Cavitt (2008) found that brine fly larvae comprised 20-100% of the diet (by volume) of American avocets (*Recurvirostra americana*) sampled at different sites prior to or during nesting at Great Salt Lake. The highest proportion of larvae in the diets occurred at Antelope Island where biostromes and dense brine fly populations occur close to shore. The lowest proportion of brine fly larvae in the avocet diets occurred in Ogden Bay where the mud flats are distant from biostromes and where fresher water allows other prey to be abundant. A small sample of black-necked stilts (*Himantopus mexicanus*) at the Ogden Bay site also suggested that brine fly larvae could be an important diet item for them. *Artemia* were absent from the diets of both of these birds. In contrast, Conover et al. (2008) found that the diets of California gulls (*Larus californicus*) were composed of 45-83% *Artemia* at his three study sites, and brine flies represented a maximum of 25% of the diet. Diet sample sizes were small for the birds, so these are only approximate proportions, and they represent only the short early or pre-nesting period when selenium in prey items can be passed to eggs. Brine flies are also important component diets of eared grebes (*Podiceps nigricollis*) in saline lakes (Jehl 1988). Additionally, red-necked phalaropes (*Phalaropus lobatus*) at Mono Lake feeding on brine flies maintained their weight, whereas those feeding only on *Artemia* lost weight (Rubega & Inouye 1994). The high fat levels and energy content of the Mono Lake brine flies

makes them a good prey item for birds, but Caudell and Conover (2006) found that brine flies from Great Salt Lake had lower caloric densities than did *Artemia*. The dominant brine fly in Great Salt Lake (*E. cinerea*) is also considerably smaller than the *E. hians* at Mono Lake, so it is possible that the flies in Great Salt Lake may not be utilized as extensively by birds as are the brine flies in Mono Lake.

Selenium concentrations in birds that feed extensively on brine flies are moderately high and may potentially cause physiological impairment. The diet of common goldeneye ducks is composed of approximately 70% brine fly larvae and pupae (Vest et al. 2008; J. Vest, personal communication). Mean selenium concentrations in the livers of these birds increased from < 3 to nearly 10 μg g⁻¹ during their winter residence at Great Salt Lake (Vest et al. 2008). Selenium concentrations of 3 μg g⁻¹ and 10 μg g⁻¹ are respective thresholds for reproductive and health-impairment in mallards (Heinz 1996; Heinz et al. 1989). Goldeneye do not breed at Great Salt Lake and since selenium is lost quickly from the body, the high concentrations of this metal would not likely impair reproduction in the spring. The relative sensitivity of goldeneye and mallards to selenium is not known, so it is uncertain whether the high concentrations of this toxicant in the birds late in the year could be impairing their health. Goldeneye also accumulate very high concentrations of

mercury while residing at Great Salt Lake, suggesting that brine fly larvae and pupae may be a pathway for the uptake of this metal. American avocets in the lake have moderately high concentrations of selenium in their blood and livers (Cavitt 2008), and there was a negative correlation between selenium concentrations in the liver and the mass of the bird. Reproductive success of the birds, however, was high and apparently not impaired by their moderately high selenium concentrations in their tissues. Additionally, the concentrations of selenium in brine flies in our study (mean, $1.5 \mu\text{g g}^{-1}$), and the similar concentrations found at most study sites by Cavitt (2008), were below thresholds reported by Lemley (1996) to cause impairment if eaten by birds. Additional physiological and diet data for the birds, as well as more thorough analysis of seasonal changes in the metal concentrations in their prey are warranted to explain these discrepancies.

In conclusion, this study demonstrated that biostromes and their associated brine fly grazers are an important component of the food web in Great Salt Lake. In contrast, the open mud and sand substrates appear to produce few brine flies, but more work in different parts of the lake is

needed to confirm this. The brine flies produced on the biostromes are an important component of the diet of some birds that may be impacted by selenium, and for a number of other species that utilize the lake. Although these conclusions appear sound, our results were based on relatively few samples collected primarily in the spring. Additional research is needed to better characterize the seasonal and spatial variability in the benthic habitats in Great Salt Lake. Whereas *Artemia* and their phytoplankton food resources in the lake have been studied extensively over the past decades, very little work has been done to understand the brine flies and their biostrome habitats. Increasing eutrophication of Gilbert Bay may alter light penetration and influence the relative contribution of benthic and pelagic algae to the food web. Specific projects that need to be considered include: (1) detailed mapping of the benthic characteristics in the lake, and in particular, the distribution of different types of biostromes in the lake; (2) analysis of the spatial and temporal distribution of brine flies over an annual cycle, and (3) additional analyses of linkages between the periphyton, brine flies and the birds that feed on them.

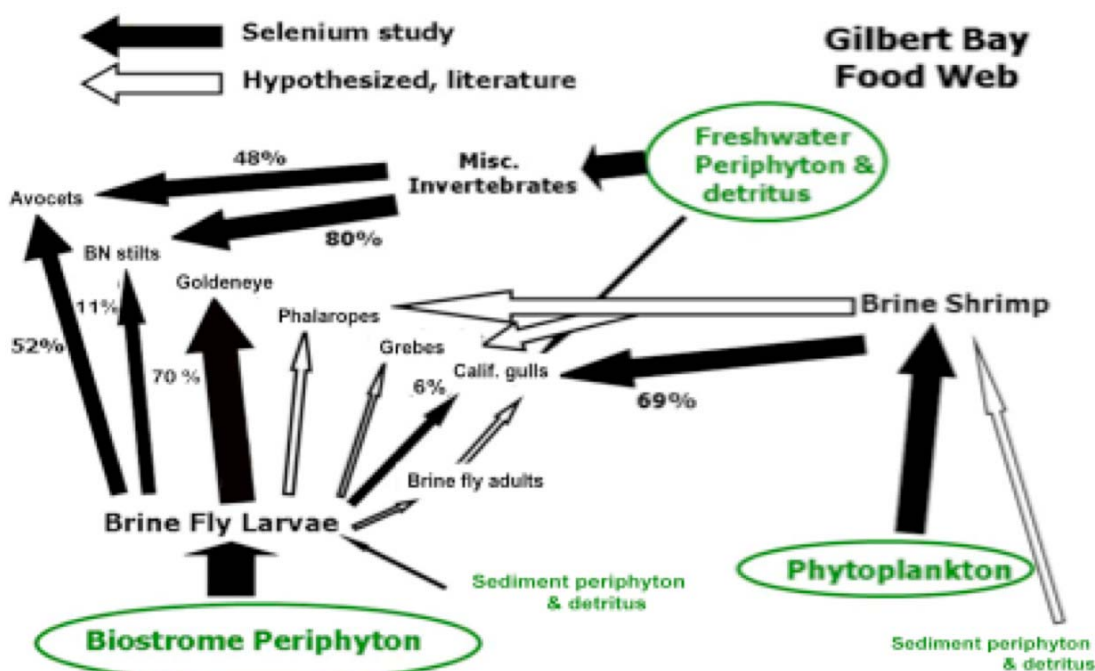


Figure 8—The food web in Gilbert Bay with an emphasis on pathways leading to birds that utilize the lake. The width of the arrows indicates the importance of a pathway. Solid arrows show data taken during the 2006–2007 selenium study (Conover et al. 2008; Cavitt et al. 2008; Vest et al. 2008). Open arrows are hypothesized pathways based on studies in other saline lakes and ponds. The Freshwater Periphyton and Detritus pathway occurs on the mud flats of Gilbert and Farmington Bays. Species codes and sample sizes: Avocets—American avocets (12); BN stilts—Black-necked stilt (4); Goldeneye—Goldeneye ducks (> 100); Calif. gulls—California gulls (53); Grebes—Eared grebes. Note the small sample sizes for some species.

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Appendix 1–Selenium and ash-free dry mass content (AFDM) of benthic substrates at two sites in Gilbert Bay of Great Salt Lake. Some dredge samples included the upper 50 mm of material, but some samples were sectioned to determine if Se content varies with depth. The visual estimate made by SCUBA divers of the percentage of biostrome, sand and mud is given. Some samples were acidified to remove carbonates. The data from 2007 are considered more reliable.

Site	Sample Date	Latitude	Longitude	Depth (m)	Substrate Type	Sediment strata (mm)	% Bio-strome	% Sand	% Mud	Acidified	AFDM %	µg Se/g dry wt
Bridger Bay	16-Jun-06	41.036	112.323	3.9	Mud	0-50	2	98	0	Y	2%	9.80
Bridger Bay	16-Jun-06	41.034	112.325	5.0	Mud	0-50	2	10	88	Y	6%	3.10
Bridger Bay	16-Jun-06	41.034	112.325	5.0	Mud	0-3	2	10	88	Y	7%	3.30
Bridger Bay	16-Jun-06	41.034	112.325	5.0	Mud	0-50	2	10	88	Y	10%	1.40
Bridger Bay	14-Jun-06	41.043	112.276	1.0	Biostrome		95	5	0	N		0.40
Bridger Bay	14-Jun-06	41.043	112.276	1.0	Biostrome		95	5	0	Y	56%	2.10
Bridger Bay	14-Jun-06	41.043	112.276	1.0	Biostrome		95	5	0	Y		0.90
Bridger Bay	14-Jun-06	41.043	112.276	3.0	Biostrome		95	5	0	N		0.60
Bridger Bay	14-Jun-06	41.043	112.276	3.0	Biostrome		95	5	0	Y	61%	2.20
Bridger Bay	14-Jun-06	41.043	112.276	3.0	Biostrome		95	5	0	Y		1.30
Gilbert South	15-Jun-06	40.802	112.163	2.7	Biostrome		100	0	0	N	58%	3.10
Gilbert South	15-Jun-06	40.802	112.163	2.7	Biostrome		90	10	0	N	59%	-
Gilbert South	15-Jun-06	40.810	112.183	3.2	Biostrome		90	10	0	N	52%	0.40
Gilbert South	15-Jun-06	40.810	112.183	3.2	Biostrome		40	60	0	Y	60%	1.30
Bridger Bay	28-Apr-07	41.041	112.279	2.5	Mud	3-40	0	0	100	N	15%	0.90
Bridger Bay	28-Apr-07	41.041	112.279	2.5	Mud	0-3	0	5	95	N	14%	0.60
Bridger Bay	28-Apr-07	41.043	112.276	3.0	Mud	3-30	0	0	100	N	9%	0.70
Bridger Bay	28-Apr-07	41.043	112.276	3.0	Mud	0-3	0	0	100	N	14%	0.70
Bridger Bay	28-Apr-07	41.043	112.271	1.0	Sand	3-50	0	100	0	N	3%	0.20
Bridger Bay	28-Apr-07	41.043	112.274	1.0	Sand	3-50	0	100	0	N	4%	0.30
Bridger Bay	28-Apr-07	41.043	112.274	1.0	Sand	0-3	0	100	0	N	4%	0.30
Bridger Bay	28-Apr-07	41.043	112.271	1.0	Sand	0-3	0	100	0	N	3%	0.30
Bridger Bay	28-Apr-07	41.043	112.275	1.0	Biostrome		100	0	0	N	32%	0.3
Bridger Bay	28-Apr-07	41.041	112.279	2.1	Biostrome		100	0	0	N	27%	0.3
Bridger Bay	28-Apr-07	41.043	112.276	3.0	Biostrome		100	0	0	N	29%	0.4
Bridger Bay	28-Apr-07	41.043	112.275	1.0	Biostrome		100	0	0	Y	69%	0.6
Bridger Bay	28-Apr-07	41.041	112.279	2.1	Biostrome		100	0	0	Y	71%	1.1
Bridger Bay	28-Apr-07	41.043	112.276	3.0	Biostrome		100	0	0	Y	73%	1.2

Microbial Biodiversity of Great Salt Lake, Utah

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ABSTRACT

Microbial biodiversity is difficult to measure in extreme environments due to the inability to culture many of the species, especially from hypersaline environments. Great Salt Lake (GSL), Utah, USA offers a unique ecology to study microbial diversity across a salt gradient. GSL has increasing salt from South to North that varies from marine salt concentrations to saturation, respectively. We used three methods to examine the biodiversity of the GSL—traditional cultivation on solid media, 16S rRNA gene sequencing, multiplexed 16S rRNA gene hybridization to the phylochip, and DNA hybridization to the Geochip for metabolic diversity estimates. Over 40 isolates from the North Arm were obtained, while six were selected for identification. Isolates included gammaproteobacteria, bacilli, and actinobacteria. Sequencing the 16S rRNA genes for identification yielded 350 clones. Rarefaction curves indicated that this did not represent the bacterial diversity of the GSL, while estimation of the diversity with the Affymetrix phylochip produced over 1000 different genera in 31 different families. Estimation of the metabolic diversity found that genes for each activity were present in all three locations. The gene abundance was similar in all locations, except for metal use where the gene abundance declined as the salt gradient declined. This study provides the first evidence of the large microbial diversity supported by GSL to provide a large metabolic potential independent of the salt concentration.

INTRODUCTION

Approximately 1.5×10^4 years ago, Lake Bonneville, a late Pleistocene lake, reached a size of 5.2×10^4 km² before suddenly discharging an immense volume of water to the north into what is now Utah and Idaho. This flood was caused by capture of the Bear River, which greatly increased the supply of water to the Bonneville Basin. Today's Great Salt Lake (GSL) is a large remnant of the ancient Lake Bonneville, and occupies the lowest depression in the Great Basin. GSL is the largest U.S. lake West of the Mississippi River, the 4th largest terminal lake in the world, and the world's second most saline lake

(Hassibe & Keck 1993; Stephens 1997; Aldrich & Paul 2002; Gwynn 2002).

Much of the salt contained in GSL was originally in the water of Lake Bonneville. Today, about two million tons of dissolved salts enter the lake each year. While GSL is typically 3- to 5-times more saline than the ocean, with the exception of sulfate, it contains roughly the same mixture of salts (Gwynn 2002). In contrast to the divalent cation-rich Dead Sea (Post 1977), GSL is a sodium chloride lake with an exceptionally high sulfate concentration (10-20 g/l) (Whelan 1973).

Industrialization of the Wasatch Front, a 100 mile-long urban corridor stretching from the cities of Provo to Ogden, led to utilization of GSL for recreation, agriculture, mining, oil exploration, railroad connection, and brine shrimp harvests. The GSL ecosystem receives industrial, urban, mining, and agricultural discharge from a 3.8×10^4 km² watershed inhabited by more than 1.8 million people. To meet the demands of industrialization, an East-West railroad causeway was completed across the lake in 1959 (Waddell & Bolke 1973; Cannon & Cannon 2002). The causeway restricts the movement of the water between the North and South arms, effectively dividing the lake into two ecosystems based on the salt gradient.

Freshwater flows into the lake in the South Arm via three rivers. Adjacent to the causeway, the South Arm has a salinity of ~17‰ - about four times saltier than the ocean. The North Arm has no significant freshwater influx and is become saturated with the salt concentration nearing 27-30‰. With construction of the causeway, GSL has become at least two different ecosystems linked through breaches in the causeway to create a halocline in two dimensions. The NaCl concentration changes from North to South as well as with depth, known as the “deep brine layer”. The size of the deep brine layer changes with local environmental conditions, such as temperature and wind speed. The fluid dynamics of the halocline restricts the mixing, thereby creating multiple non-homogeneous environments of varying NaCl concentrations across the Lake from North to South and from surface to sediments.

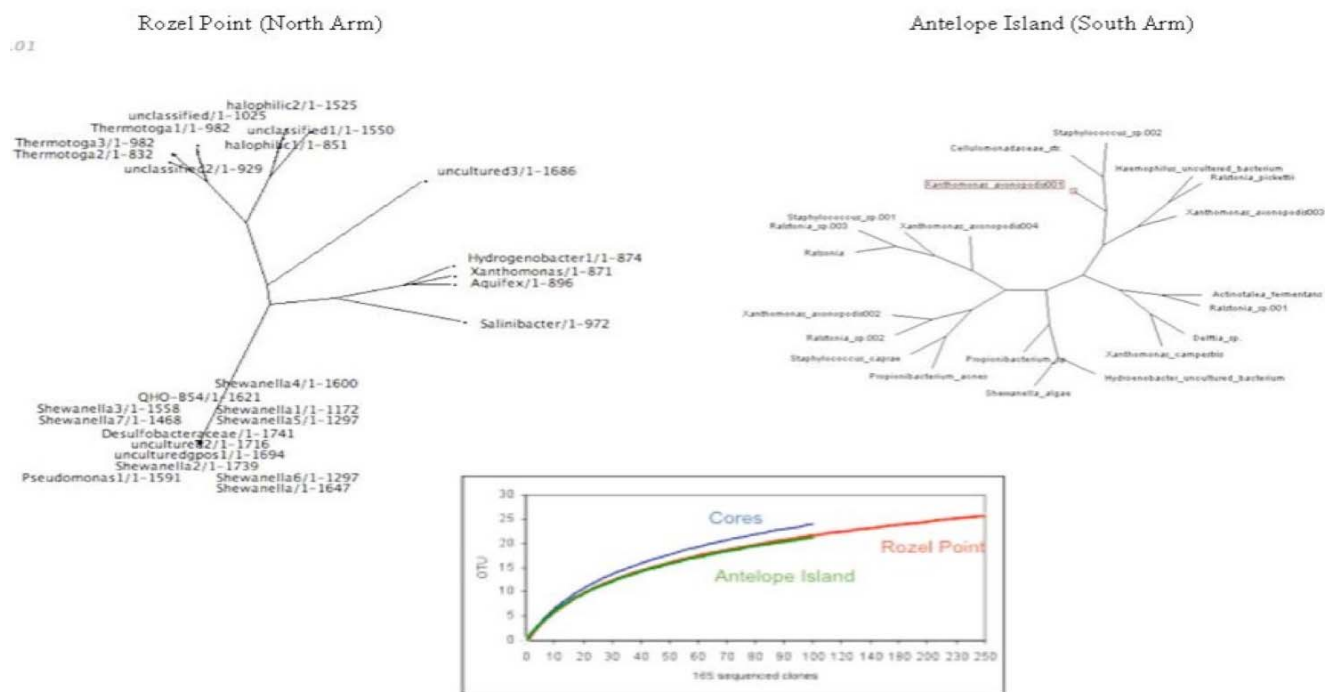


Figure 1–Diversity of the organisms determined using a DNA library from the total DNA isolation from two locations in GSL based on the salt concentration. Each sequence was determined using a full-length sequence of the 16S rRNA gene. The rarefaction curve was done for each location and compared to the GSL core sample from 100 cm. In all cases, the diversity was not adequately sampled to accurately determine the microbial diversity in the location.

The lake offers a variety of unique microenvironments (e.g. petroleum seeps, thermal springs, salt and freshwater springs, mudflats, deep brine layers, and deep sediments that date to prehistoric Lake Bonneville). Each of these conditions found in the lake offers interesting combinations of physical, chemical, and biological features that are linked to salinity as a spatial and temporal variable that creates gradient and boundary effects. Together, these physical and biological features shape the overall flora and fauna of GSL today.

The extent of human interaction with GSL also adds a new dimension to the study of the microbial ecology of GSL. These features make GSL a unique environment that is understudied with respect to the microbiota that underpin the ecological web in the lake, especially the geochemical cycling specific to the heavy metal and sulfur content of this unique ecosystem. The diverse conditions and gradients of salinity, temperature, and other physical features are the motivations to examine the GSL nutrient and element cycles in the context of the lake's genetic potential and metabolic interactions between the living communities of the lake.

We initiated experiments to determine the microbial diversity in GSL with the larger goal of understanding

microbial metabolism and survival across the salt gradient. We hypothesized that the microbial diversity would be low and dominated by relatively few types of organisms. However, we determined the microbe population to be rich and diverse with little variation along the salt gradient between families of microbes. Specific strain diversity changed between the study sites with an increase in archaea as the salt content increased. This study found that GSL supports a diversity of microbes with the metabolic diversity to maintain metabolic activity at all study sites around the lake.

MATERIALS AND METHODS

Sample Collection

In the summer of 2007, water samples were collected from different sites throughout GSL: in the North Arm at Rozel Point (saturated salt), in the South Arm at Antelope Island (17% salt) and at Farmington Bay (10% salt). Samples were collected in sterile 4 l plastic bottles (Nalgene, Fisher Scientific, CA). In addition, 50 ml of sample from each site was collected in sterile tubes with minimal headspace for subsequent compound analysis. Within 6 hours of collection, samples were refrigerated at 4°C until processing.

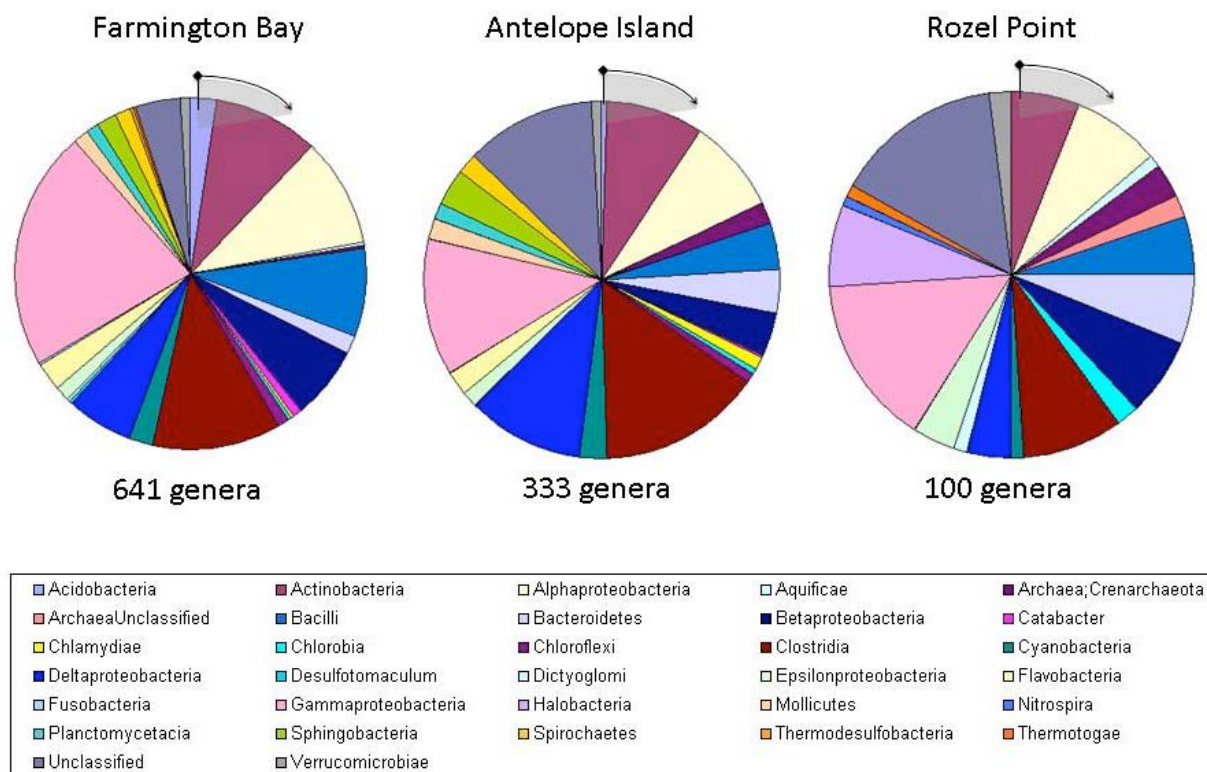


Figure 2—Microbial diversity of three locations in GSL using the phylochip. The arrows indicate the start point (short bar) and the direction of the legend (arrow around the pie chart). The legend is organized from left to right in rows.

Bacterial Isolation

The water and sediment samples from each location were plated on LB (Difco, MI) agar and incubated overnight at 30°C. Colonies on the plates were picked, re-streaked on new LB agar, and checked for purity by performing a Gram-stain and visual inspection of individual colonies.

DNA Isolation

Total genomic DNA from the hypersaline waters of GSL was extracted utilizing a modified protocol published by Griffiths et al. (2000). Briefly, 1 gallon (3.85 l) of water collected from GSL was centrifuged (10000 x g, 40 min, 4°C) in a Sorvall high-speed centrifuge and the cell pellet resuspended in 500 µl of modified CTAB (hexadecyltrimethylammonium bromide) extraction buffer (equal volumes of 10% CTAB in 0.7 M NaCl with 240 mM potassium phosphate [pH 8]). Bead beating was used to lyse cells and DNA was extracted with chloroform and precipitated with sodium acetate and ethanol. The extracted community DNA was purified through a Sephadryl S-300 column. Briefly, the column was constructed by plugging a 5 ml syringe with sterile glass wool, pouring 5 ml of resin suspended in 24% ethanol into the syringe and centrifuging 10 minutes at 1000 x g at room temperature. The column

was washed twice with sterile ddH₂O. Community DNA samples were added to the column and purified by centrifugation for 10 minutes at 1000 x g at room temperature.

16S rRNA Gene Sequencing

To assess microbial diversity DNA was extracted using a modified protocol described by Griffiths et al. (2000). The extracted DNA was purified by passing it through a Sephadryl S-300 column and used to amplify the 16S rRNA gene using Archaea or Bacteria universal primers. The products were sequenced at the Center for Integrated BioSystems Genomics Core (CIB) (Logan, UT).

Phylochip Analysis

We used the 16S Phylogenetic Array (phylochip; Affymetrix, Santa Rosa, CA, USA) containing probes for 8741 Bacterial and Archaeal taxa as described by Brodie et al. (2007). Hybridization of the phylochip is achieved using slightly modified Affymetrix protocols. Briefly, the 16S rRNA genes were amplified by PCR with Bacteria (F: 5'-AGAGTTTGATCCTGGCTCAG-3', R: 5'-ACGGCTACCTTGT AGCACTT-3') or Archaea (F: 5'-GACGGGCGGTGTGTCA-3', R: 5'-GCGGATCCGCGGCCGCTGCAGAYC-3') specific

primers. To minimize the primer bias, PCR amplification was performed with a temperature gradient from 48°C to 58°C for the annealing temperature. The PCR products from the different amplification reactions were collected, purified and quantified. The rRNA (200 ng) amplicon was fragmented by DNaseI digestion for 20 minutes at 25°C. The DNaseI was inactivated and the fragmented DNA biotin labeled for 60 minutes at 37°C following the Affymetrix protocol. The labeled DNA was added to Affymetrix hybridization solution and hybridized to a phylochip for 16 hours at 48°C rotating at 60 rpm. The chip was washed and stained following the Affymetrix protocol and scanned utilizing an Affymetrix ChiScanner 3000 at the CIB Affymetrix Core Laboratory. The hybridization intensity values and probe set annotations were merged (.gif file). The chips were normalized with R with robust multichip average (RMA) (Irizarry et al. 2003).

Statistical Analysis and Data Visualization

RMA normalized data were analyzed using SAM (Version 2.01) (Tusher et al. 2001) with a one class time course experimental design using the xCluster R module (Center for Integrated BioSystems, Logan, UT; <http://cib-xcluster.biotech.usu.edu/>). The gene expression changes were transformed to the \log_2 ratio, and were calculated by determining the difference in \log_2 intensity of a single time point with the preceding time point. Any gene with a \log_2 ratio of at least ± 0.58 , which is equivalent to a ± 1.5 fold change, and $q < 0.3$ was considered significant (Storey & Tibshirani 2003). The entire experiment was completed in two biological replicates.

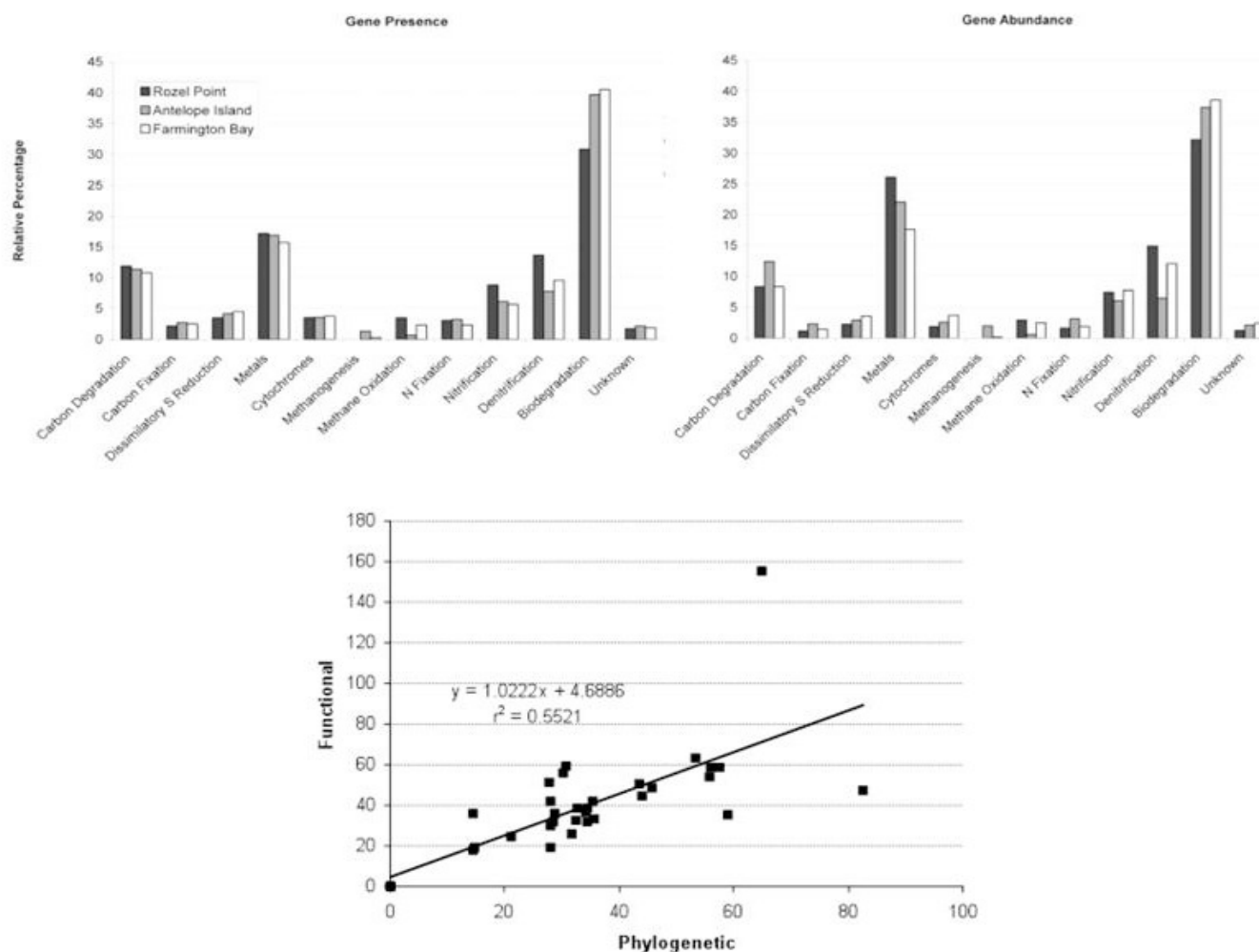


Figure 3—Estimation of the biological processes in GSL using the GeoChip. Gene presence was determined by a hybridization event, while the gene abundance was determined by measuring the hybridization intensity on the chip. Estimation of the connection between the GeoChip and phylochip is depicted as the correlation between the functional and phylogenetic assays, respectively.

Table 1—Bacterial isolates from the North Arm of GSL.

Predicted Identification ¹	Sample location	Linage	Identity to 16S rRNA sequence (%) ²	Phylochip probe set hybridization identification (FB:AI:RP ratio) ³
<i>Micrococcus luteus</i>	Rozel Point (CL1)	Actinobacteria; Micrococcaceae	99	HN2-11 (1.8:1.2:1) B-P26 (1:1:1)
<i>Salinivibrio costicola</i>	Rozel Point (MC-A)	Gammaproteobacteria; Vibrionales	99	DSM8285 (1.5:2.1:1) ATCC35508T (0.8:1:1)
<i>Morganella morganii</i>	Rozel Point (C-2)	Gammaproteobacteria; Enterobacteriaceae	98	C3 (1:1:1) AP28/C5 (1.7:1.7:1) Ju27C4 (1.4:1.2:1) Sludge (1.3:1.3:1) ATCC35200 (1.2:1.2:1)
<i>Planococcus maritimus</i>	Rozel Point (CL2)	Bacilli (Firmicutes); Planococcaceae	97	TF-9 (1.4:1.5:1)
<i>Halomonas venusta</i> -like	Rozel Point (MC-B)	Gammaproteobacteria; Halomonadaceae	77	17 <i>Halomonas</i> found. This species was not represented on the chip.
<i>Morganella morganii</i> -like	Rozel Point (RC-1)	Gammaproteobacteria; Enterobacteriaceae	54	C3 (1:1:1) AP28/C5 (1.7:1.7:1) Ju27C4 (1.4:1.2:1) Sludge (1.3:1.3:1) ATCC35200 (1.2:1.2:1)

¹Identification was based on the full-length identity from the closest match. We defined that a tentative identification was limited to the identity measure listed in the table. ²Based on full-length sequence. ³FB = Farmington Bay, AI = Antelope Island, RP = Rozel Point; Probe set identifications are denoted as annotated on the Phylochip. In all cases the probe set was used for the lineage identification.

Geochip Analysis

To determine the functional genomic capabilities of the microbial communities within the GSL, we used the GeoChip functional gene array (Zhou et al. 2002; He et al. 2007). Extracted community DNA (no amplification step) was labeled with cystidine-5 (Cy-5) dye prior to hybridization. Briefly, approximately 2 mg of genomic DNA was denatured for 5 minutes at 99.9°C in solution with 0.1 mM spermidine and random octamer mix (Invitrogen, Carlsbad, CA, USA) and snap chilled on ice. Following denaturation, 2.5 mM dithiothreitol (DTT), 0.25 mM dATP, dCTP and dGTP, 0.125 mM dTTP, 0.125 mM Cy5-dUTP, and 80 U Klenow fragment (Invitrogen) were added. Reaction mixtures were incubated at 37°C overnight. Labeled target DNA was purified with a QIAquick PCR kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Labeled DNA was measured on a ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and dried using a speed-vac at 45°C for 45 minutes. Dried, labeled DNA was resuspended in a solution of 50% formamide, 5 x sodium saline citrate (SSC), 0.1% sodium dodecyl sulfate (SDS), 0.1 µg µl⁻¹ salmon sperm DNA and 0.02 mM spermidine and incubated at 95°C for 5 minutes. Labeled reactions were kept at 60°C until hybridization. Community DNA hybridizations were performed using a HS4800

Hybridization Station (TECAN US, Durham, NC) and hybridization conditions were followed as indicated elsewhere (Yergeau et al. 2007). GeoChip microarrays were scanned using a ProScanArray microarray scanner (PerkinElmer, Boston, MA) as mentioned by Yergeau et al. (2007). Scanned images were analyzed using ImaGene 6.0 software (BioDiscovery, El Segundo, CA, USA). GeoChip array results were normalized as established by Yergeau et al. (2007).

RESULTS AND DISCUSSION

Bacterial Isolation

The initial experiments attempted to isolate organisms from multiple locations in GSL. We obtained 40 bacterial isolates from all locations that varied in colony color, size, and morphology. Six of the isolates were selected for identification using 16S rRNA gene sequencing (Table 1). Full-length sequence indicated that two new isolates were found that were identified to be *Halomonas venusta* and *Morganella morganii* (Table 1). The isolates represented members of the Actinobacteria, Gammaproteobacteria, and Firmicutes. Isolation attempts provided only organisms that we capable of growing on common, but nutrient restricted agar-LB or marine agar. Since the media and growth conditions were limited, the number and type of colonies

likely under-represented the total number of microbes capable of growth from GSL. This is also supported by the fact that what we isolated did not match isolates from studies, but are possible from this environment.

Phylogenetic Diversity Measure

The ease with which we isolated different organisms from the North Arm led us to question the diversity estimate of our sample locations in GSL—Rozel Point (North Arm) and Antelope Island (South Arm), and Farmington Bay (South Arm). To provide a larger view of the microbe population in GSL we turned to growth-independent methods. Examination of the microbial community in each location was done using total DNA isolation, cloning, and 16S rRNA gene fragment sequencing. The 16S rRNA gene sequences showed presence of numerous distinctly different organisms between the North Arm and the South Arm, but only different types of organisms were found at each location (Figure 1). This led to an estimation of the diversity using rarefaction curves for each location. The operational taxonomic unit (OTU) estimation was rising after 100 samples from the South Arm and 250 samples from the North Arm, suggesting our hypothesis that the total diversity was underestimated by the sequencing effort. The diversity of GSL was unexpectedly high, even in saturated NaCl of the North Arm. Interestingly, none of the organisms we isolated were found in the 16S rRNA gene sequencing experiment. Based on these observations, we determined that the amount of DNA sequencing needed to estimate the total diversity in the GSL was not possible with this approach. Consequently, we used an alternative approach to measure the bacterial diversity on a larger scale.

To re-estimate the microbial diversity in GSL we used the phylochip that contains over 8500 probe sets, which estimates the diversity based on hybridization of the 16S rRNA genes to the chip. The estimate for microbial diversity was done at three locations with varying salt concentrations—Farmington Bay (6% NaCl), Antelope Island (12% NaCl), and Rozel Point (30% NaCl). Similar microbial families were represented in each location, but individual genera representation decreased with increasing salinity from 641 genera to 100 genera at Rozel Point (Figure 2). In all cases the families for the isolates found in this study were represented in all locations. The organisms found with 16S rRNA gene sequencing were also observed based on hybridization of the total DNA isolated from each location to the phylochip. Since all the previous data were included in these data we concluded that the phylochip more accurately represented the community in GSL, while the isolated cultures and 16S rRNA gene sequence alone substantially underestimated the overall diversity of GSL.

In all sample locations the diversity was larger than expected. To adequately estimate the true diversity it would be better to use a metagenomics approach, which we are doing as part of the larger project.

A number of different patterns were observed in the community dynamics due to changes in salt concentration. Most of the phyla were found in each location, such as actinobacteria, cyanobacteria, and proteobacteria; yet the hybridization intensity for specific genera changed with increasing salt. The signal intensity is a measure of the population proportion as increasing amounts of the 16S rRNA gene leads to an estimation of the proportion of the community. Consequently, we estimated the ratio of the isolates found in each location based on the signal intensity between the locations relative to Rozel Point (Table 1). In all cases the salt concentration is inversely proportional to the signal intensity ratios. At the family level, this trend did not hold true. For example, Deltaproteobacteria contained the most genera at Antelope Island and as the salt concentration increased genera of this class decreased.

New phyla appeared with increasing salt, such as Chlorobia, which was found in Farmington Bay in a small amount and it increased with the salt concentration. In other cases some families were only found in the North Arm sample (Rozel Point) - Archaea and halobacteria, as expected. In other cases specific families were at specific locations. For example, large populations of Chlamydiae were only found at Antelope Island, while *Desulfotomaculum* and *Thermodesulfobacterium* were found only in Farmington Bay. Other specific examples were found where individual organisms changed differently to that of the entire family. The community dynamics observed by investigating individual probe sets within a specific family was far more extensive than culture isolation or 16S rRNA gene sequencing predicted and we expected. We suspect that these changes represent the specific gene content of a species or strain that represent the unique metabolic processes that allow survival and growth within the stress of salt concentration.

Metabolic Diversity

The amount of community membership change observed with the phylochip led us to question the amount of metabolic diversity in the community across the salt gradient, which may begin to explain changes of individuals within a family. To broadly measure the metabolic potential of the total community DNA was hybridized from the sample locations to the Geochip. Hybridization indicated presence of the metabolic gene as

represented on the chip. In some cases, the chip contained extensive genetic diversity for single genes, while other genes for intermediary metabolic processes were only partially represented (He et al. 2007). We did not estimate the amount of expression in this study, but rather presence of the gene as an estimate of the metabolic potential. The magnitude of the signal was used to estimate the relative abundance of that gene in the community.

In all locations the metabolic presence and abundance were similar (Figure 3). Abundance of the Geochip signal provided an estimate of the number genes in a broad metabolic category. This is highlighted by the striking similarity between the gene abundance and the gene presence (Figure 3). Metal metabolism is the only category that was counter to this trend. Genes for metal use were present in all locations, but the number of genes declined ~25% as the salt concentration declined, with the lowest abundance observed in Farmington Bay. This observation suggests that the diversity of the genes for metal use become more homogenous as the salt concentration declined.

Rozel Point contained more genes for metal and nitrogen metabolism compared to the other locations. Genes for methanogenesis were not detected at Rozel Point, but were found at low intensity values in the other two locations. The similarity in presence and abundance indicates that the entire community has about the same metabolic potential in the gene content. Realization of the metabolic potential depends on the gene expression regulation for each location and the specific local conditions, which was not measured in this study.

Predictive value of the phylogenetic and metabolic estimates in combination was limited. The correlation between the two factors explained just over half of the variation ($r^2 = 0.55$), indicating that the phylogenetic diversity was not strongly correlated to the metabolic capability. Consequently, the community metabolism based on the community membership was not estimated. This observation is congruent to that of Zhou et al. (2002) where use of the Geochip in soil failed to predict the microbial diversity as well.

In conclusion, this study determined that culture methods found only a very small limited number of the families present at Rozel Point. Estimation of the phylogenetic diversity with 16S rRNA gene sequencing was limited to the depth of coverage and inadequate to fully predict the biodiversity of GSL. Use of high throughput chip

technologies found extensive diversity of organisms and metabolism. Estimation of the metabolic diversity of the community found an unexpected amount of metabolism occurring at Rozel Point, indicating that GSL has a very active and diverse microbial community that has broad capabilities to consume and produce compounds from microbial metabolism.

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Great Salt Lake, Utah east of the Wasatch Mountains, Utah. This photo is taken from the International Space Station from approximately 380 km. The railway clearly separates the north and south sections of the lake, which have different salt concentrations due to the barrier. The water level of the lake is very low: note how the northeast arm of the lake has disappeared, and how Antelope Island isn't an island at all. Date: 19 August 2003. Wikimedia Commons at en.wikipedia.org. Accessed February 2009.

Development of a Site-Specific Standard for Selenium in Open Waters of Great Salt Lake, Utah

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ABSTRACT

Great Salt Lake is a unique terminal lake located adjacent to Salt Lake City, Utah. Beneficial uses of Great Salt Lake are protected through application of a narrative clause in the state water quality standards. The Utah Division of Water Quality initiated a process in 2004 to develop a site-specific water quality standard for selenium for open waters of Great Salt Lake in response to specific concerns expressed by the public. The process the Division of Water Quality initiated included formation of a stakeholders' Steering Committee and a Science Panel to identify the required studies, manage those studies, and recommend a site specific standard. Studies were recently completed to assess concentrations and effects of selenium in five species of birds; measure selenium concentrations in water, seston, brine shrimp (*Artemia* sp.), and brine flies (*Ephydra* sp.); measure selenium loads entering Great Salt Lake; and measure flux of selenium from water to sediment, atmosphere and the food web. Information from these studies was used to "populate" the elements of a comprehensive conceptual model for Great Salt Lake that is being used to establish the site-specific standard for selenium.

INTRODUCTION

Great Salt Lake is of vital importance to resident and migratory birds, local recreation, and the brine shrimp and mineral industries. In response to this importance, and in response to increasing development pressures within the lake's watershed, the State of Utah (through the Department of Environmental Quality [UDEQ], Division of Water Quality [DWQ]) initiated a program to support the development of a site-specific, numeric water quality standard for selenium for the open waters of the lake. Those waters are currently protected for their beneficial uses through the application of the narrative standard in the state water quality standards (State of Utah 2007).

In this paper we describe the overall program for development of the selenium standard and focus specifically on the research program conducted to provide information to support that standard (CH2MHill 2008a).

STUDY AREA

Figure 1 shows the study area referred to as the "open waters of Great Salt Lake" for this project. This area is commonly referred to in the literature as Gilbert Bay or the South Arm and includes Ogden Bay and Carrington Bay. Farmington Bay, Gunnison Bay (also known as the North Arm), Bear River Bay, Willard Bay, and Stansbury Bay are not included in the study area.

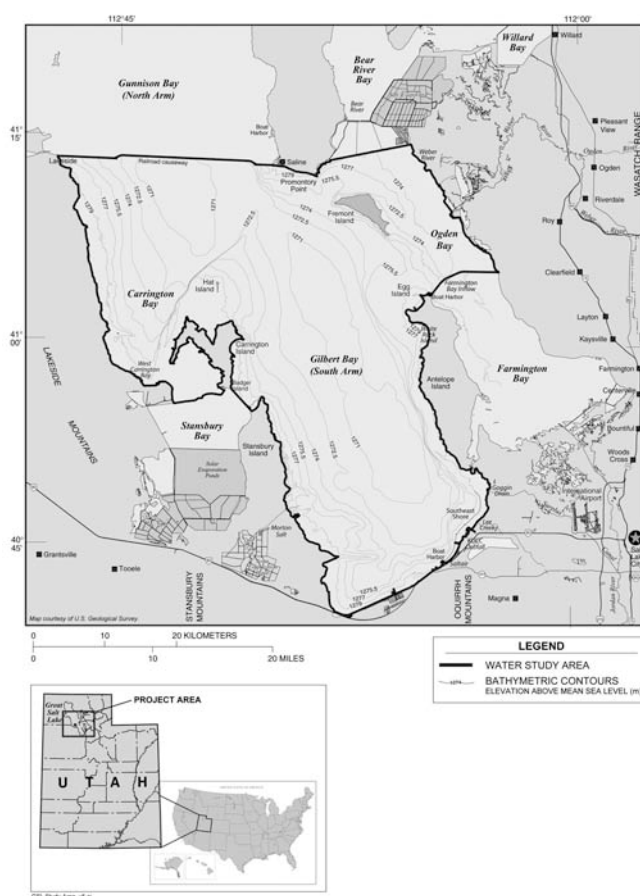


Figure 1—Great Salt Lake study area.

Need for a Site-Specific Standard

The Division of Water Quality has specified appropriate beneficial uses for waters of the State and protects those uses through the development and enforcement of water quality standards. Due to the unique geochemistry of Great

Salt Lake, the application of national fresh-water selenium water quality criteria to Great Salt Lake is inappropriate (U.S. Environmental Protection Agency [USEPA] 1987, 2004). The open waters of Great Salt Lake have instead historically been protected for their beneficial uses through the application of a narrative clause in the State water quality standards (State of Utah 2007). Any discharges directly to the lake are required to meet background concentrations in the lake, or the State has required the discharger to complete site-specific studies to establish a numeric standard that is protective of the lake's beneficial uses (Ostler 2004).

Kennecott Utah Copper Corporation (KUCC) completed studies from 2000 to 2002 that recommended a site-specific water quality standard for selenium to be included as part of their Utah Pollution Discharge Elimination System (UPDES) discharge permit to Great Salt Lake (Brix et al. 2004). These studies identified a proposed “de facto” chronic numeric standard for selenium in Great Salt Lake of 27 micrograms of selenium per liter ($\mu\text{g Se/l}$). The Division of Water Quality currently uses this selenium concentration in assessing and enforcing the Kennecott UPDES discharge permits to Great Salt Lake (UDEQ, DWQ 2004).

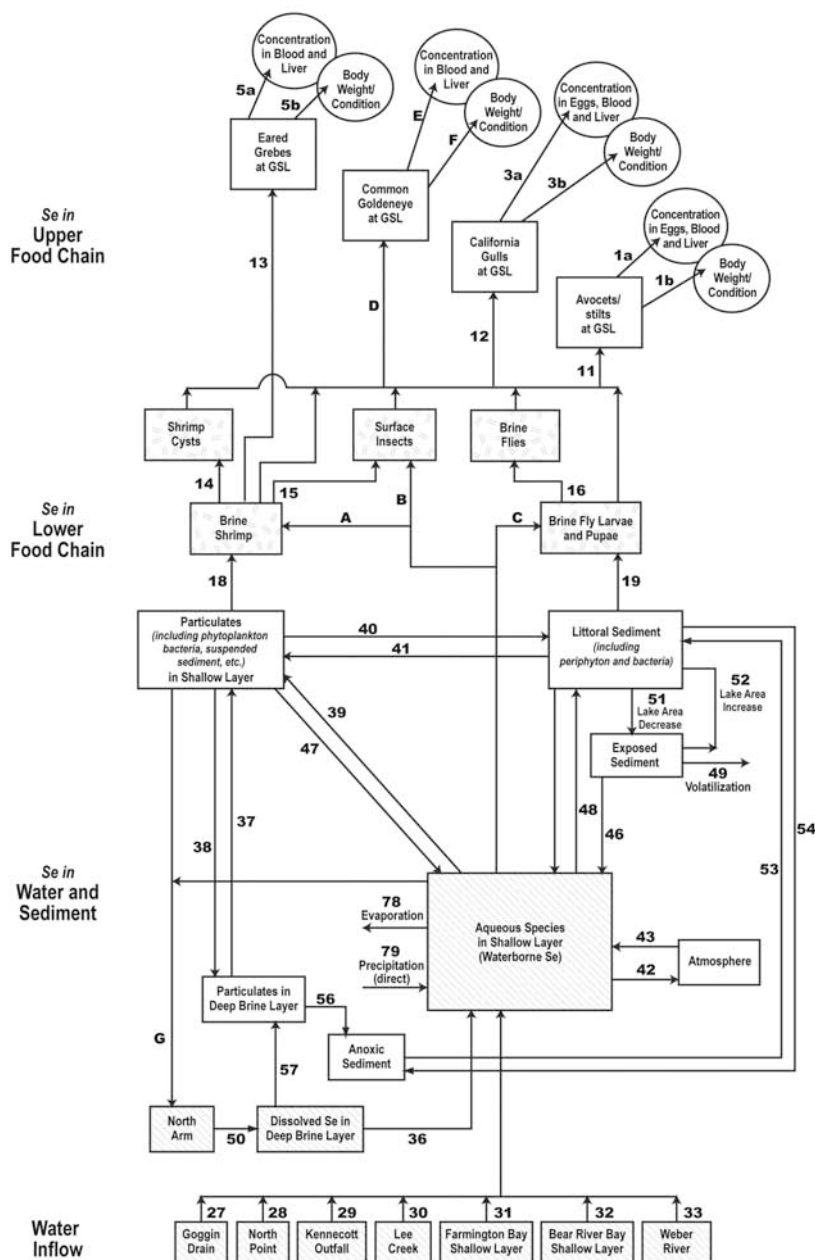


Figure 2–Simplified conceptual model for selenium (Se) cycling in Great Salt Lake. Letters and numbers along the arrows refer to linkages and transfers from one medium or trophic level to another, as described in the detailed conceptual model (Johnson et al. 2006).

Recent proposals for new discharges of wastewater to Great Salt Lake led to a recommendation that the Division of Water Quality complete additional research to verify that the discharge of wastewaters containing selenium is not harmful to the Great Salt Lake ecosystem. The Division of Water Quality convened the Great Salt Lake Water Quality Steering Committee, consisting of key stakeholders, and an expert Science Panel in 2004. Their role was to investigate and recommend a new, site-specific water quality standard for selenium for the open waters of Great Salt Lake to the State Water Quality Board, which has the authority to establish the recommended water quality standard as law.

PROGRAM DEVELOPMENT

The Division of Water Quality developed a public involvement, consultation, and coordination program (including the stakeholders Steering Committee, the Science Panel, and a public involvement program) and developed a technical program (including analytical methodologies, a conceptual model for selenium in Great Salt Lake, threshold values, and the research program) to address the need for a site-specific standard. Development of analytical methodologies and a conceptual model that characterizes selenium cycling in the study area were completed first. These were essential precursors to the research program because of the need to be able to analyze for selenium in the highly saline waters of the lake (historic measurements of waterborne selenium concentrations ranged from 20 to 200 µg/l, which are almost certainly not correct, given the results of more recent, accurate measurements) and to provide a framework for definition of research for establishment of the water quality standard.

The simplified conceptual model for selenium cycling in the open waters of Great Salt Lake (Figure 2) includes three primary components: (1) selenium in the upper food chain, (2) selenium in the lower food chain, and (3) selenium in the water and sediment. Due to the bioaccumulative nature of selenium, selenium in the system is generally recognized to originate at the “bottom” of the conceptual model (that is, from selenium in the water and sediment [abiotic component]) and move “up” through the lower food chain (food web component) and into the upper food chain (birds).

Through development of the conceptual model (Johnson et al. 2006), the Science Panel concluded that successful reproduction and body condition of birds were the two most sensitive, or critical, endpoints to be protected in preventing impairment of the beneficial uses of the study area. These critical endpoints, as represented by the reproductive success of California gulls (*Larus californicus*), American avocets (*Recurvirostra americana*) and black-necked stilts (*Himantopus mexicanus*) (species using Great Salt Lake for nesting) and the body condition of eared grebes (*Podiceps*

nigricollis) and common goldeneyes (*Bucephala clangula*) (species using Great Salt Lake during fall migration and over-wintering, respectively), would be the focus for the research program.

Toxicity threshold values for the exposure of birds to selenium at Great Salt Lake (i.e., the concentration where effects of selenium are observed) are necessary for the development of a water quality standard that is protective for them. Based on available information, the Science Panel agreed that the most significant exposure of birds occurs through their diet (brine shrimp and/or brine flies), and that the best documented and most readily monitored effects are those on reproductive success (particularly egg hatchability). The Science Panel agreed in November 2006 to define a range of selenium concentrations in bird diet items and eggs that could serve as the basis for evaluation in the research program and development of the water quality standard.

The range of values the Science Panel recommended for consideration for the water quality standard is defined by the EC₁₀ for bird diet items and eggs as defined in Ohlendorf (2003). This summary of toxicological studies showed that we can have 95 percent confidence that a 10% reduction (called an “EC₁₀”) in egg hatchability of mallards (*Anas platyrhynchos*) will occur at dietary concentrations between 3.6 and 5.7 milligrams per kilogram (mg Se/kg) with the highest probability that it will occur at 4.9 mg Se/kg (mg/kg equals µg/g or parts per million [ppm]). There is only a very small chance (2.5%) that the low or high values in the ranges provided are the true concentration where a 10% effect, or reduction in egg hatchability, occurs. A similar 10% reduction in egg hatchability in mallards will occur at egg selenium concentrations between 6.4 and 16 mg Se/kg with the highest probability that it will occur at 12 mg Se/kg. Table 1 shows these ranges of selenium concentrations in the diet and eggs and the associated best estimates for percent reduction in egg hatchability for mallards for each selenium concentration (see Table 1).

The Science Panel determined that selection of the actual water quality standard within these ranges is a question of what level of protection the State of Utah wishes to afford. It is a question of philosophy rather than science and should be determined by the Steering Committee and State Water Quality Board rather than the Science Panel. The Science Panel and Steering Committee agreed that the Science Panel would not provide an outright recommendation for a water quality standard but would (1) recommend a range of values with associated levels of reduction in hatchability and (2) provide individual recommendations of Science Panel members for a water quality standard. These items would be offered by the Science Panel for consideration by the Steering Committee and State Water Quality Board.

Table 1–Selenium concentration ranges and associated reduction in mallard egg hatchability.

Selenium Concentration (mg Se/kg dw)	Best Estimate of Reduction in Mallard Egg Hatchability		
	Most Likely	Best Case (2.5% chance of occurring)	Worst Case (2.5% chance of occurring)
For Egg			
6.4	2%	<1%	10%
12	10%	4%	26%
16	21%	10%	38%
For Diet Items			
3.6	3%	<1%	10%
4.9	10%	4%	24%
5.7	18%	10%	32%

OBJECTIVES

Using the conceptual model for selenium, the Science Panel developed a series of specific questions that would further their understanding of selenium cycling in Great Salt Lake and help them develop their recommendation for a selenium water quality standard. The central question the research program sought to resolve was stated as: “What is the acceptable waterborne concentration of selenium that prevents impairment of the beneficial uses of the open waters of Great Salt Lake?” Figure 3 illustrates five study questions that were developed to answer the central question and how they relate to the development of the research program.

RESEARCH PROJECTS

Seven projects were completed in 2006, 2007, and 2008. Detailed project data quality objectives, workplans, and standard operating procedures are found in the Selenium Program Manual (CH2MHill 2006). Detailed project background, objectives, methods, and results were documented in each project’s final report. Data and observations were integrated into a quantitative model and synthesis report as described in this document. The following projects were initiated in 2006:

- Project 1A–Determine the concentration and effect of selenium in shorebirds through the sampling of adult birds, eggs, diet, water, and sediment.
- Project 1B–Determine the concentration and effect of selenium in California gulls through the sampling of adult birds, eggs, diet, water, and sediment. Determine the concentration and effect of selenium in eared grebes and common goldeneyes through the sampling of adult birds when they arrive at Great Salt Lake and before leaving the lake.

- Project 2A–Synoptic survey of selenium in periphyton and brine fly larvae from the benthic zone (that is, lake bottom).
- Project 2B–Synoptic survey of selenium in water, seston (that is, suspended material including algae), and brine shrimp.
- Project 3–Measurement and modeling of selenium loads to Great Salt Lake.
- Project 4–Measurement of selenium flux to and from sediment and atmosphere.

A review of initial data collected for each of the projects in 2006 identified the need for additional studies to be completed in 2007. These included:

- Project 1A–Repeat a subset of the 2006 sampling program in 2007 with the addition of analysis of samples for mercury.
- Project 1B–Repeat a subset of the 2006 sampling program in 2007 with the addition of analysis of samples for mercury and the sampling of a gull colony at a freshwater location.
- Project 2B–Continue 2006 sampling program through July 2007.
- Project 4, Volatilization–Directly measure volatilization on the open waters of Great Salt Lake to verify estimates of selenium loss to the atmosphere.
- Project 4, Sedimentation–Collect additional shallow and deep sediment cores to verify sedimentation rates and permanent burial of selenium in sediment, and also to identify any variation in sedimentation rates throughout the South Arm (i.e. sediment focusing processes).
- Project 5–Complete kinetic studies in the laboratory to define the transfer of selenium from water and diet to brine shrimp.

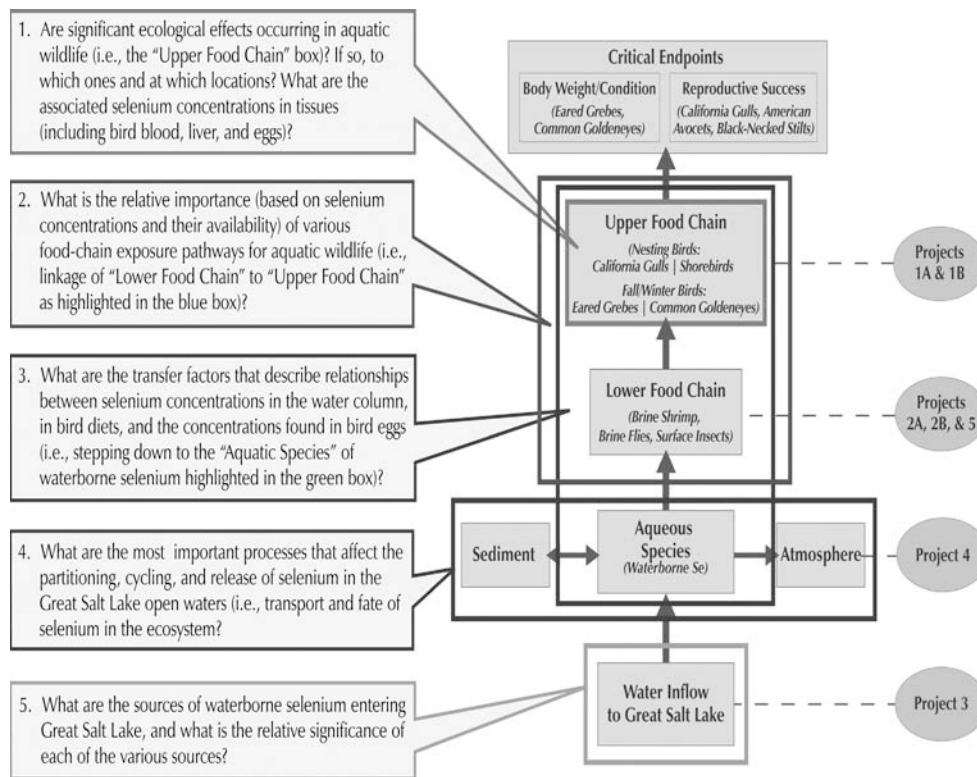


Figure 3—Program questions relative to projects.

RESULTS

The following represents a brief summary of key results from each project.

PROJECT 1—UPPER FOOD CHAIN

Shorebirds

American avocets and black-necked stilts were found to have a mixed diet of invertebrates from both fresh water and saline water sources along the shoreline of Great Salt Lake (Cavitt 2008a, 2008b). Brine fly larvae were found to be the most likely food chain link for selenium in open waters; selenium concentrations in food items ranged between 0.3 and 3.8 micrograms of selenium per gram ($\mu\text{g Se/g}$), with an overall mean concentration in food items of $1.7 \mu\text{g Se/g}$ (all selenium concentrations for tissue and sediment are expressed on dry weight basis in this paper).

Selenium concentrations found in shorebird blood (means up to $36 \mu\text{g Se/g}$ and individual concentrations up to $68 \mu\text{g Se/g}$) and livers (means up to $24 \mu\text{g Se/g}$ and individual concentrations up to $40 \mu\text{g Se/g}$) were higher than expected based on concentrations found in food sources and bird eggs (selenium concentrations in bird blood and livers are generally expected to be more similar to those found in the birds' diet and eggs than indicated by

results for these shorebirds). Further investigation and analysis of the datasets (CH2MHill 2008a) concluded that the most likely explanation for the higher-than-expected blood selenium concentrations was exposure to elevated mercury concentrations in Great Salt Lake, which had been measured previously in water and biota from the lake by Naftz et al. (2005). Selenium may play a role in mercury detoxification (that is, it counteracts the toxic effects of mercury) for individuals with high mercury levels.

Despite elevated levels of selenium found in adult tissues, concentrations in eggs were relatively low and ranged between 1.2 and $9.2 \mu\text{g Se/g}$ in individual eggs, with an overall mean egg concentration of $2.7 \mu\text{g Se/g}$ (68 eggs collected). Breeding (nest) success ranged between 94 and 97%. These success rates were considered consistent with what would be expected for non-contaminated sites.

The data collected during the 2006 and 2007 breeding seasons suggest that the selenium concentration found in water samples, food chain invertebrates, and eggs at Antelope Island and Ogden Bay were low and within typical background levels reported elsewhere. Elevated selenium levels found at Saltair were likely due to freshwater inflows from the KUCC outfall, located in the southeast portion of the study area (Figure 1).

Gulls, Grebes, and Ducks

Most California gulls collected from three colonies on Great Salt Lake (68%) consumed exclusively brine shrimp (Conover et al. 2008a). Others ate a mixture of other invertebrates, fish, and garbage. Adult brine shrimp were found to be the most likely food chain link for selenium in gulls. Egg selenium concentrations ranged from 2.0 to 4.3 $\mu\text{g Se/g}$, with an overall mean selenium concentration in eggs of 2.9 $\mu\text{g Se/g}$. Of 72 eggs collected, only one had no embryo development and none exhibited embryo malposition or deformities. None of the 100 chicks that were examined exhibited teratogenesis (deformities). Similar to the shorebirds, selenium concentrations found in gull blood and livers were higher than expected based on concentrations found in their food sources and eggs. Further investigation and analysis of the datasets (CH2MHill 2008a) concluded that the most likely explanation for the higher-than-expected blood selenium concentrations was exposure to elevated mercury concentrations in Great Salt Lake. Bird body weight was not correlated to blood or liver selenium concentrations. Despite elevated selenium levels found in gull blood and livers, selenium was not found to impair gull health or reproduction.

Eared grebes collected during the fall of 2006 were found to eat primarily brine shrimp (Conover et al. 2008b). Selenium concentrations found in grebe blood (means up to 25 $\mu\text{g Se/g}$ and individual concentrations up to 55 $\mu\text{g Se/g}$) and livers (means up to 16 $\mu\text{g Se/g}$ and individual concentrations up to 28 $\mu\text{g Se/g}$) were higher than expected. Further investigation and analysis of the datasets concluded that the most likely explanation for the higher-than-expected blood selenium concentrations was exposure to elevated mercury concentrations in Great Salt Lake. Mass of liver, pancreas, and spleen were not correlated to selenium or mercury concentrations. There was a positive relationship between selenium and body mass (which is sometimes used as an indicator of body condition) that is undoubtedly a result of physiological characteristics of the grebe. The birds gain weight during their fall migration stay on the lake (mean body weight of 381 g in September when they arrive, and 591 g in November shortly before departure); while they are there, they also accumulate more selenium from their food. Although no deleterious effects were observed in the birds, the confounding variables (physiological changes with concurrent bioaccumulation of selenium) and insufficient interpretive information specific to physiological effects of selenium in grebes did not allow a determination to be made regarding the effect of selenium and mercury on the body condition of eared grebes.

Selenium and mercury levels in common goldeneye collected in 2005 and 2006 were higher than expected based on selenium concentrations in diet (Conover et al. 2008c). Similar to the results for other species previously described, further investigation and analysis of the datasets (CH2MHill 2008a) concluded that exposure to elevated mercury concentrations in Great Salt Lake was the most likely explanation for the higher-than-expected blood selenium concentrations. Body mass and liver mass were not correlated to selenium or mercury in the blood or liver. Fat mass was negatively correlated with selenium concentrations in liver and mercury concentrations in liver and blood. Selenium and mercury concentrations were found to increase during the wintering period. Although no deleterious effects were observed in the ducks, the confounding variables (seasonal weight changes in fat reserves and concurrent bioaccumulation of selenium) and insufficient interpretive information specific to physiological effects of selenium in common goldeneyes did not allow a determination to be made regarding the effect of selenium and mercury on the body condition of these birds.

PROJECT 2—LOWER FOOD CHAIN

Benthic Zone (Bottom of the Lake)

Brine fly larvae and pupae were sampled from biostromes (also called stromatolites, which are hard underwater structures) and shore-zone sediments from locations near the northern and southern ends of Antelope Island (Wurtsbaugh 2007). Samples of biostromes, sediment, and adult brine flies were also collected. Brine flies were found to be much more abundant on biostromes than on nearby sand or mud substrates. Concentrations were found to increase from larvae (1.3 $\mu\text{g Se/g}$) to pupae (1.5 $\mu\text{g Se/g}$) to adult flies (1.8 $\mu\text{g Se/g}$). The limited number of samples did not provide adequate information to develop a predictive relationship between selenium in brine fly food sources, sediment, and the brine fly tissue.

Pelagic Zone (Water Column of the Lake)

Data on brine shrimp and lake characteristics included data on water quality, seston chemistry, chlorophyll concentrations, algal cell counts, complete density estimates of brine shrimp by life stage, and brine shrimp and seston selenium content (Marden 2007, 2008). Brine shrimp and phytoplankton exhibited characteristics indicative of a generally “healthy” population. Dissolved selenium concentrations in water were not significantly variable spatially but changed somewhat seasonally and from year to year. The lowest “monthly” mean was in late June-early

July 2006 ($0.45 \mu\text{g Se/l}$, $n = 12$) while the highest was late August-September 2006 ($0.70 \mu\text{g Se/l}$, $n = 12$). In May-June 2006, the overall mean was $0.56 \mu\text{g Se/l}$ ($n = 18$), in May-June 2007 it was $0.54 \mu\text{g Se/l}$ ($n = 18$), and in July-August 2007 it was $0.56 \mu\text{g Se/l}$ ($n = 10$). Thus, the net difference was small for the lake water column for the period of study. The mean waterborne selenium concentration for 2006 and 2007 was $0.6 \mu\text{g Se/l}$. Similarly, seston and brine shrimp selenium concentrations increased over the period of study. No statistically significant relationships were found between brine shrimp selenium concentrations and those in water or seston. The geometric mean for selenium concentrations in adult brine shrimp in 2007 was $4.3 \mu\text{g Se/g}$ and for brine shrimp nauplii/cysts was $2.4 \mu\text{g Se/g}$.

PROJECT 3–SELENIUM LOADS

Six gages were operated on tributaries to Great Salt Lake for water quality sampling and flow measurements, and standard U.S. Geological Survey (USGS) models (LOADEST) were used to produce daily loading estimates over the period of record (Naftz et al. 2008). Total estimated selenium load was 1540 kilograms (kg) over the full 15 month study period, with an annual (May 2006 to April 2007) load of 1480 kg. The KUCC outfall and Goggin Drain contributed the greatest proportion of loads among sites (27% each), although the Bear River contributed an almost equal amount (25%). Loads from Farmington Bay, Weber River, and Lee Creek comprised the remaining measured load. The greatest total loads over time at all sites occurred during May 2006. Most of the influent selenium was in the dissolved phase as selenate (Se^{6+}). Measurements at the railroad causeway separating the North and South Arms of the lake indicated a possible net positive flow and selenium load from south to north over the period of record with a mean loss from the South Arm of about $2.4 \text{ kg Se per day}$ (800 kg per year).

The mean waterborne selenium concentration for the study area increased over the 15 month period of the study and exceeded the change in concentration ($0.17 \mu\text{g Se/l}$) that could be expected from the simple addition of influent loads or lowering of the lake water level. The mean waterborne selenium concentration for unfiltered lake water samples collected as part of this project was $0.60 \mu\text{g Se/l}$. Additional unmeasured sources of selenium could account for as much as 1500 kg of additional load during the 2006 through 2007 period. Potential unmeasured sources include (1) unmeasured surface inflows, (2) submarine groundwater discharges, (3) lake sediment pore water diffusion into the overlying water column, and (4) wind-blown dust that is deposited directly on the lake surface.

PROJECT 4–SELENIUM FLUX

Data collected in this project provided a great amount of detail about in-lake geochemical processes and yielded estimates of important losses of selenium from the water column (Diaz et al. 2008a, 2008b, 2008c; Beisner et al. 2008; Oliver et al. 2008). The project provided baseline characterizations of selenium in all vertical strata of the water column, including the generally warmer upper layer; a deeper, cooler mixed layer; and the lower, most dense, deep brine layer. During times of wind-driven mixing, which are common, the lake is often divided into an upper mixed layer in combination with the underlying brine layer. The project also characterized the selenium in surface sediments, in the deeper layers of sediment cores, and as volatile compounds exiting the lake in vapor phase.

Measurements of selenium in lake water showed that most of it was present in the dissolved phase but that selenium concentrations were relatively higher in the particulate fraction of the deep brine layer. The average selenium concentration for unfiltered water samples collected as part of this project was $0.64 \pm 0.28 \mu\text{g Se/l}$ and for filtered water samples $0.49 \pm 0.25 \mu\text{g Se/l}$. Volatilization of selenium from surface waters was discovered to be a major loss process for selenium from the water column and, although highly variable, probably accounts for a net loss of selenium more than four fold greater than that attributed to sediment burial. The total selenium estimated to be lost to the atmosphere was 2108 kg (estimated uncertainty range is 1380 to 3210 kg per year).

The permanent sedimentation flux was estimated to be 520 kg per year with an uncertainty range of 45 to 990 kg per year. Downward sedimentation fluxes were highest where influenced by the Bear River inflow, and were lowest in the shallow brine layer located near the northwest-southeast axis of the study area. Sediment accumulation rates were greater in the deep brine layer than in shallow brine layer areas, suggesting that re-suspension accounted for most of the sediment accumulation at depth.

Combined volatilization and sedimentation fluxes out of Great Salt Lake total to about 2628 kg per year based upon the geometric means. Volatilization was demonstrated to be the major mechanism of selenium removal from Great Salt Lake. The measured loss fluxes more than balanced the measured annual load (1480 kg per year) during the study period. The observed increase in total selenium concentration during the study period indicates that some selenium loads have not yet been measured or that some of the losses may be overestimated. Further monitoring is needed to better define the selenium mass balance in Great Salt Lake.

PROJECT 5—BRINE SHRIMP KINETICS STUDY

Detailed laboratory studies were completed to determine selenium accumulation rates in brine shrimp from water and diet (Grosell 2008). Initial studies found that higher salinities reduced feeding by the brine shrimp and reduced their uptake of selenium directly from water, so a salinity of 100 g/l was used for experiments.

The results revealed clear saturation kinetics response at waterborne concentrations below 10 $\mu\text{g Se/l}$, where tissue concentrations increased proportionally to waterborne exposure; between 10 and 20 $\mu\text{g Se/l}$ in water there was a “knee” (inflection point) in the curve of the brine shrimp response pattern. Above those waterborne concentrations, higher values of bioaccumulation remained positively associated (but with a different slope) with water concentrations up to 40 $\mu\text{g Se/l}$, but even higher water values (up to 80 $\mu\text{g Se/l}$) demonstrated decreased bioaccumulation, possibly due to selenium regulation by the brine shrimp. The studies also showed that low food concentrations (below 10 $\mu\text{g Se/g}$ in algae) produced selenium assimilation efficiencies as high as 90%. Higher selenium concentrations in algae produced slightly lower assimilation efficiencies.

The final result of the study was a two-part model that adds waterborne and dietary exposures to produce an estimate of bioaccumulated selenium in brine shrimp. This model is described in the final report for the program (CH2MHill 2008a).

Quantitative Conceptual Model Development

A quantitative model was developed to integrate project data into the conceptual model developed previously by Johnson et al. (2006). The quantitative conceptual model was developed with two components—a Mass Balance Model and a Bioaccumulation Model.

Mass Balance Model

A modified mass balance approach was used to link measured and estimated Great Salt Lake concentrations of selenium in various media into a model that would be responsive to changing ambient conditions. The basic concept is to sum all input and removal mechanisms to estimate a waterborne selenium concentration for the study area. Measured lake and influent selenium concentrations and loads were compiled as monthly geometric mean values, whenever possible. Modeled water column loads and concentrations step through time on a monthly average

time step. The model is meant to predict water column concentrations and therefore relies on both external loads (measured or modeled loads from tributaries, assumed atmospheric deposition) as well as internal loading (estimates of remineralization from seston and sediments), as described in CH2MHill (2008a).

The technique of sequentially computing mass balances produced a relatively good match to measured values. At the end of the 15 month measurement period, the predicted water column monthly total selenium concentrations were low by an average of 0.04 $\mu\text{g Se/l}$ (7%). A remaining unmeasured total was noted in the reports of Johnson et al. (2008) and Naftz et al. (2008) as evidence for a significant, unmeasured load. In particular, lake water column concentrations during the 2006 through 2007 period were generally observed to rise during a relatively dry year of reduced stream loading. Data are insufficient to resolve the uncertainty in the dataset and resolve questions about long term patterns of lake assimilation of selenium. The Science Panel recommended that additional monitoring be conducted to build and improve upon the current model (potentially building a fully dynamic model) to allow for more accurate examination of scenarios for future conditions.

Figure 2 provides a schematic view of inputs, transport, and fate as well as linkages among “compartments” for the Mass Balance Model, though this was not fully developed into a quantitative model because of various data limitations.

Bioaccumulation Model

A Bioaccumulation Model was developed from data collected from Great Salt Lake to describe the transfer of selenium from water and sediment up through the food web and into bird eggs (CH2MHill 2008b). The model allows the user to estimate diet and egg selenium concentrations from an assumed waterborne selenium concentration. The model also allows the user to back calculate a waterborne selenium concentration from an assumed diet or egg selenium concentration. Resulting waterborne, diet, and egg concentrations are listed and plotted upon egg and diet toxicity curves to illustrate potential effects of selenium on egg hatchability (Ohlendorf 2003).

The Bioaccumulation Model is composed of a series of relationships that describe the transfer of selenium from water up through the food chain. The transfer factors and regression equations that represent these relationships were

developed from data collected from Great Salt Lake as part of the research program. The user has the flexibility to select from numerous options to evaluate the sensitivity and results from alternative transfer relationships and bird diet combinations. Figure 4 illustrates inputs, outputs, and the general flow of logic of the Bioaccumulation Model.

KEY OBSERVATIONS

The Science Panel made the following observations to answer the questions identified in Figure 3.

1. Are significant ecological effects occurring in aquatic wildlife? If so, to which ones and at which locations?

The Science Panel rephrased this question as follows to account for the two critical endpoints previously described:

1.a. Have any adverse effects been observed in the reproductive endpoints for aquatic wildlife due to selenium that were investigated as part of this program?

- No egg hatchability or teratogenic effects were observed in gulls, avocets, or stilts using the open waters of Great Salt Lake. The geometric mean selenium concentration observed for gulls was $2.89 \mu\text{g Se/g}$ and for shorebirds it was $2.72 \mu\text{g Se/g}$. These values are similar to the 85th to 90th percentile of background levels and consistent with

a non contaminated site (Skorupa & Ohlendorf 1991). We did find one egg (out of total number of 133 sampled) with a selenium concentration of $9.2 \mu\text{g Se/g}$ at the KUCC outfall that is above the lower 95 percent confidence limit ($6.4 \mu\text{g Se/g}$) but below the median ($12 \mu\text{g Se/g}$) of the EC10 for mallard egg hatchability.

1.b. Have any adverse effects been observed in non-reproductive endpoints (for example, body condition) in aquatic wildlife due to selenium?

- A determination cannot be made at this time due to confounding variables and insufficient data; however, elevated concentrations of selenium and mercury were found in bird blood and livers. This may indicate that some of these birds are using selenium to detoxify mercury.
- The Science Panel determined that the reproductive endpoint is considered the most sensitive endpoint for selenium on Great Salt Lake and will be the basis for the selenium water quality standard for open waters of the lake. Non-reproductive endpoints will require additional research before they can be used in assessing the water quality standard.
- Selenium concentrations in water; sediment; food chain items; and bird liver, blood, and eggs were measured and summarized in Section 5.0 of the final report (CH2MHill 2008a).

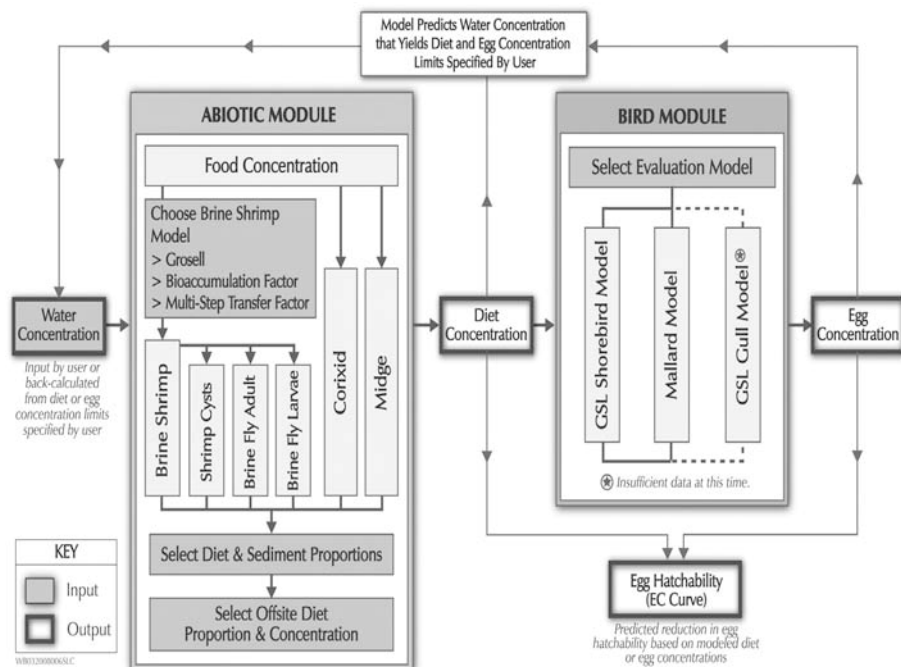


Figure 4–Bioaccumulation Model flow chart.

2. What is the relative importance of various food-chain exposure pathways for aquatic wildlife?

- Bird diets were determined by Project 1 (Cavitt 2008a; Conover 2008a). Brine flies were determined to be the most likely food chain link for selenium for shorebirds and brine shrimp were determined to be the most likely food chain link for selenium for gulls.
- Although some birds (such as gulls and goldeneyes) are known to consume food items from offsite locations (such as fresh water sources along Great Salt Lake), the assumption in the Bioaccumulation Model is that all birds consume only items they can obtain from the open waters of Great Salt Lake. This represents a conservative scenario where birds are consuming the food item with the most likely food chain link for selenium.
- It is assumed that California gulls consume a diet of 100% brine shrimp and shorebirds consume a diet of 100% brine fly larvae. Shorebirds are also assumed to consume shore-zone sediment as 5% of their diet.
- Various alternatives were incorporated into the Bioaccumulation Model to allow the user to explore and evaluate effects from various combinations of bird diets.

3. What are the transfer factors that describe relationships between selenium concentrations in water column, in bird diets, and the concentrations found in bird eggs?

- Transfer factors, regression equations, and other methods were developed to describe these relationships. The recommended transfer relationships are incorporated into the Bioaccumulation Model. The Model allows the user to select from various relationships and/or change transfer factors if desired.
- The Multi-step, Transfer Factor (MSTF) model should be used to model uptake of selenium by brine shrimp. This model was developed using site-specific data that follow the uptake of selenium by brine shrimp through seston.
- Until more data are collected, the estimate of selenium in brine fly larvae and adults should be determined through a ratio relating brine fly selenium concentrations to adult brine shrimp concentrations.
- Relationships for shorebirds are site-specific and are the best understood from information we have. For implementation of the water quality standard, relationships for shorebirds should be used. Specifically, the Shorebird Regression Model should be used to model selenium transfer between bird diet and eggs for shorebirds and the Gull Transfer Factor (GTF) Model for gulls. These models represent site-specific conditions.

4. What are the most important processes that affect the partitioning, cycling, and release of selenium in the Great Salt Lake open waters?

- Volatilization was demonstrated to be the major mechanism of selenium removal from Great Salt Lake (geometric mean of 2108 kg/yr [could range between 820 and 5240 kg/yr]). Permanent sedimentation follows as the second-most-important mechanism for selenium removal (geometric mean of 520 kg/yr [could range between 45 and 990 kg/yr]). Other mechanisms include shallow zone particulate sedimentation, deep brine layer dissolution and resuspension, and brine shrimp cyst removal.
- A possible loss of about 800 kg per year (geometric mean [could range from 0 to 1600 kg/yr]) through the railroad causeway from the South Arm to the North Arm was estimated from a few, discrete sampling events. This estimate is uncertain and warrants further work to verify.
- Most selenium was present in the dissolved phase but selenium concentrations were relatively higher in the particulate fraction of the deep brine layer.
- The measured loss fluxes more than balance the measured annual load (1480 kg per year) during the study period. The observed increase in total selenium concentration during the study period indicates that some selenium loads have not yet been measured or that some losses are overestimated and further monitoring is needed.
- Long-term cycling of selenium within Great Salt Lake was not fully addressed by this program due to the insufficient length of the study period.
- Significant variability in results was observed, but these data represent the best available information. Further work will be required to allow for accurate predictions of future waterborne selenium concentrations.

5. What are the sources of waterborne selenium entering Great Salt Lake, and what is the relative significance of the various sources?

- Water quality sampling and flow measurements for six tributaries to the Great Salt Lake identified the following selenium loads to the lake (total of 1540 kg over the 15 month study period; Note, however, that the study period was during the drought of 2006–2007). See Figure 5.
- A review of the literature identified the possibility that dry and wet atmospheric deposition could contribute a significant load of selenium to Great Salt Lake. No data from Great Salt Lake are available; however, this load could be as high as 596 kg/yr using relationships from the literature. Therefore, the selenium load attributable to

atmospheric deposition could be greater than any single tributary.

- While lake water levels generally decreased during the study period, waterborne selenium concentrations were observed to increase. This, in combination with the fact that estimates of selenium flux from the Great Salt Lake were greater than estimates of selenium loading to the lake, indicates that potential selenium sources have not yet been measured or that some of the losses are overestimated. Possible additional sources could be: (1) unmeasured surface inflows, (2) submarine groundwater discharges, (3) lake sediment pore water diffusion into the overlying water column, and (4) wind-blown dust that is deposited directly on the lake surface.

- Because of the anomalies observed in the overall mass balance of selenium in Great Salt Lake, further work is needed to better understand the mass balance of selenium in the lake.

RECOMMENDATIONS

The Science Panel made the following recommendations:

1. The water quality standard should be a tissue-based standard, based upon the selenium concentration found in the eggs of birds using the open waters of Great Salt Lake. The standard should be evaluated based upon the geometric mean of eggs sampled in the course of one nesting season at locations where birds are dependent upon the open waters of Great Salt Lake.

2. A selenium water quality standard that prevents impairment for aquatic wildlife of Great Salt Lake lies within the range of 6.4 to 16 mg Se/kg for bird eggs (UDEQ, DWQ 2008).

3. Each Science Panel member prepared a brief position statement providing their individual recommendation for a water quality standard. This statement includes the recommended basis for the standard (all are tissue-based) selenium concentration, associated level of protection, and brief rationale for the recommendation. These position statements were forwarded to the Steering Committee and Water Quality Board for consideration.

Individual recommended values were as follows:

- 12–13 mg Se/kg—6 Science Panel members (most likely value for EC₁₀)
- 10.4 mg Se/kg—1 Science Panel member
- 5 mg Se/kg—1 Science Panel member
- Abstained—1 Science Panel member, agency policy did not allow member to make recommendation.

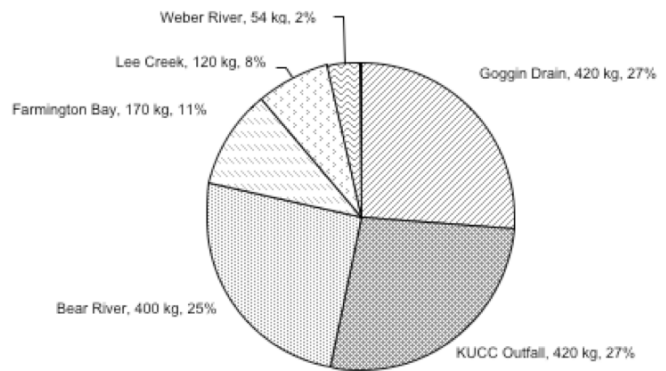


Figure 5—Tributary selenium loads.

4. For implementation, the waterborne concentration of selenium associated with the water quality standard will be derived from the Bioaccumulation Model.

5. Given the uncertainties of the current understanding of selenium cycling in Great Salt Lake, the bioaccumulative nature of selenium, the need to incorporate both waterborne and tissue-based selenium concentrations, and the desire to proactively protect and manage the water quality of Great Salt Lake, the Science Panel has developed a concept for a tiered approach to implementing the selenium water quality standard. The approach assumes the use of the Bioaccumulation Model developed as part of this program to relate water, diet and egg concentrations. The Science Panel recommends that the State of Utah implement a similar tiered approach for monitoring, assessment and management options to ensure the selenium water quality standard is not exceeded. The objectives of the approach are to perform the following:

- Monitor Great Salt Lake to assess trends in selenium concentrations and determine whether they are approaching or exceeding the water quality standard in eggs, using water and diet (measured in brine shrimp and estimated in brine flies by a “translation factor”) as indicators of whether the standard is likely to be exceeded in the egg.
- Address current uncertainty in modeled bioaccumulation relationships by validating expected bioaccumulation with new data for water or diet concentrations and, if appropriate, egg selenium and hatchability.
- Evaluate trigger selenium concentrations that initiate various monitoring, assessment and management actions identified in the assessment framework.
- Evaluate the lake with respect to the numeric water quality standard for selenium.

- Initiate management actions to mitigate further increases in selenium concentration if an upward trend is observed.

The approach implements various trigger concentrations for water, diet, and egg selenium that increase monitoring levels and management options if and when actual selenium concentrations increase.

6. The final water quality standard that prevents impairment of the beneficial uses of the open waters of Great Salt Lake should represent a level of protectiveness (that is, not exceeding a specified level of predicted reduction of egg hatchability) recommended by the Steering Committee and selected by the Water Quality Board.

7. Given the uncertainties of the current understanding of the Great Salt Lake ecosystem, it is prudent to identify potential actions DWQ could take to verify and validate the current model, the new water quality standard, and future permit limits. It is recommended that the DWQ consider the following, noting that some of these recommendations are incorporated into the proposed assessment framework:

- The highest priority research need identified by the Science Panel was to verify the transfer of selenium between the water column and brine shrimp for waterborne concentrations of 0.5 to 5.0 $\mu\text{g Se/l}$. The current Bioaccumulation Model includes two relationships (BAF and MSTF models) developed from Great Salt Lake data that describe this transfer; however, both were created from a dataset represented by waterborne concentrations of 0.4 to 0.8 $\mu\text{g Se/l}$. Further studies would verify these site-specific relationships for higher ranges of waterborne selenium concentrations.
- Periodically reassess the current conceptual model and update it with any new scientific information, as appropriate. The objective of continual reassessments of the model is to improve upon the accuracy of current relationships used in the Bioaccumulation and Mass Balance Models to minimize current uncertainties.
- Monitor brine shrimp selenium concentrations and waterborne selenium concentrations at predetermined intervals throughout Great Salt Lake. The objective is to improve on the current understanding of the transfer of selenium from the water to these diet items and assess long-term trends.
- Complete additional collocated sampling of brine fly larvae and adults and sediment and water. Current brine fly levels are based on a “translation factor” developed from limited brine fly data and brine shrimp data.
- Complete additional egg sampling studies that relate transfer of selenium from diet to eggs. The objective is to provide additional data points that will improve the statistical power of the current Great Salt Lake Shorebird Model (that is, the regression equation developed from data collected to date) and Great Salt Lake Gull Model (currently not used for lack of observed relationship).
- Continue monitoring tributary inflows and selenium loads to Great Salt Lake in conjunction with lake water column concentrations. The objective is to understand long-term trends, identify other potential selenium sources, and improve upon the current mass balance model. Special emphasis should be placed upon understanding flow inputs/outputs to the North Arm as very little information describing these processes is currently available.
- Evaluate other potential sources of selenium to Great Salt Lake.
- Sample atmospheric deposition of selenium to verify assumptions made in the Mass Balance Model. The objective of this study is to measure both wet and dry atmospheric deposition of selenium and other pertinent meteorological parameters at Great Salt Lake to quantify actual atmospheric selenium loads to Great Salt Lake.
- Conduct a one-time study to determine selenium concentrations in phalaropes when they arrive at Great Salt Lake and before their departure during their season of peak abundance at the lake. The objective of this study is to identify any potential effects of selenium upon their body condition and ability to migrate.
- Conduct further studies to evaluate the potential effects of selenium on non-reproductive endpoints in birds. Confounding variables and insufficient information available during the completion of this project did not allow for a determination of effects due to selenium on those endpoints for Great Salt Lake birds.
- Conduct further studies to understand the potential interaction of selenium and mercury and their effects on aquatic birds using open waters of Great Salt Lake.
- Verify waterborne selenium concentrations at the outer limit of point-source discharge mixing zones at predetermined intervals. The objective is to verify current mixing zone assumptions and potential effects to beneficial uses in these zones.
- Continue verifying discharge concentrations per permit requirements.

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- Steering Committee members included Walt Baker (Chairman), Utah Division of Water Quality; Robert W. Adler, University of Utah; Richard Bay, Jordan Valley Water Conservancy District; Nathan Darnall, U.S. Fish and Wildlife Service; Dave Grierson, UDNR/Forestry Fire and State Lands; Karen Hamilton, U.S. Environmental Protection Agency, Region 8; Jim Huizingh, Morton Salt; Don Leonard, Utah Artemia Association; Delane McGarvey, Davis County Health Department; Chris Montague, The Nature Conservancy of Utah; Leland Myers, Central Davis Sewer District; David L. Naftz, U.S. Geological Survey; Kelly Payne, Kennecott Utah Copper; Maunsel Pearce, Great Salt Lake Alliance; Clay Perschon, UDNR/Division of Wildlife Resources; Richard Sprott, Utah Department of Environmental Quality; and Richard West, West Side Associated Duck Clubs.
- Principal Investigators, who completed the essential research for the program, were John F. Cavitt, Weber State University; Michael Conover, Utah State University; Martin Grosell, University of Miami; William P. Johnson, University of Utah; Brad T. Marden, Parliament Fisheries, LLC; David L. Naftz, U.S. Geological Survey, and Wayne Wurtsbaugh, Utah State University. They were assisted by many students and field, office, and laboratory assistants.
- Earl Byron, Dan Moore, and Gary Santolo of CH2MHill served as technical advisors. In addition, Harry Ohlendorf was the overall senior technical advisor and Jeff DenBleyker was Project Manager for the team that included the Principal Investigators working as part of the overall team.

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Mercury Inputs to Great Salt Lake, Utah: Reconnaissance-Phase Results

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ABSTRACT

In response to increasing public concern regarding mercury (Hg) cycling in Great Salt Lake (GSL) ecosystem, a series of studies were initiated to differentiate between the mass of Hg from riverine versus atmospheric sources to GSL. Cumulative riverine Hg load to GSL during a 1 year time period (April 1, 2007 to March 31, 2008) was 6 kg, with almost 50% of the cumulative Hg load contributed by outflow from Farmington Bay. Comparison of cumulative annual atmospheric Hg deposition (32 kg) to annual riverine deposition (6 kg) indicates that atmospheric deposition is the dominant input source to GSL. A sediment core collected from the southern arm of GSL was used to reconstruct annual Hg deposition rates over the past ~ 100 years. Unlike most freshwater lakes, small changes in water level in GSL significantly changes the lake surface area available for direct deposition of atmospheric Hg. There is good agreement between lake elevation (and corresponding lake surface area) and Hg deposition rates estimated from the sediment core. Higher lake levels, combined with sediment focusing processes, result in an increase in Hg accumulation rates observed in the sediment core. These same combination of processes are responsible for the lower Hg accumulation rates observed in the sediment core during historic low stands of GSL.

INTRODUCTION

Great Salt Lake (GSL), in the western United States, is a terminal lake with a surface area that can exceed 5100 km² (Figure 1). The lake is bordered on the west by desert and on the east by the Wasatch Mountain Range. Completion of a railroad causeway in 1959 divided GSL into a North and South Arm (Figure 1) and significantly changed the water and salt balance (Loving et al. 2000). More than 95% of the freshwater surface inflows enter GSL south of the railroad causeway resulting in consistently higher salinities in lake water north of the railroad causeway. A similar rock-filled automobile causeway separates Farmington Bay from the main body of GSL (Figure 1).

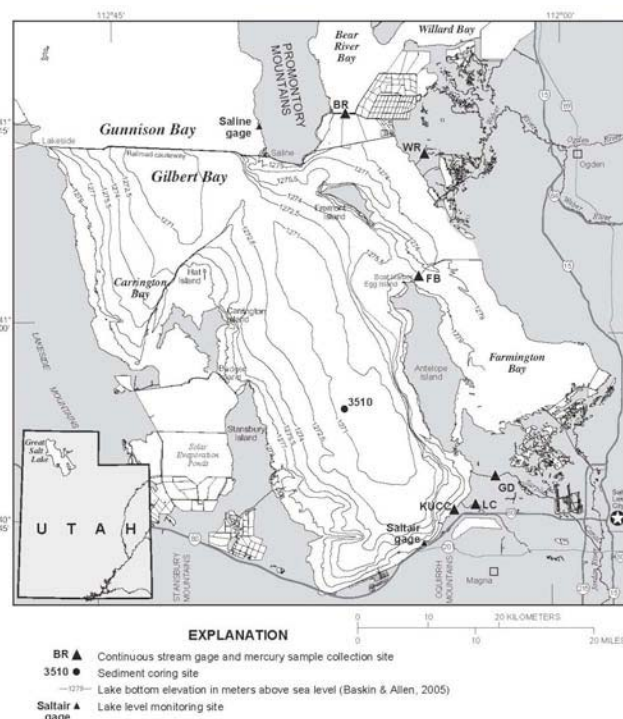


Figure 1—Location of stream gages, lake elevation monitoring sites, and sediment core site, Great Salt Lake, Utah.

The GSL ecosystem receives industrial, urban, mining and agricultural discharge from a 3.8×10^4 km² watershed with a population exceeding 1.7 million people. The open water and adjacent wetlands of the GSL ecosystem support millions of migratory waterfowl and shorebirds from throughout the Western Hemisphere (Aldrich & Paul 2002). In addition to supporting migratory dependent waterbirds, the brine shrimp population residing in GSL supports a shrimp industry with annual revenues as high as 60 million US dollars (Isaacson et al. 2002). Other industries supported by GSL include mineral production (halite, K salts, Mg metal, Cl₂, MgCl₂, and nutritional supplements) and recreation that includes waterfowl hunting (Anderson & Anderson 2002; Butts 2002; Isaacson et al. 2002; Tripp 2002).

Despite the ecological and economic importance of GSL, little is known about the input and biogeochemical cycling of Hg in the lake and how increasing anthropogenic pressures may affect its cycling. Reconnaissance-phase sampling and analysis of water samples from GSL by the

U.S. Geological Survey (USGS) from 2003 to 2007 found elevated (median concentration = 24 ng/l) concentrations of methyl Hg (CH_3Hg) (Naftz et al. 2006, 2008). In response to elevated CH_3Hg levels in water samples from GSL, the State of Utah collected and analyzed breast tissue for total Hg from seven duck species that utilized GSL and surrounding wetlands (Utah Department of Health 2005). These reconnaissance-phase analyses found that a number of the breast muscle samples exceeded the U.S. Environmental Protection Agency (USEPA) screening level of 0.3 mg/kg Hg (wet weight), the concentration at which limited human consumption of bird breast muscle is recommended (USEPA 2000). As a result of the reconnaissance-phase Hg assessment of the duck population in GSL, a health advisory was issued by the Utah Department of Health in September 2005 warning against unlimited human consumption of Northern Shoveler and Common Goldeneye ducks harvested from GSL.

Additional investigations into Hg cycling in GSL by the USGS (Naftz et al. 2008) found that separation of GSL into two distinct hydrologic and geochemical systems from the construction of a railroad causeway in the late 1950s created a persistent and widespread anoxic layer in the southern part of GSL. This anoxic layer, referred to as the deep brine layer (DBL), has high rates of SO_4 reduction, likely increasing the Hg methylation potential. High concentrations of CH_3Hg (median concentration = 24 ng/l, $n = 15$) in whole-water samples were found in the DBL with a significant proportion (31–60%) of total Hg in the CH_3Hg form. Furthermore, Naftz et al. (2008) used hydroacoustic and sediment-trap data to show that turbulence introduced by internal waves generated during sustained wind events can temporarily mix the elevated CH_3Hg concentrations in the DBL with the more biologically active upper brine layer (UBL).

In response to increasing public concern regarding Hg input to the GSL ecosystem and to begin to consider possible remediation alternatives, the Utah Department of Environmental Quality (UDEQ) and USGS initiated additional studies to differentiate the relative amounts of Hg from riverine versus atmospheric sources to GSL. Specific objectives of this study were to (1) accurately measure stream discharge and whole-water Hg concentration at all major inflow sites to GSL; (2) utilize data collected in objective one in combination with regression modeling techniques to determine daily and annual Hg loads to GSL; and (3) utilize sediment-core data collected from GSL to reconstruct riverine and atmospheric Hg input to GSL over the last 100 years. This chapter presents the results related to these objectives.

METHODOLOGY

Field Methods

Stream discharge at the Goggin Drain (GD), Weber River (WR), and Lee Creek (LC) gages (Figure 1) was measured using standard USGS methods (Buchanan & Somers 1968, 1969; Carter & Davidian 1968) using a continuous record of water stage calibrated to periodic measurements of streamflow. Due to the low channel gradients and wind influence on inflow rates at the Bear River (BR), Farmington Bay (FB), and Kennecott Utah Copper Corporation (KUCC) gage sites (Figure 1), normal stage-to-discharge relationships did not exist. Instead, hydroacoustic instrumentation in combination with velocity index methods (Simpson 2001) was used to accurately gage streamflow at those sites. Discharge from FB enters into GSL via an opening in the rock-filled causeway that artificially separates Farmington Bay from the main body of GSL (Figure 1).

During the study period (April 1, 2007 to March 31, 2008), whole-water samples from the six inflow sites were collected at the centroid of flow directly into pre-cleaned Teflon bottles provided by the U.S. Geological Survey Mercury Research Laboratory in Middleton, Wisconsin. Sample bottles were double bagged during storage and shipping. Because of the low-gradient conditions at selected inflow sites, the direction of inflow was noted during sample collection. Within 8 hours after collection, water samples were acidified with ultra-pure, 50% HCl in a fully enclosed processing chamber to prevent the introduction of airborne Hg to the water samples. During sample collection, personnel wore arm-length, powder-free gloves to minimize sample contamination. The sediment core from the top 0.5 m of sediment underlying GSL was collected using a box-coring device (Van Metre et al. 2004) suspended from a davit attached to the USGS Research vessel D. Stephens. After collection, the core was chilled to 4°C during transport to shore for processing. Once on shore (< 8 hours after core collection), the top 10 cm section of the sediment core was sliced into 1 cm intervals and transferred into 200 ml, wide-mouthed plastic containers and frozen until the samples could be freeze dried prior to chemical analysis. Weights of sediments before and after drying were recorded for determining bulk density, ρ (g dry sediment per cm^3 wet sediment). Particle dry density, ρ_b and salt content were determined by measuring displacement of dry sediment added to deionized water in volumetric flasks and resulting dissolved salt concentration. Sediment porosity (ϕ) was determined from weight loss on drying sediment samples and ρ_b with correction for salt contribution to dry sediment weight following the method described in Van Metre et al. (2004). The total dry sediment

mass (corrected for salt content) in each interval in g/cm^2 was calculated by multiplying the dry sediment porosity ($1-\phi$, cm^3) by ρ_b (g/cm^3) and the thickness for each interval thickness, and summing over the length of the core from the surface downward.

Laboratory Methods

All water analyses were performed at the USGS Hg Research Laboratory in Middleton, Wisconsin. Total Hg (Hg_t) in whole-water samples was determined using cold vapor atomic fluorescence spectrometry (CVAFS) (Olson & DeWild 1999). The CH_3Hg in whole-water samples was determined using distillation/ethylation/gas-phase separation with CVAFS detection (DeWild et al. 2002). Primary standards for Hg_t were obtained commercially and certified against a NIST standard reference material. No reference materials are currently available for CH_3Hg . Standards for CH_3Hg were prepared in the laboratory. Known reference samples were analyzed at the beginning of each analytical run, after every 10 samples and at the end of each run. Method blanks were prepared by adding SnCl_2 to 125 ml of Hg-free water and purging for 20 minutes to ensure removal of any residual Hg. Method blanks were run periodically during each sample run and used to calculate the daily detection limit (DDL). The accepted value for the DDL is < 0.04 ng/l. Matrix spikes were analyzed during each run or every 10 samples. Percent recovery of matrix spikes had to fall between 90% and 110% for the sample run to be accepted. Three field replicates and two process blanks were collected and analyzed for Hg_t and CH_3Hg . Field replicate results were in close agreement, with replicates ranging from 3.0% to 5.5% for Hg_t and 2.7% to 15.9% of the routine sample value for CH_3Hg . Process blanks had low Hg_t (0.08 and 0.10 ng/l) and CH_3Hg (< 0.04 ng/l) concentrations. Additional details on Hg laboratory methods and quality assurance and quality control procedures can be found at <http://infotrek.er.usgs.gov/mercury/>.

The Hg_t in sediment samples was extracted and analyzed according to the methods outlined in Olund et al. (2004). Each sediment sample was extracted by room-temperature acid digestion and oxidation with aqua regia. The samples were then brought up to volume with a 5% BrCl solution to ensure complete oxidation and then heated at 50°C in an oven overnight. Samples were then analyzed for Hg_t with an automated flow injection system incorporating a cold vapor atomic fluorescence spectrometer. A method detection limit of 0.3 ng of Hg per digestion bomb was established using multiple analyses of a solid-phase environmental sample. Based on the range of masses processed, the minimum sample reporting limit for this method varied from 0.6 to 6 ng/g.

Activities of ^{210}Pb , ^{226}Ra , and ^7Be were measured simultaneously in freeze-dried sections of the sediment core by gamma spectrometry (Fuller et al. 1999; Van Metre et al. 2006). Subsamples of dried sediment samples were sealed in 7 ml scintillation vials and counted using a high resolution intrinsic germanium well detector. The upper 3 cm of the core were counted within two weeks of collection for determining ^7Be (half life 53 days) as an indicator of recent sediment deposition and reworking by mixing or resuspension processes. ^{210}Pb was determined from the 46 keV gamma emission line correcting for sample self absorption following the method of Cutshall et al. (1983). The supported ^{210}Pb activity, defined by its long-lived progenitor, ^{226}Ra activity, was determined on each interval from the 352 keV and 609 keV gamma emission lines of ^{214}Pb and ^{214}Bi daughters of ^{226}Ra , respectively. Self absorption of the ^{214}Pb , ^{214}Bi , and 474 keV ^7Be gamma emission lines was negligible. The difference between ^{210}Pb and ^{226}Ra is defined as unsupported or excess ^{210}Pb ($^{210}\text{Pb}_{\text{XS}}$). Detector efficiency for each isotope was determined from NIST traceable standards. NIST and IAEA reference materials were used to check detector calibration. The reported uncertainty in the measured activity was calculated from the random counting error of samples and background spectra at the one standard deviation level was typically within $\pm 10\%$. The measured activities of replicate analysis of material from the same interval agreed to within $\pm 15\%$.

Mass Loading Estimation Method

The USGS loading software, LOADEST (Runkel et al. 2004), was used to estimate the mass loading of whole water Hg_t and whole water CH_3Hg at each gage site. The automated model selection in LOADEST was used to select the best regression model from the set of nine predefined models (Table 1). Under the automated selection option, adjusted maximum likelihood estimation (AMLE) (Cohn 1988; Cohn et al. 1992) is used to determine model coefficients and estimates of log load. The predefined model with the lowest value of the Akaike Information Criterion (AIC) statistic was then used for final load estimation (Judge et al. 1988).

RESULTS AND DISCUSSION

Simulation of Hg Loadings from Inflow Sites

The LOADEST software requires a minimum of 12 water quality samples to model chemical loadings. Currently (May 2008), only seven Hg_t and CH_3Hg samples have been collected at each of the six inflow sites. To account for the sample deficit, synthetic water-quality samples were input to the LOADEST calibration files during a similar time

period (± 7 days) and flow regime ($\pm 10\%$) as the actual water-quality samples that were collected. Although the insertion of synthetic values into the LOADEST calibration file weakens the annual loading simulation for each input site, this approach allows for a preliminary estimate of annual, riverine Hg_t and CH_3Hg loads to GSL. Future water-quality sampling will allow for the replacement of synthetic water-quality samples with actual samples in the LOADEST calibration file, thereby providing refined annual estimates of Hg loads (dissolved + particulate). Additional water-quality samples collected during varying hydrologic conditions, including peak discharge events, will further improve the annual Hg loading estimates. Details of LOADEST model calibration results are provided for each inflow site.

Because of the low-gradient conditions associated with the Bear River Bay and Farmington Bay outflow sites, wind events can temporarily reverse the normal direction of flow into GSL. When reverse flow conditions were recorded, a near zero ($< 0.0001 \text{ m}^3/\text{s}$) discharge was used in the LOADEST calibration files.

Lee Creek near Magna, Utah: The LOADEST model calibration file contained seven observations for Hg_t and CH_3Hg during the time period of April 2007 through March 2008 (Figure 2). The LOADEST estimation file contained 397 measurements of mean daily discharge ranging from 0.8 to $3.6 \text{ m}^3/\text{s}$ during the time period from March 2007 through March 2008. Regression model 9 (Table 1) was found to best simulate daily Hg_t loads ($R^2 = 0.9717$) and CH_3Hg loads ($R^2 = 0.9718$) from Lee Creek to GSL.

Comparisons between the measured and simulated loads of Hg_t at the Lee Creek gage indicate good agreement (Figure 2). The difference between measured and simulated Hg loads ranged from -4.5 to $+6.1\%$ for Hg_t and -23.7 to $+10.4\%$ for CH_3Hg loads. Annual load from Lee Creek to GSL during the monitoring period was 0.30 kg for Hg_t (Figure 3) and 9.2 g for CH_3Hg .

Goggin Drain near Magna, Utah: The LOADEST model calibration file contained seven observations for Hg_t and CH_3Hg during the time period of April 2007 through March 2008 (Figure 4). The LOADEST estimation file contained 397 measurements of mean daily discharge ranging from 0.2 to $30.9 \text{ m}^3/\text{s}$ during the time period from March 2007 through March 2008. Regression model 9 (Table 1) was determined to best simulate daily Hg_t loads ($R^2 = 0.9998$) and CH_3Hg loads ($R^2 = 0.9995$) from Goggin Drain to GSL.

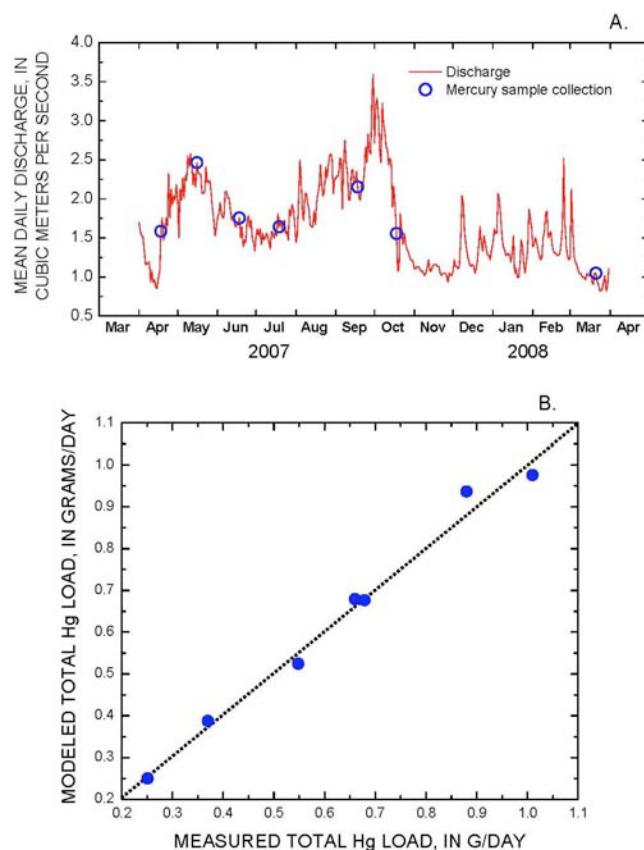


Figure 2—Stream discharge and dates when mercury samples were collected at the Lee Creek gaging station (A) and comparison between measured and modeled total mercury loads (B). Dashed line indicates one-to-one correspondence between measured and modeled mercury loads.

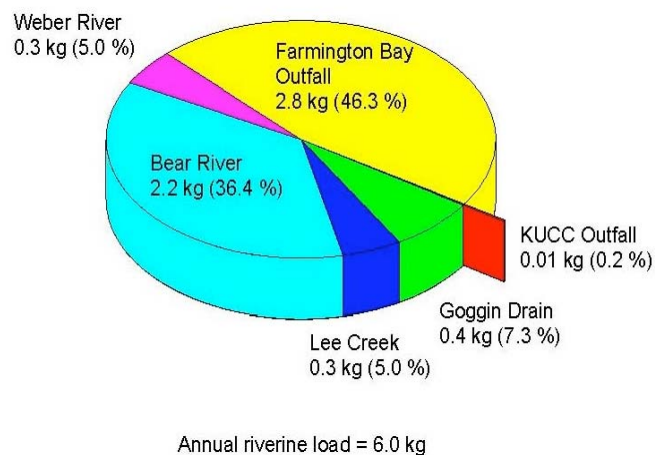


Figure 3—Distribution of Hg_t (dissolved + particulate) loads contributed to Great Salt Lake from each inflow site during April 1, 2007 to March 31, 2008. KUCC is the abbreviation for Kennecott Utah Copper Corporation.

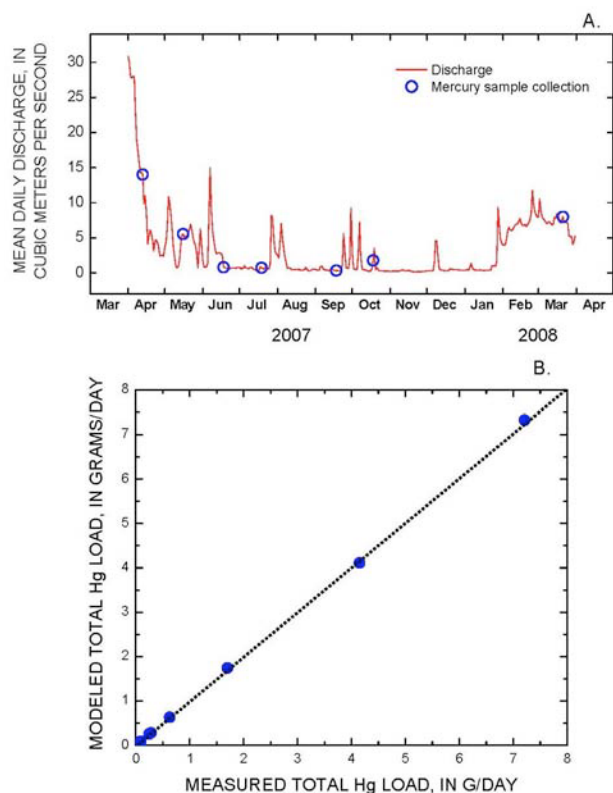


Figure 4—Stream discharge and dates when mercury samples were collected at the Goggin Drain gaging station (A) and comparison between measured and modeled total mercury loads (B). Dashed line indicates one-to-one correspondence between measured and modeled mercury loads.

Comparisons between the measured and simulated loads of Hg_t at the Goggin Drain gage indicate good agreement (Figure 4). The difference between measured and simulated Hg loads ranged from -1.1 to +2.8% for Hg_t and -2.9 to +6.0% for CH_3Hg loads. Annual load from Goggin Drain to GSL during the monitoring period was 0.44 kg of Hg_t (Figure 3) and 13 g of CH_3Hg .

Weber River near West Warren, Utah: The LOADEST model calibration file contained seven observations for Hg_t and CH_3Hg during the time period of April 2007 through March 2008 (Figure 5). The LOADEST estimation file contained 397 measurements of mean daily discharge ranging from 0.2 to 2.6 m^3/s during the time period from March 2007 through March 2008. Regression model 9 (Table 1) was determined to best simulate daily Hg_t loads ($R^2 = 0.9987$) and CH_3Hg loads ($R^2 = 0.9964$) from Weber River to GSL.

Comparisons between the measured and simulated loads of Hg_t at the Weber River gage indicate good agreement (Figure 5). The difference between measured and simulated Hg loads ranged from -28 to +1.2% for Hg_t and -5.0 to +3.7% for CH_3Hg loads. Annual load from Weber River to GSL during the monitoring period was 0.3 kg of Hg_t (Figure 3) and 7.8 g of CH_3Hg .

Kennecott Drain near Magna, Utah: The Kennecott Drain had only intermittent flows during the monitoring period (Figure 6) which did not allow for a sufficient number of water-quality samples to be collected for Hg load simulation using LOADEST. The two water-quality samples that were collected from the Kennecott Drain in October 2005 and May 2007 contained very low concentrations of Hg_t (1.8 and 1.4 ng/l) and CH_3Hg (< 0.04 and < 0.04 ng/l). An annual Hg load from the Kennecott Drain was estimated by assuming a constant Hg_t (1.4 ng/l) and CH_3Hg concentration during the annual monitoring period from April 1, 2007 through March 31, 2008. Since both of the CH_3Hg samples were below the method detection limit (MDL) of 0.04 ng/l, a concentration value of 0.75 times the MDL was used to estimate CH_3Hg concentration. The constant Hg concentration data was then combined with the mean daily discharge data during the same monitoring period to estimate annual loads. Annual Hg load from the Kennecott Drain to GSL during the monitoring period was extremely low (0.01 kg of Hg_t and 0.2 g of CH_3Hg) (Figure 3).

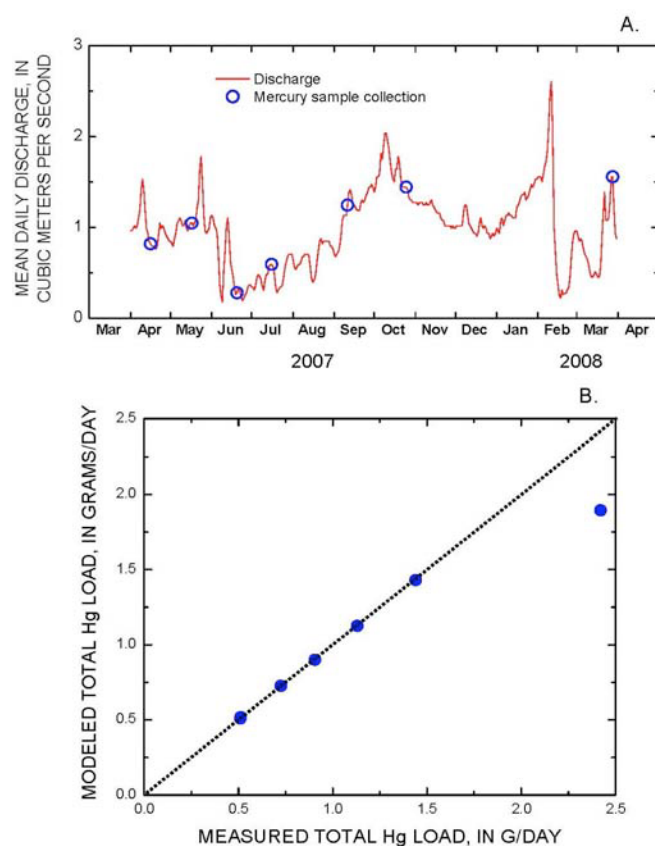


Figure 5—Stream discharge and dates when mercury samples were collected at the Weber River gaging station (A) and comparison between measured and modeled total mercury loads (B). Dashed line indicates one-to-one correspondence between measured and modeled mercury loads.

Bear River Bay Outflow at GSL Minerals Corp. Bridge:

The LOADEST model calibration file contained seven observations for Hg_t and CH_3Hg during the time period of April 2007 through March 2008 (Figure 7). Because of equipment failure and seasonal gage removal due to ice conditions, daily discharge measurements for the time periods of March 1, 2007 through April 17, 2007 and July 5, 2007 through March 31, 2008 were not made. Instead discharge was estimated from an adjacent upstream gage (Bear River near Corinne, Utah). Mean daily discharge for the missing time period was estimated from the linear relationship between measured discharge at both sites from March 21, 2006 through September 30, 2006. The regression equation developed from this comparison explained 80% of the variance in discharge ($p < 0.0001$, $N = 194$) between the two gage sites. The LOADEST estimation file contained 397 actual and estimated measurements of mean daily discharge ranging from < 0.1 to $85.0 \text{ m}^3/\text{s}$ during the time period from March 2007 through March 2008. Regression model 4 (Table 1) was determined to best simulate daily Hg_t loads ($R^2 = 0.9977$) and model 7 (Table 1) was determined to best simulate daily CH_3Hg loads ($R^2 = 0.9993$) from Bear River to GSL.

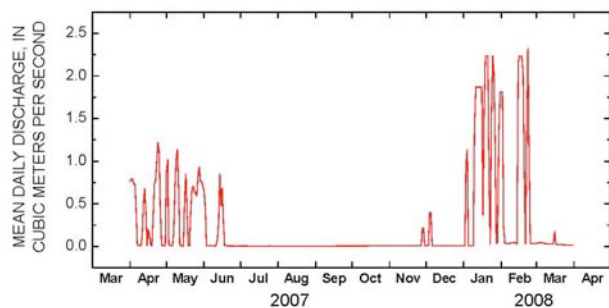


Figure 6—Discharge from the Kennecott Drain gaging station during April 1, 2007 through March 31, 2008.

During the periods of measurable discharge, agreement between the measured and simulated loads of Hg_t at the Bear River gage was good (Figure 7). The difference between measured and simulated Hg_t loads during measurable discharge ranged from -8.9 to $+0.5\%$ for Hg_t and $+0.3$ to $+2.9\%$ for CH_3Hg loads. Annual load from Bear River to GSL during the monitoring period was 2.2 kg of Hg_t (Figure 3) and 695 g of CH_3Hg .

Farmington Bay Outflow at Causeway Bridge: The LOADEST model calibration file contained seven observations for Hg_t and CH_3Hg during the time period of April 2007 through March 2008 (Figure 8). The LOADEST estimation file contained 397 measurements of mean daily discharge ranging from < 0.1 to $41 \text{ m}^3/\text{s}$ during the time period from March 2007 through March 2008. Regression model 1 (Table 1) was determined to best simulate daily

Hg_t loads ($R^2 = 0.6771$) and model 4 (Table 1) was determined to best simulate daily CH_3Hg loads ($R^2 = 0.8597$) from Farmington Bay to GSL.

Measured and simulated loads of Hg_t at the Farmington Bay outflow gage are shown in Figure 8. Simulated total Hg loads did not consistently agree with measured loads. The difference between measured and simulated Hg loads ranged from -90.4 to $+39.1\%$ for Hg_t and -83.7 to $+40.0\%$ for CH_3Hg loads. Annual load from Farmington Bay to GSL during the monitoring period was 2.8 kg of Hg_t (Figure 3) and 330 g of CH_3Hg .

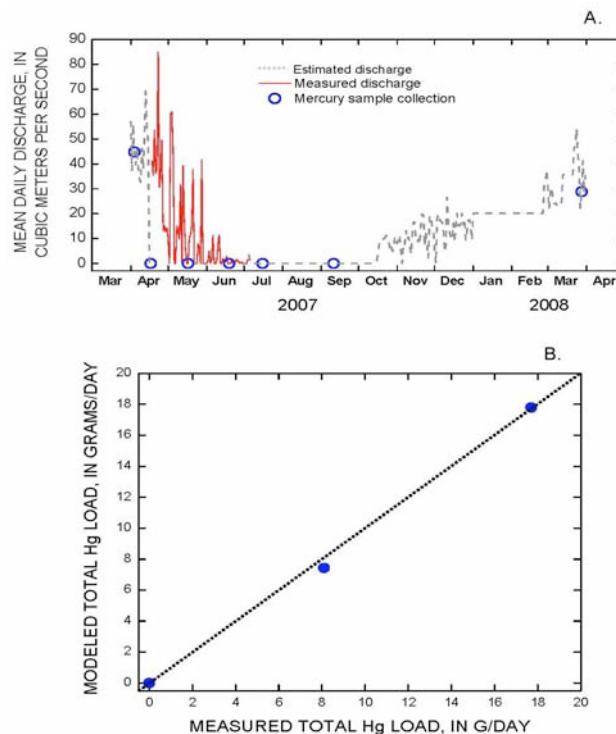


Figure 7—Stream discharge and dates when mercury samples were collected at the Bear River gaging station (A) and comparison between measured and modeled total mercury loads (B). Dashed line indicates one-to-one correspondence between measured and modeled mercury loads.

Cumulative Hg Loadings

The Hg input models developed for each gage site were used to estimate the cumulative total (dissolved + particulate) Hg_t load to GSL from April 2007 through March 2008 (Figure 3). Total estimated Hg_t load to GSL during this 1 year time period was 6 kg . Almost 50% of the annual Hg_t load was contributed by outflow from Farmington Bay (2.8 kg). The second major contributor of Hg_t to GSL was from the Bear River (36%). Minor Hg_t loads ($< 18\%$) were contributed by the four remaining inflow sites (Goggin Drain, Lee Creek, Weber River, and KUCC outfall).

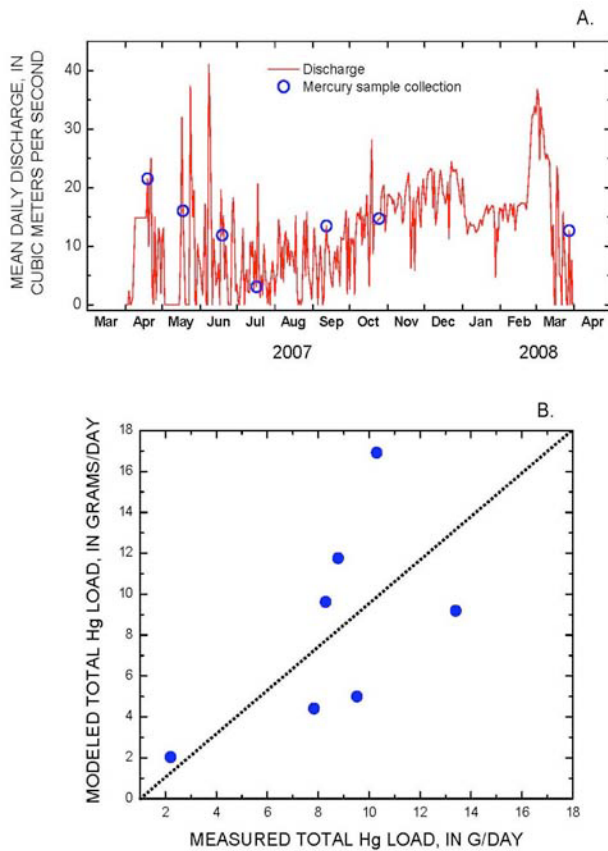


Figure 8—Stream discharge and dates when mercury samples were collected at the Farmington Bay outflow gaging station (A) and comparison between measured and modeled total mercury loads (B). Dashed line indicates one-to-one correspondence between measured and modeled mercury loads.

Bodaly et al. (1998) found that sewage treatment typically removes 88% of Hg_t from raw sewage, likely discounting this as a large source of additional Hg_t . Alternatively, Hg_t inputs from untreated urban sources such as stormwater runoff could contribute to the elevated Hg_t loads observed

in the Farmington Bay drainage basin. Fulkerson et al. (2007) found that stormwater runoff from impervious surfaces (roads, parking lots, etc.) contained elevated concentration of Hg_t that was mostly derived from dry deposition. Approximately 85% of the particulate Hg_t was removed from impervious surfaces during rainfall events. In a similar study of urban runoff from impervious surfaces, Eckley & Branfireun (2008) found that the highest Hg_t concentration in runoff were observed during the rising limb of the hydrograph and was dominated by particulate bound Hg_t .

It is possible that the elevated Hg_t loads observed in the Farmington Bay outflow could be related to urban runoff within the drainage basin. Box plots showing the concentration of Hg_t in five of the six inflow sites to GSL indicates that inflow from Weber River had the highest median concentration of Hg_t , followed by inflow from Farmington Bay (Figure 9). The higher median discharge at the Farmington Bay inflow relative to Weber River accounts for the significantly higher annual Hg_t loads contributed to GSL from Farmington Bay. Additional study evaluating the transport, settling, and resuspension of particulate bound Hg_t derived from impervious surfaces would be an important component of future work.

It is also possible that the elevated Hg_t loads from Farmington Bay and Bear River Bay outflows could be partly related to atmospheric deposition directly on the water surface. Both gage sites have large surface areas of slow-moving water that could receive direct atmospheric deposition of Hg (Figure 1).

Table 1—Regression models considered during the automated selection option in LOADEST (Runkel et al. 2004). [a_0 thru a_6 , model-determined regression coefficients; \ln , natural log; Q , discharge; $dtime$, decimal time; π , 3.141593].

Model Number	Regression Model
1	$a_0 + a_1 \ln Q$
2	$a_0 + a_1 \ln Q^2$
3	$a_0 + a_1 \ln Q + a_2 dtime$
4	$a_0 + a_1 \ln Q + a_2 \sin(2\pi dtime) + a_3 \cos(2\pi dtime)$
5	$a_0 + a_1 \ln Q + a_2 \ln Q^2 + a_3 dtime$
6	$a_0 + a_1 \ln Q + a_2 \ln Q^2 + a_3 \sin(2\pi dtime) + a_4 \cos(2\pi dtime)$
7	$a_0 + a_1 \ln Q + a_2 \sin(2\pi dtime) + a_3 \cos(2\pi dtime) + a_4 dtime$
8	$a_0 + a_1 \ln Q + a_2 \ln Q^2 + a_3 \sin(2\pi dtime) + a_4 \cos(2\pi dtime) + a_5 dtime$
9	$a_0 + a_1 \ln Q + a_2 \ln Q^2 + a_3 \sin(2\pi dtime) + a_4 \cos(2\pi dtime) + a_5 dtime + a_6 dtime^2$

Comparison of Atmospheric Deposition of Hg to Riverine Loadings

Atmospheric deposition can be one of the major sources of Hg to aquatic environments (Krabbenhoft & Rickert 1995); therefore, determination of the relative proportions of riverine versus direct atmospheric Hg inputs to the lake surface of GSL will be important for understanding Hg cycling in the lake and developing future remediation strategies. Atmospheric Hg deposition can be from both dry and wet deposition directly to the surface of GSL. The surface area of GSL used in the atmospheric deposition calculations was calculated for the highest mean monthly lake elevation recorded at both the south (1279.4 m) and north arm (1279.2 m) of GSL during the spring of 2007. Based on lake area tables developed for GSL by Baskin (2005, 2006), a maximum lake surface area of $3.2 \times 10^9 \text{ m}^2$ was available (not including Farmington or Bear River Bays) during the study period for atmospheric Hg deposition.

Measurements and associated modeling of dry deposition of reactive gaseous mercury (RGM) to the surface of GSL was conducted over a one year period (2006–2007) by Peterson & Gustin (2008). Annual dry deposition of Hg modeled by Peterson & Gustin (2008) was $4.4 \mu\text{g}/\text{m}^2$. Using the cumulative surface area of GSL during the spring of 2007 of $3.2 \times 10^9 \text{ m}^2$, approximately 14 kg of Hg would be deposited to the lake surface during 2006–2007.

Wet deposition of Hg to the surface of GSL was estimated using the newly installed Mercury Deposition Network (MDN) site located near GSL (Latitude: $40^\circ 42' 42.48''$; Longitude: $111^\circ 57' 39.23''$; Elevation: 1297 m). This MDN site has been operating since May 2007 and wet deposition Hg data currently (2008) exist for 13 months during May 2007 through May 2008 (Table 2) (National Atmospheric Deposition Program 2008). Wet deposition data collected during the 12 months from May 2007 through April 2008 resulted in a cumulative Hg deposition of $5.5 \mu\text{g}/\text{m}^2$ (Table 2). Combining the cumulative surface area of GSL during the spring of 2007 of $3.2 \times 10^9 \text{ m}^2$ with the estimated annual wet deposition value, approximately 18 kg of Hg would be deposited to GSL via wet deposition processes.

Comparison of annual cumulative atmospheric (32 kg) versus riverine (6 kg) deposition of Hg_t to GSL indicates that atmospheric deposition processes are the major input source to GSL by over 5:1. Additional atmospheric and riverine Hg data are needed to further confirm and refine these annual deposition amounts. The combined annual atmospheric and riverine input masses of Hg were compared to longer-term Hg deposition rates estimated from sediment records in GSL. These results are presented in the following section.

Table 2—Precipitation and mercury data collected from the Mercury Deposition Network monitoring site located near Great Salt Lake, Utah (site UT97) (National Atmospheric Deposition Program, 2008). [SVOL, sample volume; HgConc, total mercury concentration reported by the contract lab; HgDep, total mercury deposition; mm, millimeters; ml, milliliters; ng/l, nanograms per liter; ng/m^2 , nanograms per square meter]

Month and Year	SVOL, ml	HgConc, ng/l	HgDep, ng/m^2
May 2007	75.0	28.50	131.1
June 2007	112.1	39.60	764.3
July 2007	185.5	67.90	286.2
August 2007	61.7	46.80	454.0
September 2007	238.9	80.50	444.9
October 2007	328.8	48.80	896.8
November 2007	159.0	22.30	103.5
December 2007	314.8	16.50	450.7
January 2008	255.3	40.90	360.5
February 2008	261.0	22.70	329.1
March 2008	279.0	36.20	355.3
April 2008	321.4	309.90	938.2
May 2008	218.9	96.30	671.0

Sediment-Core Records of Cumulative Hg Inputs

The upper 10 cm section of a sediment core collected from site 3510 (Figure 1) during 2006 was used as a proxy record to reconstruct long-term records of Hg_t deposition in GSL. Sediment accumulation rates ($\text{g}/\text{cm}^2\cdot\text{yr}$) were determined from ^{210}Pb and ^7Be profiles versus cumulative mass instead of depth to account for sediment compaction.

The ^{210}Pb activity decreases with increasing depth in sediment to the supported activity defined by ^{226}Ra at the 10 cm depth (Figure 10). The decrease in unsupported activity was exponential as illustrated by plotting the natural log of unsupported ^{210}Pb versus cumulative sediment mass (Figure 10), resulting from decay of the unsupported ^{210}Pb activity over time by its characteristic decay rate with a half life of 22.3 years (Bierman et al. 1998). Unsupported ^{210}Pb results from emanation of ^{222}Rn from continental land masses, decay in the atmosphere to ^{210}Pb , and subsequent deposition onto the lake surface and watershed, followed by scavenging to sediment particles and/or erosion from the watershed that are subsequently deposited on the lake bed. The zone of near constant ^{210}Pb activity between 0 and 3 cm may reflect a period of

increased accumulation or mixing of the sediment due to physical processes, such as episodic resuspension and redeposition of surface sediments during wind events (Beisner et al. 2008). Biological mixing of sediments is unlikely because of the anoxic conditions in the DBL.

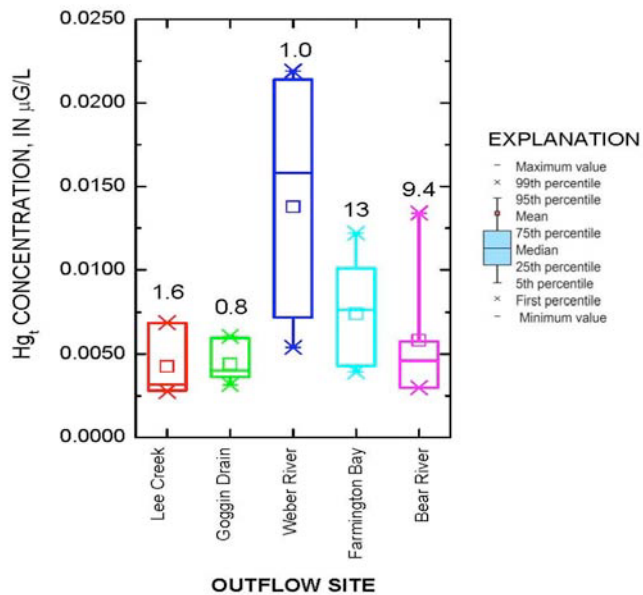


Figure 9—Box plots of whole water Hg_t concentration in water samples from selected inflow sites to Great Salt Lake compared to median daily discharge in m^3/s during April 1, 2007 through March 31, 2008. With the exception of Bear River, analytical results from seven water samples were used for each box plot. Data from six water-quality samples were used for Bear River.

The presence of 7Be in the top 2 cm (Figure 11) is consistent with rapid deposition of sediments. The source of 7Be is atmospheric deposition to the lake surface and rapid scavenging to suspended particulate material. Because of its 53 day half life, the 7Be in the 1-2 cm depth interval can only occur if some fraction of the sediment was in the water column within the past 200 days to accumulate 7Be . The presence of 7Be is likely the result of non-biological resuspension and redeposition processes in GSL.

Sediment mass accumulation rate (MAR) was determined from the ^{210}Pb profile using the constant flux–constant sedimentation rate, CF-CS, method (Appleby & Oldfield 1992). The CF-CS method assumes a steady state accumulation of sediments and a constant unsupported ^{210}Pb activity per gram of depositing sediment particles. The sediment mass accumulation rate ($g/cm^2 \cdot yr$) was calculated from the slope of the linear regression of the \ln (unsupported ^{210}Pb , dpm/g dry sediment) versus the cumulative dry mass, g/cm^2 from 2.5 to 9.5 cm (Figure 10). The core profile was well described by a single regression ($R^2 = 0.98$) yielding an overall MAR of $0.044 g/cm^2 \cdot yr$

(Figure 10). The age of the midpoint of each sediment interval was then calculated by dividing the cumulative mass (g/cm^2) at the middle of each depth interval by the overall MAR, and subtracted from the core collection date to assign a calendar year for each interval. The resulting dates are depicted on Figure 11. These dates do not account for the processes affecting the upper 3 cm of the sediment profile. To correct for processes that resulted in the zone of constant ^{210}Pb activity (0 to 3 cm interval) and the presence of 7Be (0 to 2 cm), the sediment ages were recalculated starting at 2 cm and assuming the 0-2 cm sediment horizon is < 1 year of age. Sediment chronologies are shown as a function of depth in Figure 11 with and without the 2 cm active layer. The effect of accounting for the 2 cm active layer in deriving the sediment chronology is a shift in sediment age to about 5 years younger in each interval below the active layer. This approach assumes that sediments below 2 cm in depth are no longer available for resuspension and are effectively preserved.

The chronology of the sediment core was then combined with the salt corrected Hg_t concentration determined from each 1 cm section to calculate annual Hg_t deposition rates (Table 3). Based on the sediment record collected from site 3510, the mean annual Hg_t deposition ranged from 55 to $150 \mu g/m^2$. These reconstructed annual deposition amounts are significantly higher than the combined dry deposition ($4.4 \mu g/m^2 \cdot yr$) (Peterson & Gustin 2008) and wet deposition ($5.5 \mu g/m^2 \cdot yr$) estimated for GSL. Uniform distribution of riverine Hg_t discharge over the cumulative 2007 surface area of GSL ($3.2 \times 10^9 m^2$) would add another $1.9 \mu g/m^2 \cdot yr$ of Hg_t , resulting in a cumulative annual atmospheric plus riverine deposition of $11.8 \mu g/m^2$.

The large difference between the cumulative measured Hg_t deposition ($11.8 \mu g/m^2 \cdot yr$) and reconstructed Hg_t deposition (average = $130 \mu g/m^2 \cdot yr$) over the last ~ 100 years from the site 3510 sediment core is likely the result of sediment focusing in GSL. Sediment focusing is the preferential deposition of sediments and associated contaminants at a site from both the redistribution of sediments from within the lake and from sediments delivered from the watershed (Van Metre et al. 1997). Previous studies have found that uniform sedimentation is not present throughout the south arm of GSL. Colman et al. (2002) mapped post-Bonneville sediment thickness in the South Arm of GSL and found large variations in sediment thickness ranging from < 2 m to 10 m with a thickness of 5 to 6 m at site 3510. Oliver et al. (in review) used ^{210}Pb profiles of shallow sediment cores collected from the South Arm of GSL to investigate

sedimentation rates over the past ~ 100 years. The ^{210}Pb profiles indicated a wide range of sedimentation mass accumulation rates (MARs) from near $0.00 \text{ g/cm}^2\cdot\text{yr}$ (with no detectable unsupported ^{210}Pb) to areas with MARs up to $0.05 \text{ g/cm}^2\cdot\text{yr}$. These MARs translate into compacted linear sedimentation rates ranging from 0.00 to 0.08 cm/yr , with site 3510 near the upper end of the range at 0.05 cm/yr . Thus, the high annual Hg deposition rates determined in the

sediment core from site 3510 are likely the result of sediment focusing in GSL to this site. Mercury concentration in additional sediment cores collected from other areas of GSL are needed to further refine lake-wide rates of cumulative Hg_t deposition. In addition, a measured atmospheric deposition rate for ^{210}Pb would provide a means to correct for sediment focusing to the core site.

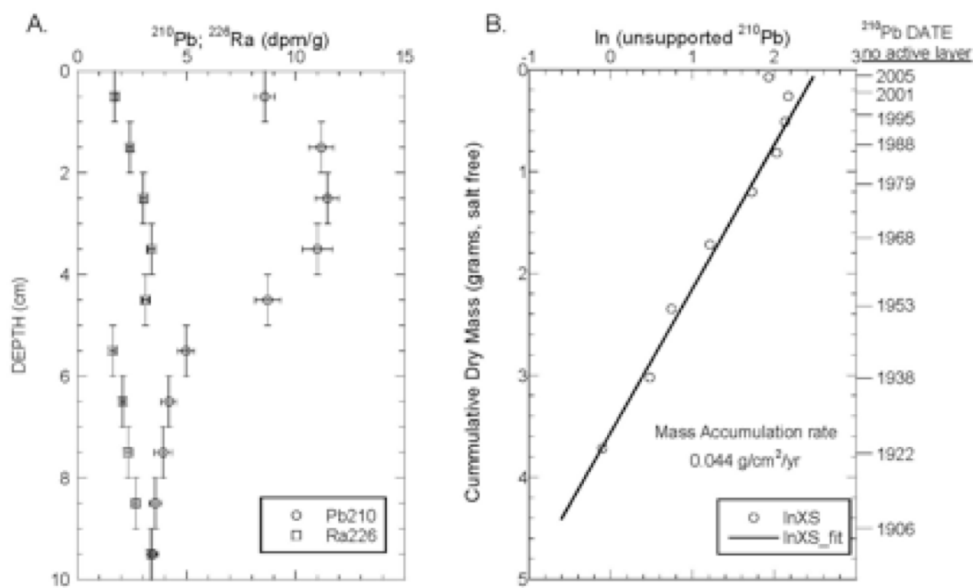


Figure 10—Total ^{210}Pb and ^{226}Ra activity, in disintegrations per minute per gram, versus depth in sediment core (A). Horizontal error bars depict 1 sigma uncertainty in measured activity based on counting statistics. Natural logarithm of unsupported ^{210}Pb activity versus cumulative dry sediment mass (B). Only data with measurable unsupported ^{210}Pb are presented. Solid line represents linear regression of the data used to derive sediment mass accumulation rate.

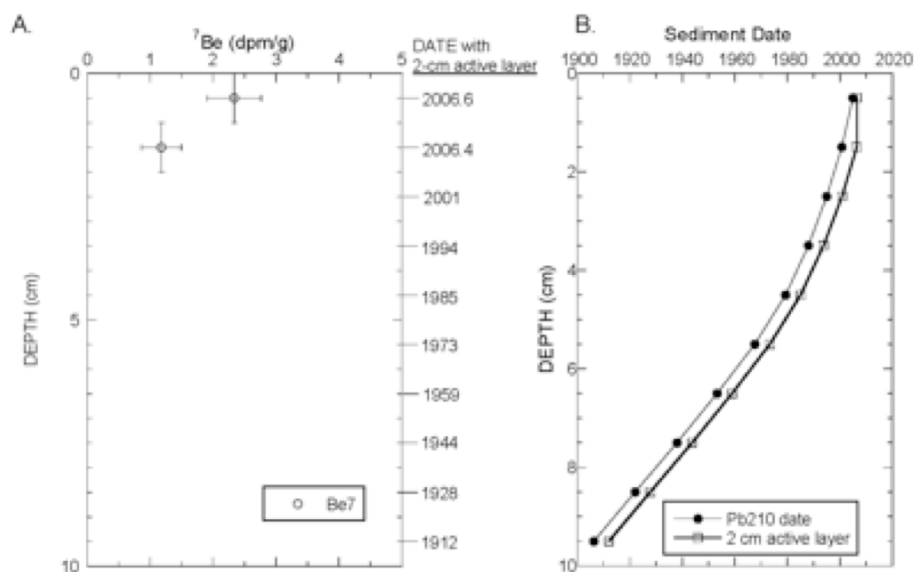


Figure 11—(A) ^7Be activity, in disintegrations per minute per gram, versus depth in sediment core. Horizontal error bars depict 1 sigma uncertainty in measured activity based on counting statistics. (B) Sediment deposition date as function of depth based on the sediment mass accumulations estimated from ^{210}Pb using the CF-CS method, with and without correction for 2 cm active layer. Non-linearity in deposition date versus depth is the result of sediment compaction.

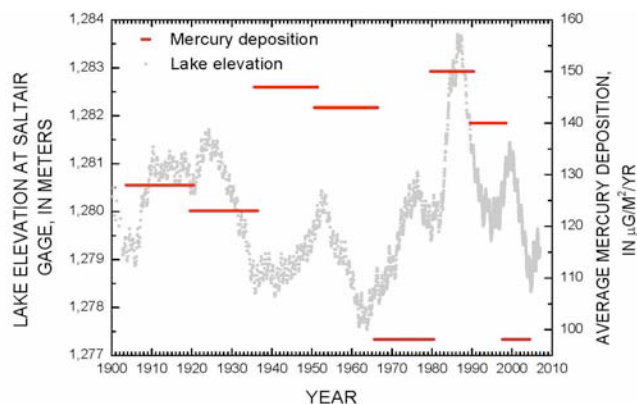


Figure 12—Comparison between historic lake elevation and reconstructed deposition rates of mercury using data collected from sediment core 3510. Because of resuspension processes, the deposition rates for the top 2 cm active layer were not plotted.

In addition to sediment focusing, the sediment core deposition rates show relatively large variations over the past ~ 100 years (Table 3). Historic changes in the lake elevation and resulting surface area of GSL could have a large effect on the total mass of Hg_t directly deposited onto GSL. As previously discussed, atmospheric deposition is estimated to contribute about 84% of the current Hg to GSL, with riverine inputs comprising the remaining 16%. Unlike most freshwater lakes, small changes in lake elevation results in large changes in the surface area “footprint” of GSL that is available to receive Hg inputs via direct atmospheric deposition (Baskin 2005, 2006). With this process in mind, historic changes in the Hg_t deposition rates recorded in the sediment core from site 3510 were compared to changes in lake elevation in the South Arm of GSL (Figure 12).

During the past ~ 60 years, there is very good agreement between lake elevation and Hg_t deposition (Figure 12). The highest Hg_t deposition rate recorded in the sediment core ($> 150 \mu g/m^2 \cdot yr$) corresponds to the highest lake level of GSL in recent history. In contrast, the lowest Hg_t deposition rate recorded in the sediment core ($< 100 \mu g/m^2 \cdot yr$) is in close correspondence with one of the lowest lake levels of GSL in recent history.

During the mid-1930s to the early 1950s the lake level was low; however, there is a relatively high Hg_t deposition rate ($\sim 147 \mu g/m^2 \cdot yr$) recorded in the sediment core (Figure 12). This high Hg_t deposition combined with a low lake elevation could reflect a period of higher global anthropogenic inputs of atmospheric Hg from increased industrial output related to World War II. An ice core collected in northwestern Wyoming (330 km northeast of GSL) showed a spike in Hg_t concentration in ice samples corresponding to snow deposited in the early to mid-1940s (Figure 13). Furthermore, global Hg production also shows a large spike during this same time period (Figure 13).

Based on these initial sediment core results, high lake levels and resulting increased surface area, combined with sediment focusing processes may increase Hg_t accumulation rates in sediment in specific areas of GSL.

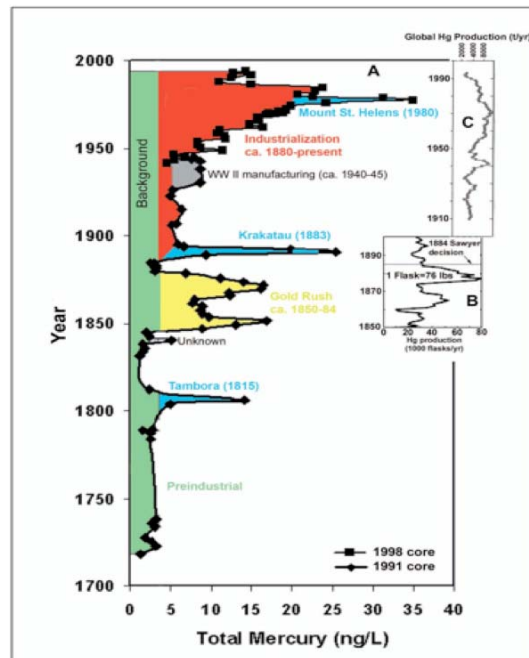


Figure 13—(A) Profile of historic concentrations of Hg_t in the Upper Fremont Glacier (used with permission from Schuster et al. 2002). A conservative concentration of 4 ng/l was estimated as preindustrial inputs and extrapolated to 1993 as a background concentration. Age-depth prediction limits are (10 years (90% confidence level); confidence limits are 2–3 years. (Inset B) Hg production during the California Gold Rush (Alpers & Hunerlach 2000). (Inset C) World production of Hg in tons per year during the last century (Engstrom & Swain 1997).

SUMMARY

Despite the ecological and economic importance of GSL, little is known about current and historic Hg inputs to the lake. Regression modeling techniques were used to estimate whole-water Hg_t and CH_3Hg loads to GSL from six major inflow sources during a one-year period from April 1, 2007 to March 31, 2008. Annual Hg_t loads to GSL ranged from 0.01 kg (KUCC outfall) to 2.8 kg (Farmington Bay outflow). Cumulative Hg_t load to GSL during this 1 year time period was 6 kg, with almost 50% of the cumulative Hg_t load contributed by outflow from Farmington Bay. According to previous research, urban runoff from impervious surfaces in the Farmington Bay watershed, which includes Salt Lake City, may be responsible for the high proportion of Hg_t inputs from Farmington Bay and should be a component of future studies.

Dry deposition of Hg measured by Peterson and Gustin (2008) was combined with the 2007 maximum surface area of GSL to estimate a cumulative dry deposition rate of 14 kg/yr. Cumulative wet deposition of Hg to the surface of

GSL was estimated to be 18 kg/yr using data collected at the MDN site near GSL (National Atmospheric Deposition Program 2008). Comparison of cumulative annual atmospheric Hg deposition (32 kg) to annual riverine input (6 kg) indicates that atmospheric deposition is the dominant input source to GSL. A sediment core collected from the southern arm of GSL was used to reconstruct annual Hg_t deposition rates over the past ~ 100 years. The large difference between the reconstructed Hg_t deposition from the sediment core (average = $130 \mu\text{g}/\text{m}^2\cdot\text{yr}$) to the cumulative measured Hg_t deposition (riverine + atmospheric) of $11.8 \mu\text{g}/\text{m}^2\cdot\text{yr}$ is likely the result of focusing of sediment to this site in GSL. Unlike most freshwater lakes, small changes in water level in GSL significantly changes the lake surface area available for direct deposition of atmospheric Hg. There is good agreement between lake elevation (and corresponding lake surface area) and Hg_t deposition rates estimated from the sediment core over the past 60 years. Changes in lake surface area during historic lake-level fluctuations combined with sediment focusing processes are proposed to explain the reconstructed variations in Hg_t deposition rates from the sediment core collected from GSL.

Table 3—Mean annual total mercury deposition in Great Salt Lake from 1904 to 2006 using sediment-core data from site 3510. Year of sediment deposition was determined using the 2 cm active layer model.

Sample interval, in cm	Year(s) of deposition	Salt-corrected total Hg concentration, in $\mu\text{g}/\text{g}$	Mean annual Hg deposition, in $\mu\text{g}/\text{m}^2$
0 to 2	2004 to 2006	*0.126	55.4
2 to 3	1998 to 2004	0.223	98.1
3 to 4	1990 to 1998	0.319	140.0
4 to 5	1980 to 1990	0.340	150.0
5 to 6	1966 to 1980	0.223	98.1
6 to 7	1951 to 1966	0.326	143.0
7 to 8	1936 to 1951	0.335	147.0
8 to 9	1920 to 1936	0.279	123.0
9 to 10	1904 to 1920	0.290	128.0
*Average concentration from two samples (0-1 and 1-2 cm)			

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Dynamics of Mercury in Eared Grebes on Great Salt Lake

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Total mercury was measured in primary and breast feather and in liver and breast muscle tissues of eared grebes (*Podiceps nigricollis*) during the fall migration at Great Salt Lake, Utah and for reference, also at Mono Lake, California in 2006. A subset of liver and breast muscle tissues was also analyzed for methylmercury and selenium. Grebes typically arrive at Great Salt Lake starting in August and into September and depart by late December.

Concentrations of total mercury in grebes from Great Salt Lake increased in all tissues except primary feathers during a 64 day period between October and December (Figure 1). The most pronounced change in total mercury occurred in the liver, increasing nearly threefold ($F_{2,27} = 20.78$; $P < 0.001$) from a mean of 10.2 mg/kg to 27.4 mg/kg on a dry weight basis, followed by breast feather ($F_{2,27} = 4.80$; $P = 0.016$) and breast muscle ($F_{2,27} = 3.48$; $P = 0.045$). Notably, mean liver methylmercury (9.7 to 22.2 mg/kg) and selenium (13.7 to 23.2 mg/kg) concentrations also increased during this same period. Mercury concentrations in breast (mean = 13.9 mg/kg) and primary (12.4 mg/kg) feathers and liver (7.3 mg/kg) and breast muscle (1.6 mg/kg) from eared grebes at Mono Lake were generally lower than those from Great Salt Lake in mid-October. The grebes at Mono Lake staged for a much shorter period than those at Great Salt Lake in 2006, thus later seasonal comparisons were not possible.

Feathers had the highest concentrations of mercury, supporting evidence that this tissue is an important mechanism for excretion of mercury. Breast muscle had the lowest mercury concentrations; however, all Great Salt Lake samples still exceeded recommended human

consumption screening criteria of 0.3 mg/kg wet weight. Mean concentrations of methylmercury comprised 72% and 96% of total mercury in liver and breast muscle of Great Salt Lake eared grebes, respectively.

When compared to historic data (1992–2000), our data indicated that liver mercury concentrations have increased in eared grebes at Great Salt Lake over the past two decades. Increases (mean = 0.34 mg/kg in 1996 - 2000 and 1.02 mg/kg in 2006) were also observed in brine shrimp (*Artemia franciscana*), the predominant food of grebes at Great Salt Lake.

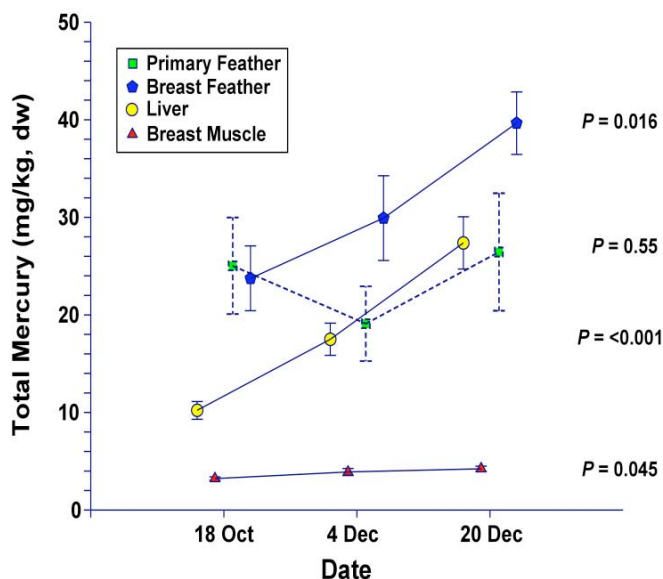


Figure 1—Seasonal concentrations (mean and standard error) of total mercury in tissues from eared grebes collected on Great Salt Lake during 2006. P-values indicate level of significance between sample dates. Concentrations are in mg/kg dry weight.

Eutrophication, Nutrient Fluxes and Connectivity between the Bays of Great Salt Lake, Utah (USA)

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Natural geography and causeways have divided Great Salt Lake into four bays interconnected by breaches (Figure 1). Farmington Bay (~250 km²) receives excessive wastewater discharges from nearby metropolitan Salt Lake City, and is hypereutrophic with massive blooms of toxic cyanobacteria when salinities are < 5%. The mean summer chlorophyll concentration in 2005–2006 was 186 µg/l, and concentrations of toxic nodularin reached 205 µg/l, exceeding the World Health Organization's human health guideline 10-fold. The entire water column in the bay is usually anoxic at night, hydrogen sulfide is abundant, and odors can be extreme. Brine shrimp production in this bay is minimal because of poor water quality and/or predation by air-breathing invertebrates (corixids).

We assessed how the massive production in Farmington Bay might impact nutrient loading and brine shrimp production in Gilbert Bay. Loading of the limiting nutrient (N) to Gilbert Bay was dominated by Bear River inflows during spring runoff (77%), but during summer, 60% of the load to Gilbert came from Farmington Bay. MODIS satellite imagery documented plumes of phytoplankton-rich water flowing out of Farmington Bay and overflowing into Gilbert Bay, particularly during summer. Isotopic analyses of ¹³C and ¹⁵N at 34 stations indicated, however, that the high algal production in Farmington Bay did not contribute substantially to the diets of brine shrimp in Gilbert Bay, at least during the May, June and November synoptic analyses. However, isotopic analyses suggested that brine shrimp utilize particulate matter exported from Farmington Bay (Naftz, D.L., C. Angerth, T. Kenney, B. Waddell, S. Silva, N. Darnall, C. Perschon & J. Whitehead. 2008. Anthropogenic influences on the input and biogeochemical cycling of nutrients and mercury in Great Salt Lake, Utah, USA. *Applied Geochemistry* 23: 1731–1744).

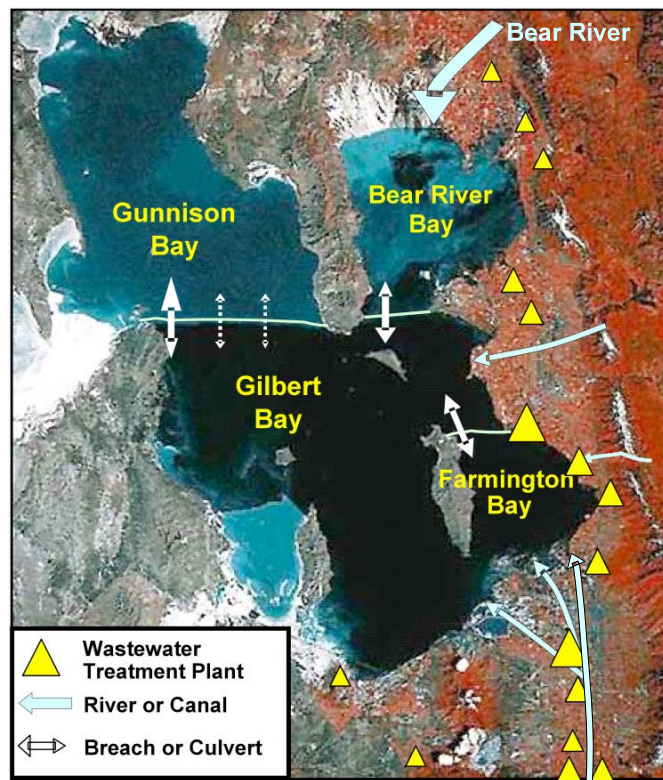


Figure 1—The four bays of Great Salt Lake showing causeways that separate the system into four bays. White arrows show flow paths between bays.

Particulate N export from Farmington Bay represented 11% of the primary production in Gilbert Bay from May to November. Preliminary estimates of nutrient loading to Gilbert Bay are > 2 g N m⁻², levels that can cause dangerous loading in freshwater lakes. Predicted population growth in the Great Salt Lake watershed is > 250% by 2050 and uncontrolled nutrient loading may consequently cause eutrophication problems not only in Farmington Bay, but in Gilbert Bay as well. The management of nutrient loading and other pollutants in Great Salt Lake will need to take into account these likely increases and the close coupling of the bays.

Competitive Exclusion of Cyanobacterial Species in Great Salt Lake

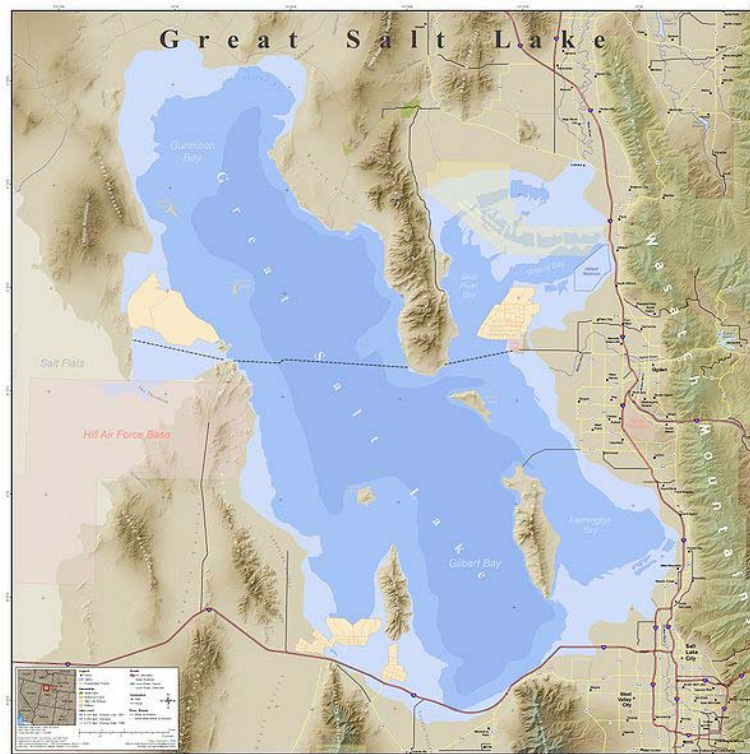
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The division of the waters of Great Salt Lake by a rail and vehicular causeway into different regions of salinity and color variation represents a natural experiment that permits examination of competitive exclusion of cyanobacteria. Cyanobacterial distributions partially follow the salinity, with *Aphanothece halophytica* proliferating in the North Arm and *Nodularia spumigena* being prominent in the South Arm. I hypothesized that cyanobacterial species abundant north of the railway causeway are competitively excluded from the south by other species, and that cyanobacterial species that thrive and bloom south of the Antelope Island causeway cannot grow in the high salinity of the north. To test this hypothesis, 129 flasks of autoclaved water from the north and south sides of each causeway were inoculated with Great Salt Lake water samples from the north and south sides of the causeway.

Four genera of cyanobacteria, *Aphanothece*, *Oscillatoria*, *Phormidium*, and *Nodularia* were identified and counted from the culture flasks using comparative differential interference contrast, fluorescence, and scanning electron microscopy. The relative abundance of cyanobacterial species was determined and differences were tested for statistical significance. *Aphanothece halophytica* was found in all inocula, but its growth was suppressed in the presence of *Nodularia spumigena*, while *N. spumigena* was found only in inocula from the less saline waters in the south, and apparently cannot survive in the extremely saline waters of the north.

These analyses suggest that both abiotic and biotic factors influence the distribution of cyanobacteria in Great Salt Lake. *Nodularia* is excluded from the north by high salinity, but *Aphanothece* is outcompeted in the south by *Nodularia*.



Map of the Great Salt Lake, Utah, showing predominant features. Created December 2008 by Justin Morris & Greg Fryer using ArcGIS.

Evidence and Implications of Movement of the Deep Brine Layer in the South Arm of Great Salt Lake, Utah

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Trace elements are found in elevated concentrations in lake bed sediments of Great Salt Lake and their periodic resuspension and dissolution into the water column may affect water quality and the biota and needs to be better understood. Lake circulation is restricted by a railroad causeway completed in 1959 and typically the South Arm of the lake is stratified with a lower dense deep brine layer (DBL) that originates in the North Arm. The DBL flows to the bottom of the South Arm of the lake where it accumulates decaying organic matter from the overlying water column and becomes anoxic. Periodic interruption of stratification in the water column has been observed at two fixed stations on the lake by monitoring vertical water temperature profiles and 3-D velocity profiles (Figure 1) using a SonTek® Argonaut™-XR Acoustic Doppler Current Profiler. Displacement of stratification is characterized by an abrupt change of the DBL temperature to the temperature of the upper brine layer (UBL) during periods where the DBL is warmer than the UBL (September–March) and where the DBL is colder than the UBL (April–August). Abrupt changes in horizontal velocity

and direction precede the temperature equilibration between the layers. Events occur over periods of 12 to 24 hours and are associated with strong sustained wind events and development of a surface seiche on the lake. The surface seiche may set up an internal seiche along the UBL/DBL interface which would place oxic UBL in contact with anoxic sediment previously overlain by DBL. Mixing or displacement of the DBL may involve trace element movement within the water column due to changes in pH and redox potentials. For example, methylmercury concentration from unfiltered whole water samples in the DBL are high (12–42 ng/l) compared to the UBL (0.4–1.6 ng/l). Laboratory experiments simulating UBL contact with anoxic lake bed sediment were conducted over 24 hours to investigate trace element desorption and (or) dissolution for selected trace elements. Results from the laboratory experiments indicate that a small percentage of selenium (1%) and arsenic (2%) associated with anoxic bottom sediments is periodically recycled into the UBL where it can potentially be incorporated into the biota utilizing the oxic, UBL of GSL.

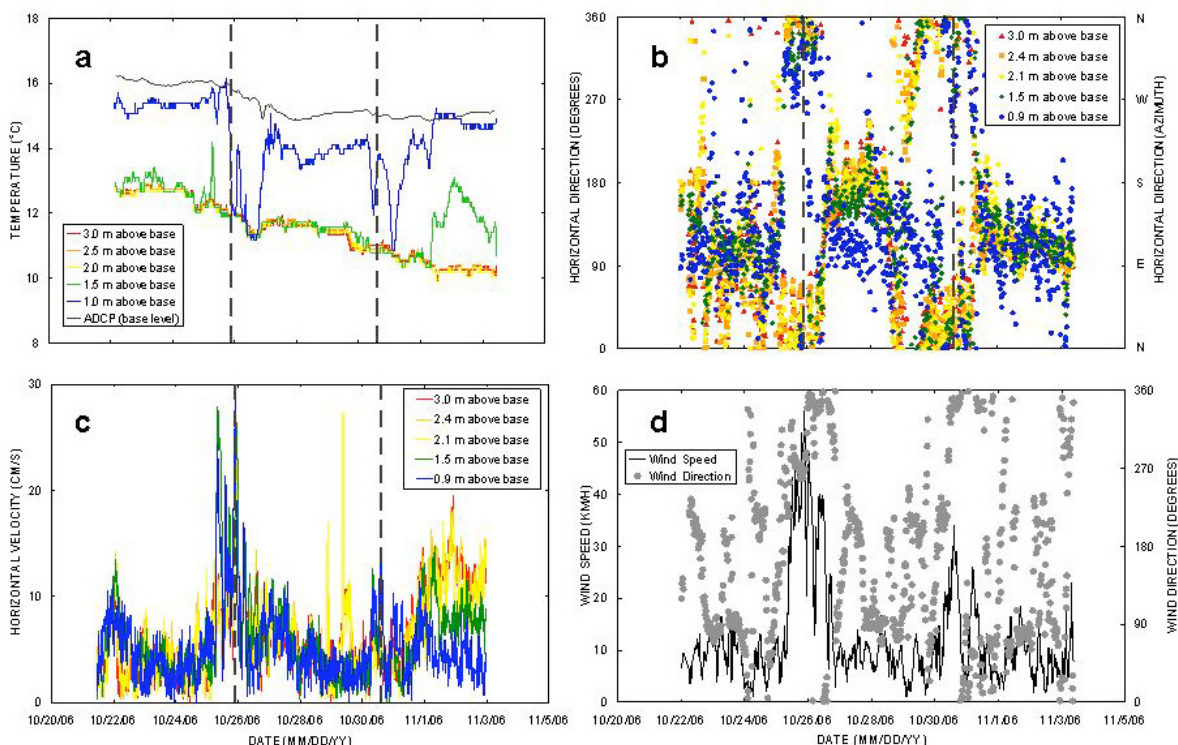


Figure 1—Variations in parameters during a strong wind event, Great Salt Lake, Utah: (a) temperature equilibration event at site 3510 associated with (b) a fluctuation in horizontal velocity from ADCP, (c) fluctuation in horizontal direction from ADCP, and (d) deviations in wind velocity and direction at Hat Island (MesoWest). Dashed lines correspond to peak wind speeds.

Production of Magnesium from Great Salt Lake, Utah, USA

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ABSTRACT

Magnesium metal has been commercially produced from the waters of Great Salt Lake since 1972. Worldwide use of magnesium has markedly increased over the last twenty years due to its unique properties of low density and high strength. Great Salt Lake is a valuable resource for the recovery of magnesium minerals due to its chemical composition, natural geography/climate and proximity to transportation and markets. US Magnesium LLC and its predecessors have overcome various technical challenges as well as political hurdles, climatic calamities and market uncertainties. This discussion will describe the extensive use of solar evaporation ponds to produce suitable raw materials for magnesium manufacture as well as significant mineral by-products. It will also include a basic technical description of the magnesium metal manufacturing process, an explanation of the operating history of the Rowley Utah plant, and briefly touch on the current commercial uses of magnesium metal.

INTRODUCTION AND HISTORY

Since the arrival of mountain man explorers in the 1830s and pioneer settlers in the 1840s, Great Salt Lake has been recognized as a valuable source of minerals. In addition to the sodium chloride “salt” implied by the name, the waters of the lake also serve as the raw material for the magnesium facility operated by US Magnesium LLC at Rowley, Utah. That facility currently accounts for all of primary magnesium production in North America and about 10% of the world’s magnesium metal production.

In 2006 the total world consumption of magnesium was estimated at about 8.0×10^5 metric tons. The plant at Rowley is among the largest in the world and represents about 10% of the world production and all of the current North American production in 2008.

The largest single use of magnesium is alloying aluminum to provide strength, malleability, and corrosion resistance. The aluminum beverage can is perhaps the largest single user, but significant quantities magnesium are present in almost every structural use of aluminum ranging from window frames to aircraft components.

The second most important market for magnesium is its use in structural applications via diecasting. The largest use of

magnesium die-castings is in the automotive industry where magnesium’s strength and lightweight are being used in an ever-increasing volume to improve fuel economy. Magnesium die-castings are also selected as a housing for many computer, cell phone, and electronic components because of magnesium’s electrical dampening properties. Another important use of magnesium die cast parts is in the manufacture of manual and power hand tools where magnesium’s lightweight improves safety and operator performance.

Magnesium’s electro-chemical potential is ideal for use in corrosion protection applications. A substantial amount of magnesium is consumed each year as high electrical potential sacrificial anodes, which are installed, for corrosion protection on oil, gas, and other pipelines, hot water heaters, and other equipment in need of cathodic protection.

Addition of magnesium to iron causes the iron to become higher strength and more ductile. Automotive crankcases, which used to be forgings, are now manufactured in ductile iron. The steel industry’s preferred method to remove embrittling sulfur compounds involves injecting powdered magnesium into molten metal so the sulfur can be removed as a slag.

Magnesium is also used as a reducing agent in the production of titanium, zirconium, hafnium, and beryllium. Other chemical uses of magnesium include production of Grignard reagent catalysts (a catalyst commonly used in the synthesis of halogenated organic chemicals) motor oil additives, pyrotechnic materials, and as a pharmaceutical material.

Production of magnesium metal from Great Salt Lake has roots from other places. In 1940, a diverse metallurgical company, National Lead Industries, began to develop technology for the production of magnesium by operating a United States government magnesium plant using the ferro-silicon process at Lucky, Ohio, during World War II. That company gained additional expertise in the production of magnesium metal in 1951 with the formation of a jointly owned company, the Titanium Metals Corporation of America (also known as Timet) at Henderson, Nevada. Magnesium metal is used as the chemical reducing agent in the production of titanium. The magnesium values are then recovered as $MgCl_2$, which is electrolyzed to recover and

recycle both magnesium and chlorine. In the early 1960s National Lead began an active investigation into the possibilities of producing and selling commercial quantities of magnesium metal. In searching for additional sources of magnesium, National Lead Industries joined with Hogle-Kearns, a Utah investment firm, and Kerr McGee, a diversified chemical company, in a venture to explore the potential of Great Salt Lake for producing magnesium metal. Acquiring rights to the lake's Stansbury Basin and its potential to be developed into an economically attractive solar pond system was the indispensable resource for the conceived operation. The Stansbury Basin is natural depression in the lakebed, which is bounded on three sides by natural terrain. The pond system as envisioned was bounded on the East by Stansbury Island, on the West by the Lakeside Mountain range, and on the South by naturally increasing contour (Figure 1). To the Northwest the envisioned solar ponds to large degree would be bounded by a mud flat that has higher in elevation than the majority of the basin. To the Northeast the distance between Badger Island and Stansbury Island was connected by a zone of relatively shallow water (Figure 1) that would facilitate the construction of an impoundment structure and still allow access to deep water that would assure a constant supply of feed brine for solar evaporation.

During 1965 and 1966, NL Industries conducted pilot operations to select the best process for use with Great Salt Lake brines. Scale model solar ponds were constructed at a location near to the proposed operation, and a manufacturing pilot plant for producing cell feed was built near Lakepoint, Utah, 45 km east of Rowley. Magnesium chloride product from this pilot plant fed a prototype cell at TIMET in Henderson, Nevada. From this program a decision was made in 1969 to build a magnesium plant at Rowley, Utah (Figure 1), utilizing brine from the Great Salt Lake. By the time construction plans were finalized, National Lead had acquired the equity of its partners and was the sole owner of the proposed magnesium operation.

Construction of the integrated facility began in 1970 with Ralph M. Parsons as the general contractor. The magnesium manufacturing plant was located on an undeveloped site 16 km north of Interstate 80 on the West side of the Stansbury Basin (Figure 1). The exact location was determined in part to take advantage of straddling power district boundaries that provided alternative sourcing of electricity which are a substantial component of the manufacturing cost. The plant site was named "Rowley" after Jeff Rowley who during the period of construction was the CEO of National Lead. In addition to normal construction costs, it was necessary to construct 24 km of paved highway, a railroad spur, a dedicated natural gas line,

and a dedicated 1.38×10^5 -Volt power line to service the new plant.

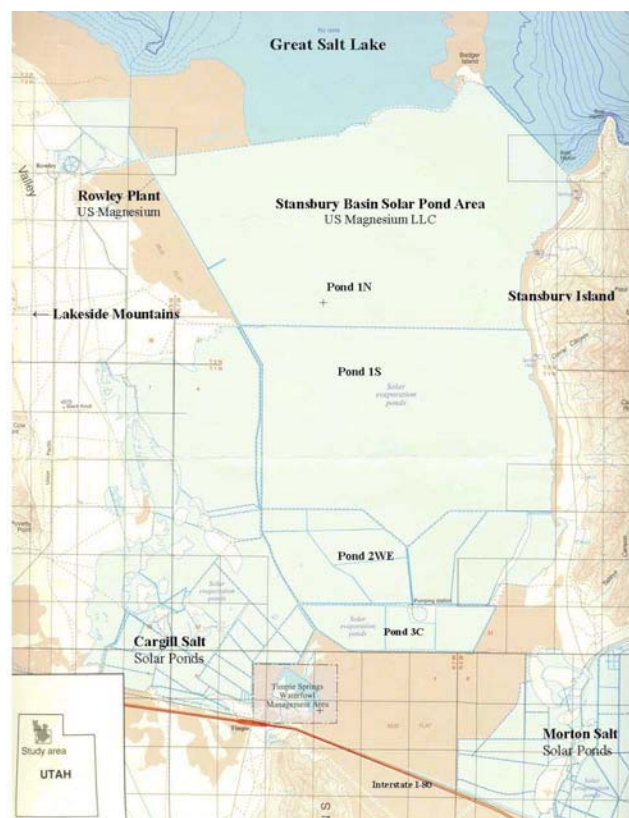


Figure 1—Stansbury Basin Solar Evaporation Ponds, Great Salt Lake, Utah.

Actual start up of the magnesium operations occurred in the summer of 1972. Initial start-up operations experienced substantial difficulties as viable operating systems were developed. It was necessary to shut down operations in 1975 to re-engineer some parts of the process. Norsk Hydro, a primary magnesium producer in Norway was contracted to assist this process. A system for the removal of boron was added to the process. Boron which is naturally occurring in the waters of Great Salt Lake had unexpected adverse implications for final magnesium product recovery and quality. Boron chemicals are surface active with liquid magnesium. If boron is not removed prior to electrolytic reduction of magnesium chloride, the surface of the magnesium metal produced tends to carry a film of chloride salts making the refining process more difficult.

Operations after the 1975 shut down approached routine, but suffered economically from the much higher than expected capitalization cost and lower than expected plant productivity. In 1980 NL Industries (National Lead changed its name to NL industries in the mid-70s) sold the magnesium operation to Amax Inc., a diversified mining and natural resource company.

Shortly after the transfer of ownership, a change in the weather pattern caused an unprecedented rise in the level of Great Salt Lake. Amax was compelled to expend millions of dollars to continue operation. Millions were spent to raise and fortify the dikes that separated the Stansbury Basin Ponds from Great Salt Lake (Figure 1). Additional expenditures were required to expand the solar evaporation area to compensate for wet weather patterns and the dilution of the mineral content of the lake caused by the increased volume. Modifications to the Rowley magnesium manufacturing process were made in an attempt to compensate for weaker than normal feed brines.

In spite of company efforts, a storm on June 7, 1986 coupled with record lake elevations, breached the main dividing dike that separated the solar ponds from the lake. Over the course of a week, the Stansbury Basin Ponds filled with lake water raising the level in the ponds by about 2.1 m (7 feet) and dropping the level in the balance of the lake by an estimated 14 cm (5.5 inches.)

Realization of the time required to bring the flooded Stansbury Basin ponds back into production, and uncertainty as to the future trend in lake levels, caused Amax to examine other alternatives. During this period, magnesium production continued at a reduced rate using brines purchased from Reilly Chemical near Wendover, Utah, located 150 km west on the Utah-Nevada state boundary and Leslie Salt located near San Francisco Bay, California.

It was determined after substantial analysis that there was an area 70 km to the west near the Knolls, Utah, comprised of mud flats interspersed with sand dunes that could be converted to solar ponds of an appropriate size at a reasonable economic cost, and at a speed of construction that could make use of Great Salt Lake brine in New Foundland Bay that was there as a result of the State of Utah's "West Desert Pumping Project". The permits to use the area of interest were obtained and construction with an expedited completion schedule commenced in May of 1987 with the engineering firm of Morrison-Knudsen acting as the general contractor. The construction included a 10 km feed canal, six pump stations, a maintenance shop and office facility and over 100 km of containment dikes. Construction was sufficiently completed by December of 1987 that the "Knolls Solar Evaporation Ponds" could start the initial filling. In addition to the construction of ponds at

Knolls, Amax also assisted the State of Utah in 1988 by extending the inlet canal to the Hogup Pump Station that provided suction head protection and capacity to the West Desert Pumping Project. This was accomplished with a large cutter-suction dredge, the Vagabond, which was then owned and operated by Amax.

The Knolls ponds were designed anticipating that the West Desert Pumping project would operate for a limited period. The operating plan at Knolls included sufficient storage that the ponds could supply and store up to 10 to 12 years of plant feed after pumping to the West Desert ceased. The West Desert Pumping Project operated into 1989 and then was shut down. Brine was generally accessible from New Foundland Bay until mid-1990. After that time brine would be available on occasion from re-dissolution of the salt deposited on the West Desert.

In 1989 Amax sold the magnesium facility to Renco Inc., a privately held company of New York. The magnesium operation was renamed Magnesium Corporation of America or "Magcorp."

In the late 1980s and early 1990s the level of Great Salt Lake receded as quickly as it had risen in the early 1980s. By 1992 the lake level had retreated to a level that allowed Magcorp to begin the process of re-commissioning the ponds in the Stansbury Basin. The first harvest brine in ten years from the Stansbury Basin ponds was brought in 1995. The Rowley magnesium operation has operated on raw materials brine produced in the Stansbury Basin solar ponds since that time.

In 2001 economic conditions led to Magcorp filing bankruptcy. In 2002 the Renco Group re-purchased the assets in a bankruptcy sale and a new company, US Magnesium LLC, was formed.

The Rowley Magnesium Process

Costs and reliability are both critical factors in any manufacturing process. The same is obviously true for magnesium operations at the Rowley plant. Decisions are made with the intent of reducing energy costs and maximizing equipment efficiency. The present Rowley process is described below.

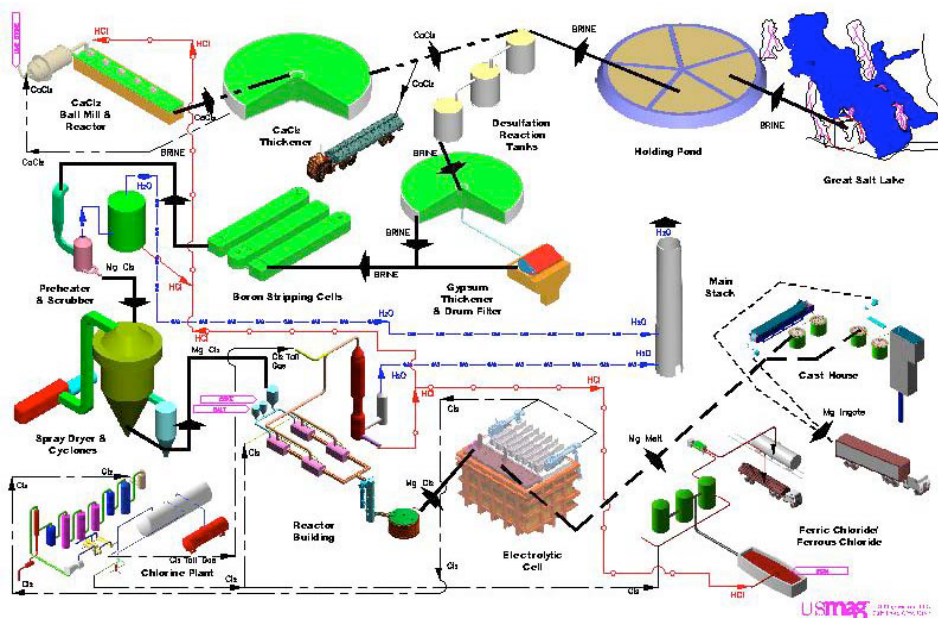


Figure 2—Simplified magnesium production process.

Solar Evaporation

The source of raw materials for the manufacture of magnesium is the magnesium chloride (MgCl_2) that occurs naturally in Great Salt Lake. The natural magnesium concentration in the South Arm of the lake has ranged as low 0.18% Mg in 1986 when the lake level was at its zenith, to approximately 1% at the lake's historic low level in 1963. The nominal concentration of magnesium is about 0.45% Mg by weight. To be an economically acceptable feed to the Rowley magnesium manufacturing process, a concentrated feed brine of greater than 8.4% Mg (by weight) is required. To achieve this concentration, US Magnesium employs the world's most extensive industrial use of solar energy. The Stansbury Basin ponds are comprised of State mineral leases that occupy approximately 300 km² (7.5 x 10⁴ acres). The actual "wet area" within earthen dikes amounts to about 225 km² (5.6 x 10⁴ acres). The Stansbury Basin ponds annually bring in between 75 and 135 billion liters of lake water dependent on the previous year's evaporative performance and inventory needs. The basin is divided into large pond segments. Efficient operation and maximum recovery is achieved by operating the ponds in a continuous mode where the brine advances like a slow moving river that becomes shallower as magnesium concentration increases rather than letting individual ponds evaporate to the desired concentration. The progressive concentration of magnesium is illustrated in Table 1, which shows the relative concentrations of Great Salt Lake and the effluent from three of the ponds in sequence. The magnitude of this evaporation step is illustrated by the fact that less than one

percent of the volume of the original Great Salt Lake brine finally reaches the plant for manufacture of magnesium. In concentrating the brine, about five million metric tons of salts are deposited in the ponds each year.

Table 1—Weight percent of each constituent.

	Great Salt Lake brine	Effluent Pond 1S	Effluent pond no. 2WE	Effluent pond no. 3C to holding pond
Mg	0.4500	2.000	4.800	8.50
K	0.3000	1.500	3.600	0.15
Na	4.0000	7.000	2.600	0.20
Li	0.0020	0.010	0.024	0.07
B	0.0018	0.009	0.021	0.06
Cl	7.0000	14.000	16.000	22.60
SO ₄	1.0000	5.000	5.300	4.20

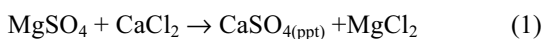
Because of the seasonal variations in weather and temperature in Utah, and because the rate of evaporation is inversely related to the concentration of the brine, it is only possible to achieve the desired final brine concentration in the two or three hottest and driest months of the year typically starting in the month of June and continuing through early September. When the target magnesium concentration is achieved, the concentrated brine is pumped to "deep storage" holding ponds, which in aggregate can store up to three years supply of brine. This deep storage is required to avoid the dilution from annual precipitation and

to assure an adequate supply of plant feed brine during years when weather conditions won't permit adequate evaporation/concentration success. In addition to the magnesium rich brine product that serves as the feedstock to the magnesium operation, sodium chloride and potassium salts are also recovered and sold as will be described later.

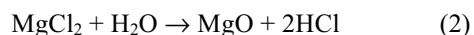
Feed Preparation

The preparation of the magnesium chloride contained in the concentrated lake brine for use as feed for electrolytic cells entails the removal of unwanted impurities, further concentrating the brine, and eliminating water. In the final steps the MgCl_2 is melted and purified. The process steps are outlined in Figure 2.

The concentrated magnesium chloride brine is pumped into the production plant from the deep storage holding ponds to a series of reaction tanks where CaCl_2 is added and gypsum is precipitated and collected in a thickener. This reaction may be simplified as follows:



This step removes most of the sulfate, which may be unstable (explosive reactions) in molten systems. The brine next passes through a solvent extraction (liquid-liquid) step where a long chain alcohol in a kerosene carrier is used to remove the naturally occurring boron from the solution. The active reagent used has varied through the life of the magnesium operation, but decanol [$\text{C}_{10}\text{H}_{19}\text{OH}$] and octanol [$\text{C}_8\text{H}_{17}\text{OH}$] have been used with good economic performance. This impurity that is "surface active" on molten magnesium surfaces must be removed as it adversely affects the magnesium recovery purity of the final product. Next, the brine passes through a preheater vessel, which utilizes available waste heat to further concentrate the brine prior to being fed to spray dryers. The spray dryers convert the concentrated brine to a dry MgCl_2 powder. The intent is to remove all of the water, but some residual amount remains. Additionally some of the magnesium chloride is oxidized to a magnesium oxide [$\text{Mg}(\text{OH})_x\text{Cl}$] in the drying process. For simplicity this group of chemical species, magnesium hydroxy-chloride is generally referred to as MgO . This MgO is formed by hydrolysis of MgCl_2 as shown in a simplified way as:



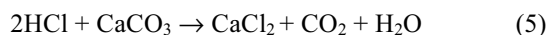
The spray drying process is energized by the exhaust gases from natural gas fired turbine/generators in a true utilization of co-generation technology. The electricity produced is used in the electrolysis of magnesium chloride described later. Gas burners are also available to operate the spray dryer when the turbines are not available for use.

The spray-dried powder is pneumatically conveyed and stored in large bins until it is fed to the cell feed preparation step that is locally referred to as the "Reactor" process. In the Reactor, the powder produced in the spray dryers is melted and further purified with chlorine and carbon to remove MgO and water. This is a continuous process where the spray dried magnesium chloride powder is first fed into a brick lined furnace known as a "melt cell" where it is melted via electrical resistance heating. The molten salt then overflows from the melt cell through a ceramic lined trough into a "reactor cell" where additional retention is provided to bring the intended chemical reactions to completion. A tertiary chlorine treatment step uses proprietary contact technology to further reduce the magnesium oxide content. The refined/purified molten salt moves to holding cells where the temperature is kept at 900°C (1550 EF). From the holding cells the purified molten salt is transported to the electrolytic cells by custom designed mobile molten material haulers.

Removal of impurities from the molten salt is complex but can be summarized by the following simplified equations:



HCl produced from the chlorination of water as shown in Equation 4 is recovered as concentrated, but not commercial grade hydrochloric acid which is subsequently reacted with limestone to produce CaCl_2 (see Equation 5) needed for desulfation and in the production of iron chlorides which are sold as byproducts.



Production and Handling of Magnesium Metal

Molten salt containing about 94% MgCl_2 is transferred to the electrolytic cells on a regular schedule. At the time of construction in 1972, the electrolytic cells at Rowley (1999) were essentially a modification of the I.G. Farben cell developed in Germany in the 1930s. Cells of this design

were also referred to as cooled cells or I.G. cells. Those cells consisted of five graphite anodes with steel cathodes on either side. Semiwalls (thin ceramic diaphragms) extended from the top of the cell down into the bath isolating chlorine gas from the molten magnesium. The gas compartments around the anode collected the chlorine gas, which was generated on the anode surface. The chlorine is drawn to the chlorine recovery plant. The cathode compartment collects the magnesium metal, which floats on the surface of the molten salt due to its lower density (sufficient air is passed over the cathode compartment to remove any fumes and then through a scrubber in order to provide a fume-free environment for workers). In addition to the cooled cells, US Magnesium also utilized “Amax Sealed Cells” which were a diaphragmless (no semiwall) design developed under the period of Amax’s ownership. These sealed cells had a single large common anode compartment and the molten magnesium is directed to a different compartment where it may be removed. The magnesium metal is removed from the cells and is sent to the foundry.

Beginning in 1995 the magnesium operation engaged in a research and development effort to modernize the design. The intent was to improve electrical efficiency, magnesium recovery and to reduce operational costs and environmental air emissions. The design developed over a six-years and eventually was named the “M-Cell” design. The first commercial M-cells were installed in April 2001. By late 2002 all other electrolytic cells were replaced by M-cells.

Efficient operation of electrolytic cells depends on maintaining temperatures and magnesium concentrations. The geometry of the M-cells physically separates the molten metal formed on the cathodes from the chlorine gas that is generated on the anode surface. Electrolytic cells frequently receive input of molten magnesium chloride from the Reactor process in order to maintain optimum operation. Magnesium metal being less dense than the molten salt electrolyte floats to the surface where it is collected by mobile vacuum equipment. In addition to magnesium chloride, the electrolytic cell electrolyte also contains sodium, potassium, calcium and lithium chlorides that are derived from the raw materials and process additions of calcium. If the magnesium concentration is sufficiently depleted, production of sodium metal commences. The electrolytic decomposition of magnesium chloride is otherwise straightforward.

The collected molten magnesium metal is transferred to the foundry via mobile transports referred to as “Glamas” or vacuum wagons that utilize a stationary vacuum system to draw molten metal from the cell surfaces into an appropriate steel vessel. Air pressure is subsequently applied to evacuate the molten magnesium to holding/refining furnaces where it is refined and/or alloyed as required. The molten magnesium is then cast into shapes appropriate for the customer’s needs. These molds may vary from as small as 7 kg (15 pounds) to as large as 800 kg (1750 pounds). Most magnesium shipments to customers are conveyed by truck with a lesser portion being shipped by rail.

By-products of Magnesium Production

In addition to manufacturing magnesium metal, US Magnesium also produces and sells a number of byproducts. A co-product of electrolytic magnesium is chlorine. Approximately three kg of chlorine are produced for each kg of magnesium. US Magnesium accounts for about 1% of the United States’ chlorine production. Part of the chlorine produced at US Magnesium’s Rowley operation is recycled into the described “Reactor” process. The balance of the chlorine is loaded into tank cars, transported by rail and sold as merchant grade chlorine. Before the modernization of the electrolytic process in 2001, the capture of chlorine at the Rowley facility ranged around 85%. With the advent of the modernization of the electrolytic process essentially all (99.9%) of the chlorine is captured as elemental chlorine or converted to hydrogen chloride and captured in scrubbing equipment. Chlorine has a variety of chemical uses and is also used in water purification. The chlorine produced at Rowley has largely been used in the production of ferric chloride, production of plastics, leaching of gold bearing minerals, and other general commercial uses.

As previously mentioned, US Magnesium produces calcium chloride using crushed limestone (previously oolitic sand) and hydrochloric acid produced in environmental control equipment (refer to Equation 5). One third of the calcium chloride produced is used in the process for the removal of sulfate from the solar pond brines. The balance is available for outside sales. Calcium chloride is used as an additive for specialized concrete, as a dust suppressant, and for heavy media purposes in oil field service.

Iron chlorides (ferrous and ferric chloride) are also produced for outside sales at US Magnesium's Rowley facility. The by-product hydrochloric acid is reacted with a variety of economically acceptable metallic iron sources to produce ferrous chloride. Ferrous chloride can be treated with chlorine to convert it to ferric chloride. These iron chlorides are sold and are principally employed as flocculating aids and odor control agents in sewage water treatment.

In the solar ponding operation a significant amount of sodium chloride salt is precipitated. Much of this is not commercially useful due to and remoteness from market locations or high content of potassium, magnesium, and sulfate. By contractual arrangement, about 25% of total brine volume in the solar pond system is diverted to an adjacent sodium chloride operation (Cargill Salt) (Figure 1) where sodium chloride is deposited in such a way that allows recovery of high quality commercial grades of NaCl. The bitterns (concentrated brines exiting the sodium chloride operation) are then returned to US Magnesium for further evaporation and inclusion with the brine used for the production of magnesium metal. Additionally US Magnesium on occasion sells raw sodium chloride salts from its pond floor when commercial opportunities occur.

As the brine approaches the final desired magnesium concentrations, the salts being precipitated from the saturated solution are composed predominately of mixtures of potassium, magnesium and sulfate. The salts deposited in the final ponds are acceptable as feed for the commercial

production of potash fertilizer. The salts in the floors of these final ponds on occasion are harvested and sold for that purpose.

The magnesium chloride solution that serves as a raw material for magnesium production is also in demand as a custom ice preventative for road and bridge surfaces. It is also a suitable dust control agent. When inventories permit, US Magnesium sells excess magnesium chloride solutions for use in those markets.

Other by-products presently being considered from the production of magnesium at Rowley, Utah include commercial grades of hydrochloric acid (HCl), lithium metal or lithium compounds, and bromine compounds.

SUMMARY

US Magnesium and its predecessors have been successfully and economically producing magnesium metal from raw materials derived from solar evaporated brine solutions for more than 30 years. Such raw materials have advantages of low impurities and allow for a high volume manufacturing process that is energy and labor efficient. Such raw materials derived solutions also have low levels heavy metal impurities compared to mined magnesium ores. To generate metal from solutions requires multiple steps of beneficiation and purification to complete the transformation of magnesium chloride to magnesium metal. Perfecting the manufacturing process required years of development and optimization.

Developing Vegetation Metrics for the Assessment of Beneficial Uses of Impounded Wetlands Surrounding Great Salt Lake, Utah, USA

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ABSTRACT

Many wetlands around Farmington Bay of Great Salt Lake are managed waterfowl habitat by means of impounding the flow at the terminus of the Jordan River. The majority of the Jordan River flow is comprised of the secondary-treated effluent of several municipal waste water treatment plants (WWTP), resulting in elevated phosphorus concentrations. This study was initiated to determine whether the assimilative capacity for phosphorus of the impounded wetlands has been exceeded, resulting in a negative impact to the submerged aquatic vegetation (SAV) in the wetlands. The majority of the SAV is sago pondweed and western fineleaf pondweed (*Stuckenia pectinata* and *S. filiformis* ssp. *occidentalis*, respectively), highly preferred food items for waterfowl. Utah Department of Environmental Quality has identified support for waterfowl and shorebirds and the aquatic life in their food chain as the primary beneficial use of these wetlands, and thus, loss or degradation of *Stuckenia* prior to waterfowl fall staging and migration may constitute a loss of this important beneficial use. Therefore, Utah needs vegetation metrics that will indicate relative health of a wetland with respect to the abundance, density and health of the SAV and the level of nutrient loading it receives. The primary goal of this ongoing study is to develop wetland assessment methods that will be used to establish water quality standards and methods for Clean Water Act §305(b)/303(d) assessments—one of the first attempts by any state of the U.S. to set wetland water quality standards through development of site-specific assessment protocols. To develop metrics that describe the relationship between nutrient gradients and biological responses, we are 1) testing potentially useful parameters for their utility in assessing wetland condition; and 2) refining condition metrics that will identify thresholds of significant change (impairment) that can be attributed to nutrients. This paper presents the first of several potentially useful vegetation metrics. Our analyses showed that percent areal cover of SAV in nutrient enriched wetlands senesced 62-84% from July through November whereas the vegetation in a non-impacted reference wetland remained stable. The fall senescence occurs at a time when migratory waterfowl rely on submerged aquatic vegetation (SAV) for sustenance.



Figure 1—Eastern shore of Great Salt Lake, USA showing all impounded wetland sites of the State of Utah Division of Water Quality's study on ecological and beneficial use assessment of Farmington Bay wetlands. Reference sites are located at the PSG (Public Shooting Grounds) and nutrient enriched sites are located at FB WMA (Farmington Bay Wildlife Management Area), NEW (Newstate Duck Club), AMB (Ambassador Duck Club), and ISSR (Inland Sea Shorebird Reserve).

INTRODUCTION

Great Salt Lake, Utah, USA (located between 40° and 41° N, 113° and 112° W) is the fourth largest terminal lake in the world (Figure 1). On average, the lake is 3 to 5 times the salinity of the ocean. Yet, the very gentle slopes at the lake's margin provide for approximately 1.82×10^5 ha (4.5×10^5 acres) of fresh to slightly brackish lacustrine wetlands leading from tributary flows. These vast acreages of wetlands, occurring in the interior, arid US west, are vitally important to the five to seven million waterfowl and shorebirds that depend on Great Salt Lake and its wetlands for nesting and migratory staging each year. About 6.1×10^4 ha of wetlands occur in Farmington Bay (situated

on the southeastern portion of the lake), of which approximately 3.5×10^4 ha are impounded and managed for waterfowl. All of these impounded wetlands are supplied by the Jordan River, the majority flow of which is provided by the effluent of four major municipal waste water treatment plants. The resulting ambient P concentration ranges from 0.9 to 1.3 mg l^{-1} (Miller & Hoven 2007). Nearly all of the Jordan River water flows through impounded wetlands before being released to the open water of Farmington Bay. It is not known how the high nutrient levels in these wetlands impact this ecosystem.

The Federal Clean Water Act requires all states to assign “beneficial uses” to all surface waters of the US and to perform frequent assessments of the water quality to determine if these beneficial uses are being met. As such, the Utah Department of Environmental Quality assigned “support for waterfowl and shorebirds and the aquatic life in their food chain for Great Salt Lake wetlands” as a beneficial use of these wetlands. The areas of concern for this study are the nutrient enriched impoundments supported by the Jordan River (Figure 1). Wetlands located at the north end of the lake (Public Shooting Grounds) where nutrient enrichment is far less serve as reference sites (Figure 1). Both nutrient enriched and reference impoundments are managed nearly exclusively for the production of submerged aquatic vegetation (SAV) and in particular, for sago pondweed (*Stuckenia* sp.), because it is the preferred forage taxon by omnivorous waterfowl (Kantrud 1990). Although the impoundments are managed to optimize SAV growth for waterfowl, the wetlands are also important for other waterbirds and wildlife (e.g., Olson et al. 2004). One wetland at the southern end of Farmington Bay (The Inland Sea Shorebird Reserve (ISSR)) is specifically managed for shorebirds but its impoundments are deep enough to grow SAV and attract waterfowl (Hoven & Miller, personal observation).

SAV has been shown to be a sensitive indicator of water quality (Kemp et al. 1983; Orthe & Moore 1983; Tomasko et al. 1996; Stumpf et al. 1999) and a sentinel accumulator (Wolfe et al. 1976; Burrell & Schubel 1977; Brix & Lyngby 1983; Ward 1987; Hoven et al. 1999) of anthropogenic stressors in shallow estuarine embayments worldwide. SAV provides a myriad of ecological functions to a watershed. It provides a protective environment and nursery function to invertebrates, fish, and shellfish; stabilizes sediments; cycles nutrients and elements; attenuates nutrients and other pollutants; and filters suspended sediments (Thayer et al. 1975, 1984; Kenworthy et al. 1982; Phillips & Meñez

1988). Because SAV have specific light requirements, they may be susceptible to shading by algae (epiphytes, macroalgal mats, and/or phytoplankton), duckweed, suspended sediments, and/or water color (Buzzelli et al. 1999; Hall et al. 1999; Stumpf et al. 1999; Zieman et al. 1999). Algal blooms are stimulated by increased nutrient loads and often associated with inputs from high human density and/or industrial areas or areas of agricultural runoff (Staver et al. 1996; Madden & Kemp 1996) and have been shown to correlate with decline in areal cover of seagrasses (Short & Burdick 1996; Valiela et al. 1997).

In the impounded wetlands of Farmington Bay we have observed several indicators of hypertrophy, including floating mats of filamentous green algae (primarily *Cladophora* spp.), cyanobacteria (primarily *Oscillatoria* spp.) and duckweed (*Lemna minor*) (Rushforth & Rushforth 2007). Biofilm was frequently observed at nutrient enriched sites on *Stuckenia* leaves, which was comprised of epiphytes (mostly diatoms), mucilaginous material and fine inorganic sediments (Rushforth & Rushforth 2007). Our study has been designed to 1) identify thresholds of adverse biological or ecological changes to gradients in nutrients and other parameters that are typically associated with hypereutrophy, and 2) identify sensitive and ecologically important responses to nutrient enrichment in Farmington Bay and its wetlands. An array of these metrics could then be incorporated into an index of biological integrity that quantifies various ecological functions against a gradient in nutrients. Ultimately, thresholds along this scoring range will be used to establish beneficial use support status. This effort represents one of the first attempts by any state to establish water quality standards and methods for Federal Clean Water Act §305(b)/303(d) assessments for wetlands.

METHODS

Five impounded sites were identified around or near Farmington and Bear River Bays of Great Salt Lake, Utah (Figure 1). Ambassador Duck Club, Newstate Duck Club, and Farmington Bay Wildlife Management Area (FB WMA) all receive water from the Jordan River and empty into a downstream duck club (Newstate Duck Club passes much of its water on to FB WMA) or releases it directly to Farmington Bay. The ISSR receives water from the Northpoint Consolidated Canal (a diversion from Jordan River water). The ISSR generally allows its impoundments to draw down (recede) via evaporation during summer months to provide forage and nesting habitat for shorebirds. Public Shooting Grounds (PSG) is situated at the north end

of the lake on Bear River Bay and receives its water from freshwater springs and some irrigation return flow. The ponds typically have a management protocol that calls for 46 cm (18 inches) of water depth to maximize SAV growth; however, during our study many ponds were frequently less than 46 cm, due to limited water availability.

Sample site selection was based on the assumption that nutrient assimilation would result in declining nutrient concentrations as nutrient enriched water flowed through successive ponds leading from the terminus of the Jordan River or from its water diversions serving the Ambassador Duck Club or the ISSR. In this manner we expected to describe co-located biological responses to a water column nutrient gradient. In reality, however, only one wetland complex, Ambassador Duck Club, exhibited a distinct nutrient gradient (Miller & Hoven 2007). Retention times in the Ambassador ponds were much greater than in the other pond systems, which most likely contributed to this uniqueness (Miller & Hoven 2007). Nevertheless, there was enough variation in nutrient concentrations among the various pond systems to address the nutrient enrichment and biological response relationship.

We measured nitrate-nitrite and total phosphorus using standard EPA methods (353.2 and 365.2, respectively). We also measured dissolved oxygen (DO), pH, temperature, and electrical conductivity (EC) using Hydrolab® or In-Situ® multiprobe sondes. Measurements and sample collections were performed at designated outlet culverts (easily identifiable landmarks) that were located near biological sample collections (indicated by site location points on Figure 1). The water quality sampling points were collected at approximately one month intervals during daylight hours from May through November, 2005. Sediment phosphorus was also determined for the upper 10 cm of sediment surface in all ponds using the persulfate digestion method EPA 365.1.

One-hundred-meter long transects were measured by pacing within each pond 50 m offshore and 100 m away from (when possible) culverts to avoid any influence of water flow patterns on the distribution of the plants. One square meter quadrats were established by laying two 2.0 m PVC poles 0.5 m apart and perpendicular to the transect line at 10 random locations along the transect. Percent cover (to the nearest 1%) as visual areal estimates at mid-canopy of the SAV was determined within each quadrat. The 2.0 m PVC poles were marked to show area designations

(e.g., 1, 5, 10, 25, 30%), a modification of the Daubenmire frame technique (Daubenmire 1959). Species composition and general observations of turbidity and algal or duckweed cover were also noted. It was not possible to use a Secchi disc for turbidity determinations due to the shallowness of the water. Percent cover was estimated during July, August, and November of 2005.

Three composite samples of the dominant species of SAV in each pond were collected for tissue carbon (as total organic carbon), nitrogen (as total nitrogen), and phosphorus (as total phosphorus) analysis. Plant samples were stored in a refrigerator in sealed plastic bags until they were processed. Processing included rinsing plants free of sediment and debris, wiping periphyton off with absorbent paper towels and hand selecting approximately 5 g (wet weight) bright green leaves with forceps. Leaves of similar length on a turion were used rather than shorter leaves on distal-most shoots in an attempt to collect similarly aged leaves. Leaves were kept under water while processing and once adequate sample was derived, the leaves were rinsed in distilled deionized water and dried in aluminum foil trays at 34°C for at least 72 hours. The dried samples were quickly placed in clean, labeled sealed plastic bags and stored in a closed box prior to chemical analysis. Total organic carbon and total nitrogen were determined using ASTM D5373 analytical methods at Timpview Analytical Laboratories of Orem, UT; and total phosphorus was determined using EPA Method 325.2 (ICP atomic emission spectroscopy) at the Brigham Young University Soils Lab. At the lab, samples from each site were composited to ensure adequate material for analysis.

Data were analyzed using multivariate factor analysis. Water quality factors were determined following the methods outlined by Madon (2006). Of the eight parameters used, TSS, conductivity, and temperature explained the least amount of variability when ordinated in the second and third factors and hence, were excluded to reduce the data to one ordination factor. All water quality data were transformed by Log10, using $(\log_{10}(x + 1))$ for zeros. Percent cover data were first composited as total SAV per quadrat (i.e., % *Stuckenia* sp. plus % *Ruppia cirrhosa* (spiral ditchgrass)) and transformed by arcsine \sqrt{x} , using arcsine (square root $((0 + 3/8) / (15 + 3/4))$ for zeros (Anscombe 1948). Univariate repeated measures were performed to assess whether the ponds were responding differently with respect to percent cover across time.

Table 1—Mean water quality parameters at the upper ponds of nutrient enriched and reference impoundments during 2005 (PSG are reference ponds). Values of 0.01 for total P or NO₂-NO₃ are reported as 1/2 of the instrument detection limit of 0.02. DO = dissolved oxygen. TDS = total dissolved solids. se = standard error. nd = do data.

SPRING	DO		NO ₂ + NO ₃		P				TDS	
	(mg l ⁻¹)	se	(mg l ⁻¹)	se	(mg l ⁻¹)	se	pH	se	(mg l ⁻¹)	se
AMB	11.1	1.10	0.66	0.2	0.50	0.05	9.0	0.1	666	n=1
FB WMA	8.6	0.10	0.02	0.0	0.20	0.05	9.5	0.4	1099	591
NEW	1.8	n=1	1.15	n=1	0.40	n=1	7.9	n=1	nd	
ISSR	16.7	0.03	0.01	0.0	0.10	0.05	10.1	0.2	1716	176
PSG	8.7	n=1	0.05	n=1	0.01	n=1	8.7	n=1	2702	n=1

SUMMER	DO		NO ₂ + NO ₃		P				TDS	
	(mg l ⁻¹)	se	(mg l ⁻¹)	se	(mg l ⁻¹)	se	pH	se	(mg l ⁻¹)	se
AMB	9.1	1.7	1.70	0.3	0.80	0.030	9.1	0.4	nd	
FB WMA	6.2	0.1	0.02	0.0	0.10	0.010	9.3	0.2	861	126
NEW	1.6	0.2	2.00	0.9	0.70	0.300	8.1	0.2	nd	
ISSR	4.2	1.2	0.01	0.0	0.20	0.100	9.1	0.1	1113	208
PSG	9.0	2.3	0.01	0.0	0.04	0.005	9.0	0.1	1576	136

FALL	DO		NO ₂ + NO ₃		P				TDS	
	(mg l ⁻¹)	se	(mg l ⁻¹)	se	(mg l ⁻¹)	se	pH	se	(mg l ⁻¹)	se
AMB	8.8	0.7	1.90	0.1	1.20	0.1	8.5	0.04	nd	
FB WMA	9.4	2.0	0.10	0.1	0.10	0.1	9.1	0.20	nd	
NEW	9.2	2.2	4.40	1.0	0.90	0.2	8.3	0.30	nd	
ISSR	9.0	1.6	0.01	0.0	0.30	0.1	9.1	0.10	1771	235
PSG	8.3	0.7	0.01	0.0	0.01	0.0	8.6	0.10	2896	275

RESULTS

Stuckenia sp. was the dominant SAV observed among the sites. *Ruppia cirrhosa* was occasionally present in the more-saline ponds and *Ceratophyllum demersum* (coon's tail) was also occasionally present in small proportions but very rare. There were varying amounts of floating mats of filamentous green algae (primarily *Cladophora* spp.), the cyanobacterium *Oscillatoria* spp., among others, and epiphytic algae and diatoms were frequently on the SAV at the nutrient enriched sites (Rushforth & Rushforth 2007).

Among all ponds, water column pH occasionally exceeded Utah's freshwater standard of 9.0 and this included reference ponds in PSG that had less than 0.02 mg l⁻¹ total P (Table 1). The daytime dissolved oxygen concentration in the PSG ponds was often 120% to 200% saturation. The source of high dissolved oxygen in this case, was likely the dense meadows of SAV and a calcareous green macroalga, *Chara* sp. Low nutrient and high dissolved oxygen concentrations with dense vegetative cover was a common condition among the impounded wetlands at our reference

site. Table 1 lists the means of water quality samples collected among the nutrient enriched and reference sites. Total P at the reference sites was often below the detection limit (0.02 mg l⁻¹) while nutrient enriched wetland sites always contained substantially more P (0.10–0.90 mg l⁻¹).

Mean sediment P concentrations measured in successive (upstream to downstream) ponds in the five different wetland complexes are summarized in Figure 2. There was at least a slight decrease in sediment concentrations in each of the systems, with the biggest declines in the Ambassador Duck Club and Public Shooting Grounds sediments.

SAV leaf tissue nutrient concentrations collected during the summer and fall months showed consistent levels of organic carbon (C) between nutrient enriched and reference ponds (37.8–41.7% C, Figures 3 and 4). Concentrations of total nitrogen (N) in tissue were generally higher in nutrient enriched ponds (3.3–4.8% N at Ambassador, ISSR and Newstate), while FB WMA and PSG nitrogen levels ranged from 1.9–2.9% N during the summer. During the fall, SAV tissue nitrogen levels ranged from 3.6–4.1 at Ambassador

and FB WMA and 3.0–3.2 at the PSG reference sites. Total phosphorus (P) levels of SAV leaves ranged from 0.41–0.70% P at nutrient enriched ponds during the summer and fall (except FB WMA T1 levels were 0.22 during the fall) whereas they ranged between 0.20–0.25 at the PSG reference sites during both sampling periods.

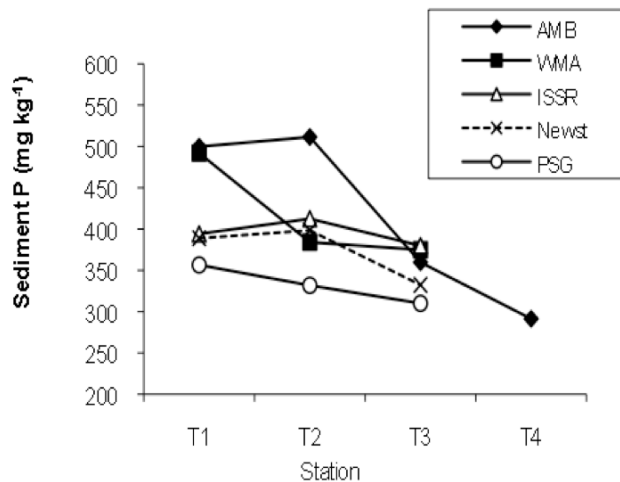


Figure 2—Mean sediment phosphorus (P) concentrations at the nutrient enriched and reference (PSG) impoundment sites. T1–T4 represent transect in sequential order of flow through the ponds; AMB = Ambassador, WMA = FB WMA, News = Newstate.

When C:N:P ratios of similarly-aged SAV leaves are compared between nutrient enriched and reference sites during July, all but one nutrient enriched site (Ambassador T2, pond 100) show carbon limitation and all sites (both nutrient enriched and reference) show nitrogen limiting ratios according to Redfield C:N:P ratios of organic matter,

106:16:1 (Table 2; Redfield 1934). By late fall, most nutrient enriched sites lacked enough plants to provide enough leaf sample for analysis or were lacking plants altogether. Those that had plants, showed even lower carbon ratios (with the exception of a gain in both carbon and nitrogen above limiting levels at FB WMA T1, Unit 1). The only samples that had SAV leaf C:N:P ratios that met or exceeded Redfield ratios were from summer PSG T2 and T3, fall FB WMA T1 and fall PSG T2.

The percent cover data indicate that SAV was impacted by nutrient loading primarily late in the season (Figure 5). The upstream or upper ponds of the impounded sites (with the exception of ISSR, which does not exhibit a flow-through series of ponds) are the first to receive flows from their respective source waters. Seasonal biological sampling revealed substantial differences in plant community responses in the upper ponds (Figure 5, $F_{(df\ 4,8)} = 75.5, 13.6$; p value < 0.0001). The highest mean percent cover of SAV in the upper ponds at the nutrient enriched sites (i.e., those around Farmington Bay: Ambassador, 75.2 ± 8.2 ; FB WMA, 9.0 ± 1.6 ; Newstate, 70.2 ± 10.6 ; and ISSR, 89.9 ± 2.4) occurred in June and July but had declined substantially by August and was nearly absent by November ($2.0 \pm 0.4, 2.4 \pm 0.4, 1.0 \pm 0.0, 5.8 \pm 1.1$, respectively; Figure 5, mean \pm se). Reference SAV percent cover at the upper pond of PSG, on the other hand, remained quite high through November ($95.8 \pm 0.6, 70.3 \pm 11.3, 93.7 \pm 2.7$ in July, August, and December, respectively).

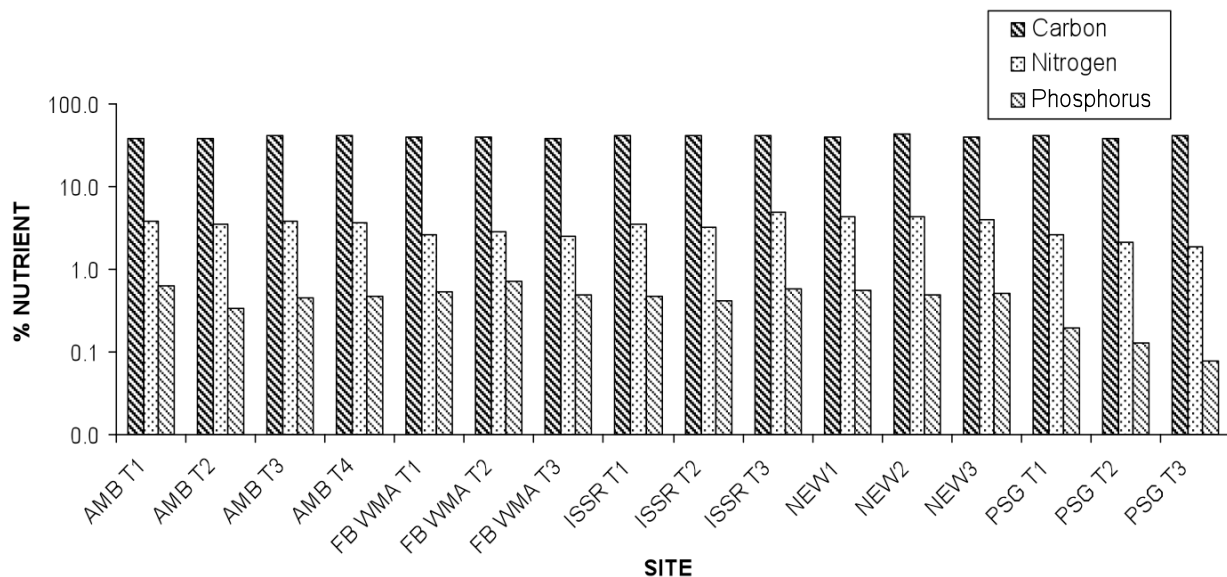


Figure 3—Percent carbon, nitrogen and phosphorus in submerged aquatic vegetation (SAV) during the summer, 2005. Numbers represent transect in sequential ponds from upstream to downstream; AMB = Ambassador Duck Club, NEW = Newstate Duck Club, PSG are reference ponds. $n = 1$ at all nutrient enriched sites, $n = 3$ at reference sites (PSG).

The multivariate factor analysis also supported the idea that the loss of SAV was related to nutrient enrichment. The principle axis determined by the analysis produced a water quality factor gradient showing nutrients and oxygen levels at one end, and high salinity (conductivity, TDS) at the other (Figure 6). Most of the impounded sites of this study showed moderate to abundant percent cover SAV in the early through late summer and there was no significant correlation with the principal multivariate gradient (data not shown; p value = 0.364). By the fall, however, most sites showed a decline in percent cover that corresponded with the water quality factor axis (Figure 6, $F_{(df 1)}$, p value = 0.026).

DISCUSSION

Seeds, tubers and vegetative parts of *Stuckenia* spp. are all preferred food by various waterfowl (Kantrud 1990) and indeed, many tens of thousands of waterfowl have been observed foraging and resting on these and other ponds each fall (Paul & Manning 2002). However, our data indicates that the *Stuckenia* density in many of the nutrient enriched ponds remained at low densities or experienced considerable senescence by the beginning of the fall waterfowl migration season, although there have been no apparent indicators of weakened birds (i.e. starvation, failure to migrate, massive downings, etc.). This suggests that either the birds are moving to more productive ponds on a regular basis or a significant amount of foraging on less preferred items or a combination thereof is occurring. Nonetheless, it remains important to elucidate waterfowl diets during this critical time on Great Salt Lake wetlands.

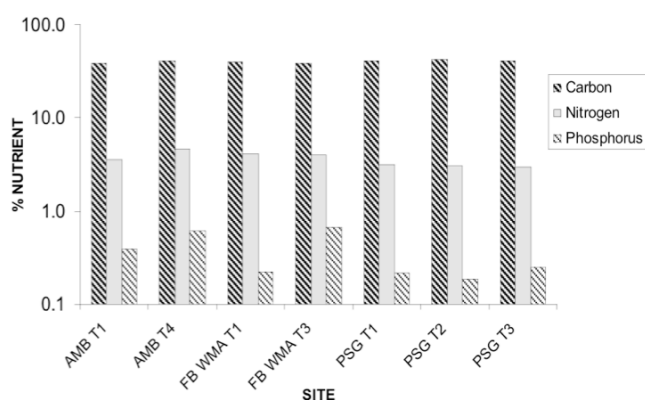


Figure 4—Percent carbon, nitrogen and phosphorus in submerged aquatic vegetation (SAV) during the fall, 2005. Numbers represent transect in sequential ponds from upstream to downstream; AMB = Ambassador Duck Club, NEW = Newstate Duck Club, PSG are reference ponds; (no plant material was available at Newstate Duck Club and the ISSR). $n = 1$ at all sites except $n = 3$ at PSG T1 and T3.

The apparent decline in vegetative productivity in the nutrient enriched ponds seem to contradict the paradigm that lower nutrient concentrations should result in less biomass and more nutrients should support greater biomass. There are two important factors that explain this contradiction:

1) Both emergent and submergent vegetation can derive all of their N and P requirements from sediments (Thiebaut & Muller 2003; Carr & Chambers 1998; Madsen & Adams 1988; Carignan & Kalff 1980). Indeed, Canfield & Hoyer 1988 and Peltier & Welch (1969) found no relationship between macrophyte growth and water-column P and N concentrations. Carignan & Kalff (1980) reported that nine common species of aquatic macrophytes, including *Stuckenia pectinata*, took all of their phosphorus from the sediments when grown in situ in both a mesotrophic and a mildly eutrophic bay. Even under hypereutrophic conditions, the sediments contributed an average of 72% of all the phosphorus taken up during growth. Therefore, submerged macrophytes in PSG obtain adequate nutrients from their associated sediments regardless of nutrient levels in the water column.

2) There is considerable evidence that the early senescence and loss of percent cover in the nutrient enriched ponds and particularly in comparison to the reference ponds at the PSG are the result of degraded water quality and related effects rather than normal seasonal changes. Total water column P in the nutrient enriched impounded sites was frequently more than an order of magnitude greater than in the PSG ponds and the resulting algal blooms may be the driving factor that is overwhelming those systems. Often heavy epiphytic biofilms (including sediment) were observed on the leaves of the SAV, and floating and entangled mats of macroalgae (Chlorophyta and Cyanophyta) and duckweed were frequently present at the nutrient enriched sites where nutrients were elevated. The “premature” senescence of SAV was likely induced by shade-related stress to the SAV by the epiphytic and macroalgal communities, and duckweed in some cases. Additionally, as percent cover of the SAV declines, suspended sediment from the wind events or carp perturbations remains in the water column for longer periods since there is no physical structure (i.e. plants) to slow water currents and facilitate settling and water clarity (Short & Short 1984; Ward et al. 1984). This turbidity may cause additional stress on the remaining SAV due to reduced light.

Table 2—C:N:P of Summer and Fall SAV Leaf Tissue for Reference and Nutrient enriched Sites, 2005; n = 3 for all sites. (Ratios with ☒ in front of them represent samples that met or exceeded the Redfield C:N:P ratios of 106:16:1, while ratios with !! in front if they approached Redfield ratios with respect to nitrogen levels. Asterisks identify sites that were dominated by *Ruppia cirrhosa*; all others were dominated by *Stuckenia* sp. Samples were taken in December, - indicates not enough above ground tissue for sampling.)

SITE	SUMMER (July)	FALL (November)
Ambassador_T1	61:6:1	-
Ambassador_T2	111:10:1	97:9:1
Ambassador_T3	89:8:1	-
Ambassador_T4*	87:8:1	67:7:1
FB WMA_T1	75:5:1	☒ 176:19:1
FB WMA_T2	55:4:1	-
FB WMA_T3	79:5:1	58:6:1
ISSR_T1	86:7:1	-
ISSR_T2*	100:8:1	-
ISSR_T3*	73:8:1	-
Newstate_T1	72:8:1	-
Newstate_T2	90:9:1	-
Newstate_T3	76:8:1	-
PSG_T1	!! 208:14:1	!! 188:14:1
PSG_T2	☒ 291:16:1	☒ 220:16:1^
PSG_T3	☒ 540:24:1	161:12:1

Although somewhat lower, sediment P concentrations in PSG were in the same range as those in the nutrient enriched ponds (approximately 300–500 mg kg⁻¹) yet SAV did not show a premature senescence as in the reference sites. Madden & Kemp (1996) simulated eutrophication in submersed estuarine plant communities. They noted several important responses under nutrient enriched conditions that may have implications for the Farmington Bay nutrient enriched sites. In their study, epiphytic algal biomass was stimulated by an order of magnitude, while SAV biomass declined severely under both N + P enrichment. Phosphorus enrichment alone has not been shown to trigger community shifts in estuarine production but when N + P enrichments are introduced to mesocosm experiments and model simulations, epiphyte production can be exponential, while attenuating light to deleterious levels to SAV (Madden & Kemp 1996; Taylor et al. 1995, 1999). Dissolved inorganic nitrogen (NO₂- and NO₃-) levels in two of the nutrient enriched ponds (Ambassador and Newstate) were high, whereas those in the other two nutrient enriched wetlands and in the reference wetland were low, and likely limiting for algal growth. However, cyanobacteria provide enough

fixed nitrogen locally to support heavy epiphytic growth (Powell et al. 1989). Additionally, N₂-fixing heterotrophs and cyanobacteria associated with duckweed mats have been found to fix as much as 15-20% of the nitrogen requirement for duckweed (Zuberer 1982), a substantial amount that could also contribute to the localized water column pool for macroalgae and diatoms. The increased density and coverage in duckweed, and filamentous and epiphytic algae in response to increased nutrients has been well documented (Vaithyanathan & Richardson 1999; Portielje & Roijackers 1995, and others). Accordingly, where nitrogen is limited, rich populations of epiphytic, nitrogen-fixing cyanobacteria most often accompany duckweed populations (Duong & Tiedje 1985, Zuberer 1982; Finke & Seeley 1978). This ability to manipulate nutrient availability provides a symbiotic relationship that favors a floating duckweed community. In turn, increased shading and concomitant increased tendency toward anoxia deeper in the water column may severely restrict health and survival of submerged vegetation (Morris et al. 2004). Further, Madden & Kemp (1996) found that in nutrient enriched treatments epiphytic growth on SAV increased and that the leaf tissue area decreased due to leaf mortality and sloughing. The periphytic growth was an important factor in the decline of SAV due to increased shading—more so than turbidity related to phytoplankton blooms.

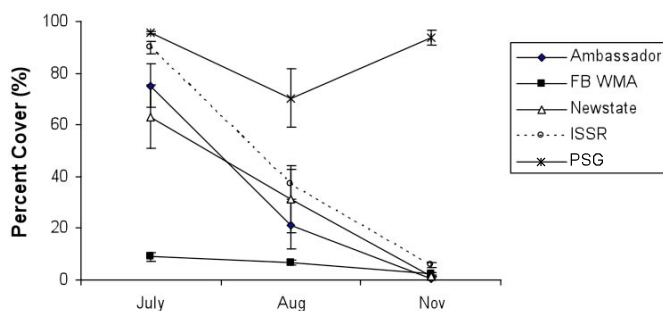


Figure 5—Mean percent areal cover of SAV during the summer and fall months of 2005 for both nutrient enriched (Ambassador, FB WMA, Newstate, and ISSR) and reference (PSG) upper ponds (n = 10; ± se; p value < 0.0001).

Another important conclusion from Madden & Kemp (1996) was that long-term shading stress to SAV in enriched environments inhibits carbon storage in root and rhizome tissues. SAV roots and rhizomes can provide a root buffering effect such that carbon stored from production periods is reserved for reproduction the following spring. When Madden & Kemp (1996) ran their model for successive years under sustained nutrient enrichment, detrimental epiphyte loads lead to negative P:R (production to respiration ratio) and resulted in reduced SAV biomass, reduced carbon stored in the roots and rhizomes, and

ultimately a decreased reproductive potential. They concluded that a “root buffering effect” is essential for long-term survival of SAV beds and to restore plants to historic levels would likely require improvements to water quality that persist for several years to allow root rhizome systems to become re-established.

The similarity of sediment P concentrations between nutrient enriched and reference sites, and yet vastly different water column P concentrations between nutrient enriched and reference sites, provides evidence for the connection between nutrient enrichment and various indicators of severe eutrophication in Farmington Bay nutrient enriched ponds. In turn, it is likely that epiphytic growth on the SAV leaves and presence of algal mats and duckweed attenuated light below critical levels required by *Stuckenia* and lead to a premature senescence of SAV. This condition was likely exacerbated as fall progressed and photoperiod and sun angle diminished. During fall collections at nutrient enriched ponds, SAV roots and rhizomes of remaining shoots were often rotting or not well developed (the only exceptions were ISSR T2 (West Pond A) and T3 (Southwest Pond South) and Ambassador T3 (W2) and T4 (W5) where *Ruppia cirrhosa* dominated; Hoven, personal observation). With reduced photosynthetic capacity and resultant reduction in oxygen transport to the roots, below ground tissue may have been susceptible to sulfide toxicity and/or infection by pathogens such as slime mold. On the other hand, plants at the PSG reference ponds grew densely and were difficult to pull (i.e. their roots and rhizomes were well developed and strong) as late as December. Although the plants are perennial, it is possible that roots and rhizomes of SAV at nutrient enriched sites lack carbon stores to regenerate each spring and rely heavily on seedlings each year to maintain the beds.

During the summer, N and P concentrations in SAV leaves reflect differences in available N and P levels in the water (Figures 3 and 4, Tables 1). In particular, SAV assimilated high levels of P at the nutrient enriched sites where P is elevated in the water and sediment, and maintained low levels of P at the reference sites both during the summer and fall. It is possible that higher levels of P in the nutrient enriched SAV samples reflected incomplete removal of periphyton from those leaves, however the majority of the periphyton community was composed of loosely attached diatoms (Rushforth & Rushforth 2007) and likely removed during sample processing. While C:N:P ratios appear to indicate nutrient imbalances in the SAV among ponds, it is difficult to identify the driving nutrient without additional information (Table 2). For example, what may appear to be carbon limitation in nutrient enriched ponds (i.e. low C:P ratios) may simply be that high P levels in the SAV are overwhelming the C levels. Alternatively, apparent high

C:P ratios in the tissue may be indicative of P starvation. For this reason, C:N:P ratios alone are not a suitable metric of wetland health but may serve as a supplementary metric. Presumably, the improved tissue nutrient (C:N:P) ratios at FB WMA Unit 1 during the fall may reflect nutrient inputs from the pond's use as a rest area for waterfowl. Waterfowl grazing on SAV in this pond may have stimulated new shoots that could have had lower epiphyte burdens in the fall and better photosynthetic capacity to fix carbon than during the summer months. However, there was limited SAV cover at that time of year (Figure 6).

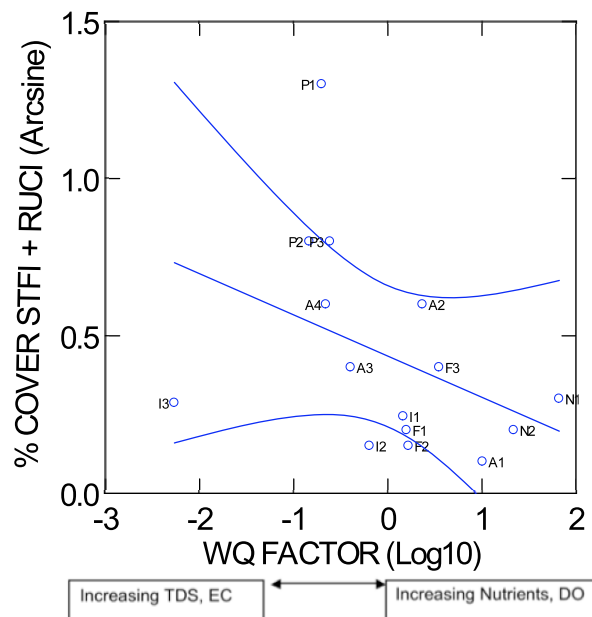


Figure 6—Percent cover (\pm 95% confidence interval) of STFI + RUCI (SAV) versus WQ factor (see text) at nutrient enriched and reference ponds during November 2005 (p value = 0.026). A = Ambassador Duck Club, F = Farmington Bay Wildlife Management Area, I = Inland Sea Shorebird Reserve, N = Newstate Duck Club, P = Public Shooting Grounds (reference site). DO = dissolved oxygen. Numerals show the successive pond at each study area.

Our study shows that SAV percent cover in nutrient enriched ponds responded differently than that of reference ponds and that the nutrient enriched response over time is occurring at different rates relative to nutrient levels in the water (Table 1, Figures 5, 6). SAV, specifically *Stuckenia* sp., demonstrated a premature senescence during August, which amounted to 62–84% loss in areal cover at nutrient enriched ponds. Notably, this was before the arrival of waterfowl migrants. The largest numbers of ducks were accounted for in managed wildlife areas of Great Salt Lake during September as they migrate southward for winter the months (Paul & Manning 2002). Extensive surface mats of filamentous algae or duckweed often developed on these ponds and heavy coatings of biofilms (composed of epiphytic algae, sediment, and possibly bacteria and fungi) were observed on the living leaves. This surface epiphytic

and biofilm shading may reduce light penetration to below optimal or even threshold requirements (Taylor et al. 1995, 1999; Madden & Kemp 1996). Further, this would expectedly be exacerbated by shorter photoperiod and lower sun angle as fall progressed. If photosynthesis rates are sub-optimal (i.e. $P < R$), there may not be adequate oxygen production to diffuse down to the roots and maintain an oxygen-rich root zone to sustain healthy plants. In turn, this could represent a decline in additional food availability during a time when waterfowl are attempting to nourish and regain energy stores.

Early senescence and its cause(s) warrant further investigation, particularly as to the seasonal timing and whether correlations between nutrient (water column and SAV tissues), light attenuation, and biomass or extensive biofilms exist. If such correlations are documented, they need to be quantified and considered for inclusion into an index of biotic integrity. In addition, the literature indicates that Photosystem II fluorescence is a useful indicator of plant stress (in this case, stress from shading), and exhibits potential as an SAV community metric of wetland condition that may help explain the premature senescence of the SAV (Baker 2008). Together, these metrics may serve to evaluate the condition of impounded wetlands relative to water quality. Ultimately, with regard to the beneficial uses, our concern is that heavy nutrient loading to Farmington Bay's wetlands are causing the disappearance of large underwater meadows of *Stuckenia* (a preferred food by waterfowl) prior to the arrival of migrating of waterfowl.

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Farmington Bay Wetlands of Great Salt Lake: Nutrient Criteria, Macroinvertebrate Studies and Beneficial Uses

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The US Federal Clean Water Act requires States to develop water quality standards for their surface waters, including wetlands. For most toxic pollutants the EPA has conducted extensive acute and chronic bioassays to establish numeric thresholds of toxicity. The States further refine these values by identifying the appropriate beneficial uses for the various types of water bodies. However, for nontoxic pollutants, including the nutrients phosphorus and nitrogen, local ecosystems characteristics such as elevation, watershed land use, geology, and other water quality parameters and even biological components of the ecosystem can drastically alter ecosystems responses to nutrient loadings. Consequently, EPA has not developed nationwide numeric criteria for nutrients. Rather, they have suggested that States adopt nutrient threshold concentrations based on a national probabilistic study or to perform site-specific water quality and ecological studies in order to develop nutrient criteria based on local chemical and biological conditions. Only a few States have proceeded to develop site-specific nutrient criteria and most of these have been in response to overwhelming evidence of, or public concern for, eutrophication and associated problems with dissolved oxygen or pH in specific water bodies. In Utah, agency, academic and public concerns over the trophic condition of Farmington Bay of Great Salt Lake (GSL) and its wetlands prompted the Utah Division of Water Quality (DWQ) to begin a project in 2004 to develop local water quality criteria for phosphorus for Farmington Bay wetlands. This effort has included developing appropriate measures that will be used to develop the site-specific criteria as well as subsequent assessment protocols that will determine whether the beneficial use of ‘support for waterfowl and shorebirds and the aquatic life in their food chain’ is being fully supported. With that objective we focused on the availability of nesting habitat and

direct measures of reproductive success for the vast numbers of shorebirds that nest on the sloped, lacustrine playa wetlands of GSL and the forage items (macroinvertebrates) that occur in these shallow waters. Several thousand American avocet (*Recurvirostra americana*) and black-necked stilt (*Himantopus mexicanus*) nests were evaluated for nest site habitat preference and monitored for nesting success and several dozen adult and juvenile birds were sacrificed to identify preferred food items. Both American avocets and black-necked stilts preferred the early-successional vegetative community of pickle weed (*Salicornia* sp.) and alkali bulrush (*Scirpus maritimus*) that develop in vast acreages of the transitional zones between freshwater tributaries and the hypersaline open water of GSL. Nesting success ranged from 93 to 98%, among the highest ever measured globally. Typically, adult birds lead their young to shallow channels or open water areas fringed by the protective cover of older bulrush, where forage and cover are in close proximity. Stomach contents from collected birds were dominated by midges (Chironomidae) and water boatman (Corixidae), followed by other insects and plant seeds. The relative abundance of organisms in stomachs was similar to the co-located macroinvertebrate samples. These insects are known to be tolerant of moderate salinity (TDS ranging from 3 to 50 g l⁻¹) and meso- to hypereutrophic conditions, including large diel swings in pH and dissolved oxygen. Despite the dominance by these pollution-tolerant taxa, our observations suggest that the shallow, brackish-water wetlands of GSL provide ideal shorebird nesting habitat that includes ideal vegetative structure and high densities of preferred forage items. Together these conditions offer excellent support for shorebird populations that use sloped wetlands in Great Salt Lake.

Landforms of Fetch-Limited Saline Lakes as Evidence of Storm-Wind Direction and Strength*

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Coastal landforms of some lakes provide evidence of relative wave energy and direction of strong storm winds. Others are evidence of relative wave energy but not evidence of direction of strong storm winds. For example, relative wave energy can be due to long fetch rather than storm-wind strength. A detailed study of elevations of 1986/87 shoreline debris of Great Salt Lake suggests that shorezone landforms of similarly fetch-limited, shallow, closed-basin, relatively equi-dimensional, saline lakes can provide evidence of direction of storm winds.

In 1986/87, Great Salt Lake reached its historic highstand lake elevation creating research opportunities for studying the lake's coastal processes. Evidence of the 1986/87 shoreline included anthropogenic trash such as lumber, plastics, and automobile tires as well as beach sediments and shorezone landforms. Shoreline superelevation (the elevation of shoreline debris above still-water lake level) was surveyed at 1228 locations along the 64 km shoreline of Antelope Island, the largest island in Great Salt Lake. One third of the surveyed debris was more than a meter above still-water lake elevation. Shores facing west and northwest into strong storm winds generally had higher shoreline superelevation than eastern shores in the lee of the island. High shoreline superelevation was associated with erosional shorezone features including rocky shores, steep beach faces, curvilinear bays, pocket beaches framed by bedrock, sea cliffs, and wave-cut platforms. Low shoreline superelevation was associated with accreting shorezone features including mudflats, salt water wetlands, sandy beaches, linear beaches, multiple beach ridges, spits, bars, cusped bars, and lagoons.

Differences in wave energy cause differences in shoreline superelevation. Research explored whether differences in energy of waves of Great Salt Lake were caused primarily by differences in wind strength or by differences in fetch. This research question was tested by examining shoreline

superelevation at locations on opposite shores of Gunnison Bay, the large northwestern part of Great Salt Lake (Figure 1). For fetch-dominated lakes, shoreline superelevation would be similar on opposite sides of a lake because the wind-generating area is great enough that moderate to strong winds generate approximately the same wave heights. For fetch-limited lakes, the wind-generating area is small enough that relative wind strength determines wave height. Shoreline superelevation on opposite sides of a fetch-limited lake would not be symmetric. Patterns of shoreline superelevation of 1986/87 debris for Gunnison Bay are asymmetric (Figure 1). Shores facing into strong winds have debris at higher elevations than those on the upwind sides of the lake. The recognizable contribution of wind strength to superelevation is explained by Great Salt Lake's fetch-limited size that limits the wave-generating areas sufficiently that wave environments do not develop into fully-arisen seas.

The hypothetical water body ideal for determining wind directions from shorezone landforms would be: fetch-limited (less than 50 km open water); circular; preferably with three equidistant islands; and water sufficiently saline to not freeze. Contrasting landforms of that lake should indicate relative strength of storm winds if other factors do not complicate the relationship of coastal landforms and wind strength. Complicating factors include: sediment influx, tectonics, and ice. Shorezone features of lakes and paleolakes similar to Great Salt Lake are logical places to look for evidence of storm-wind strength and direction.

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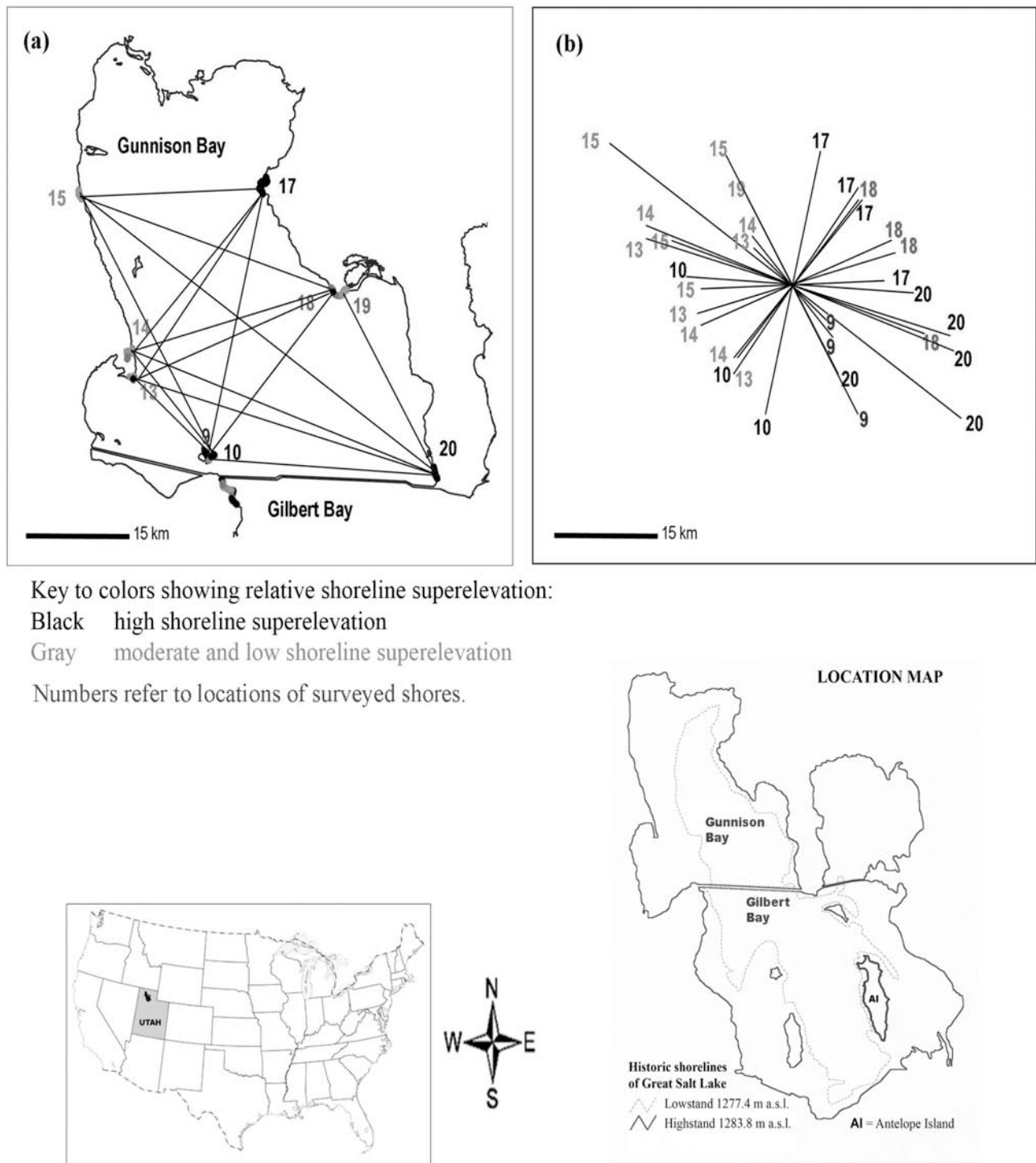


Figure 1—Shoreline superelevation, Gunnison Bay, Great Salt Lake, Utah. Shoreline superelevation patterns for Gunnison Bay indicate that differences in energy and storm waves of Great Salt Lake are primarily due to differences in wind strength rather than to differences in fetch length. (a) Pairs of surveyed shores with 1986/87 shoreline debris. Numbers refer to locations of surveyed shores. Length of line is fetch, distance across open water. Paired locations are on opposing sides of Gunnison Bay; (b) Compass rose of paired surveyed shores showing fetch length, fetch direction, and relative superelevation of shore debris. Shores facing into storm winds have high superelevation. Shores in the lee of land have relatively low shoreline superelevation.

Avian Response to Early Tidal Salt Marsh Restoration at Former Commercial Salt Evaporation Ponds in San Francisco Bay, California, USA

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ABSTRACT

Restoration of former commercial salt evaporation ponds in the San Francisco Bay estuary is intended to reverse a severe decline (> 79%) in tidal salt marshes. San Francisco Bay is a critical migratory stopover site and wintering area for shorebirds and waterfowl, and salt ponds are important high tide roosting and foraging areas. Conservation of past bird abundance is a stated goal of area restoration projects, and early adaptive management will be critical for achieving this objective. However, initial avian response at sites restored to tidal flow may not be indicative of long-term results. For example, winter shorebirds at a 529 ha pond breached in 2002 showed a marked increase in shorebird abundance following breaching. Shorebirds comprised 1% of area totals during 1999–2002 and increased to 46% during 2003–2008. These changes accompanied increased tidal range and sedimentation, but minimal vegetation establishment. Conversely, a fully vegetated, restored 216 ha pond in the same system consistently supported less than 2% of all waterbirds in the region. Early restoration may temporarily increase habitat, but managed ponds will be needed for long-term waterbird abundance within a restored pond-marsh system.

INTRODUCTION

During the past 200 years, over 79% of historic San Francisco Bay (SFB) salt marshes have been lost, resulting in diminished habitat for native marsh species and fragmentation of remaining marshlands (Goals Project 1999). The loss of salt marshes was in part due to construction of commercial salt ponds covering over 1.4×10^4 ha (Goals Project 1999) that have been a part of SFB's landscape since 1856 (Josselyn 1983). In the past decade, large areas of commercial salt ponds have been acquired to be restored back to tidal marsh systems. Four thousand hectares of former salt evaporation ponds and surrounding habitats along the Napa River northwest of Vallejo were purchased by the California Department of Fish and Game (DFG) in 1994, and the Napa Crystallizer Plant (567 ha) was purchased in 2003. Over 1.0×10^4 ha of commercial salt ponds in South SFB were transferred to the

ownership of DFG and the U.S. Fish and Wildlife Service (FWS) in 2003. These ponds are currently planned for tidal wetland restoration under the Napa-Sonoma Marsh Restoration Project, the Napa Plant Site Restoration Project, and the South Bay Salt Pond Restoration Project. About 4500 ha of salt evaporation ponds remain in salt production, but current restoration plans suggest that 36–64% of all SFB salt pond area will be restored to tidal marsh by 2058.

SFB estuary is an important staging and wintering area for migratory waterfowl and shorebirds in the Pacific Flyway (Harvey et al. 1992). It is recognized as a site of hemispheric importance for shorebirds because it supports at least 30% of some flyway populations (Page et al. 1999), and also up to 50% of many diving duck populations (Accurso 1992). Many of these migratory waterbirds use the baylands, which consist of the area between the historic high and low tide lines and comprise about 8.6×10^4 ha in the estuary, including all acquired salt ponds (Goals Project 1999). While salt ponds offer important opportunities for tidal marsh restoration, they have become an integral part of the landscape and provide essential habitats for large numbers of waterbirds during migration and winter (Anderson 1970; Bollman & Thelin 1970; Accurso 1992; Takekawa et al. 2001; Warnock et al. 2002). Salt ponds are unique hypersaline wetland habitats that provide dense forage for birds that feed on saline-specialist invertebrates such as brine shrimp (*Artemia* sp.) and brine flies (*Ephydra* sp.) (Anderson 1970; Takekawa et al. 2006a). Although large salt ponds were not a natural feature of the landscape, they have existed in the San Francisco Bay estuary for more than 150 years (Ver Planck 1958), and much smaller salt pannes provided hypersaline habitats in the historic landscape (Goals Report 1999). The artificial, nontidal hyperhaline ponds vary seasonally in salt content from brackish to saturated, range from a few centimeters to a few meters in depth, and are composed of relatively simple but productive assemblages of algae and invertebrates (Carpelan 1957; Lonzarich & Smith 1997). Due to extensive urbanization in SFB, few alternative habitats exist to support large numbers of migratory and wintering shorebirds and waterfowl (Takekawa et al. 2000, 2005; Warnock et al. 2002). Consequently, one goal of the salt pond restoration projects is to maintain existing ecological

value for waterbirds (e.g., Trulio et al. 2005; see also Stralberg et al. 2005) by creating a mixture of habitats: some ponds would be retained as managed ponds to support bird species currently using the ponds, while others would be restored to tidal marsh to support endangered marsh-dependent species such as the California clapper rail (*Rallus longirostris obsoletus*) and salt marsh harvest mouse (*Reithrodontomys raviventris*). Because very high bird densities have been observed on a few commercial ponds (Takekawa et al. 2006a), managers hope to optimize features and conditions of the managed ponds remaining after restoration to support past numbers of migratory and wintering birds with reduced salt pond area.

Adaptive management has been heavily emphasized in salt pond restoration planning because of inherent uncertainties in restoration outcomes and because of potential impacts to endangered species and to entire flyway populations of migratory birds (see Trulio et al. 2005). Restoration monitoring is critical for adaptive management to be successful because it can provide early detection of unintended project consequences, which can then be addressed with corrective management action. However, initial avian response to levee breaching may not be indicative of long-term project success in former evaporative salt pond systems, as breached salt ponds are transitional habitats. They are open to tidal flow and thus to the hydrologic processes that may bring about the re-establishment of marsh vegetation, but vegetation establishment can take many years to occur (Williams & Orr 2002).

We examined bird use at six former commercial salt evaporation ponds over a ten year period. One pond was breached and mostly re-vegetated prior to the study period and provided a reference site for a restored marsh in this system, while other ponds were breached or remained intact during the study period. This study examines how salt pond bird use may change in both restored and managed ponds during the restoration process and how avian response may guide adaptive management at salt pond restoration sites.

STUDY AREA

We examined salt ponds in the North Bay (38.12°N-38.21°N; 122.28°W-122.37°W) subregion of the San Francisco Bay estuary (Figure 1). The salt ponds of the Napa-Sonoma Marshes Wildlife Area (NSM) were located 5 km northwest of Vallejo, California and comprised about 3400 ha. They were acquired in 1994 (2917 ha) and 2003 (470 ha) by the California Department of Fish and Game and became part of the NSM. Although commercial salt production ceased in 1993, the system remained intact for

nearly a decade with lower salinities and muted tidal flow in primary ponds and higher salinity and very little tidal flow in ponds farther inland (Lionberger et al. 2004; Takekawa et al. 2006a). Salinity generally increased from lower to higher numbered ponds as evaporation caused the water to become progressively more concentrated as it moved through the system. Water historically entered the system by tidal influence through a one-way gate from the North Bay into Pond 1 (P1). Water was pumped into Pond 2 (P2) intermittently during the study period (T. Huffman, California Department of Fish and Game, personal communication). Increased water elevation in P2 supplied water into Pond 3 (P3) via an inverted siphon except when density differences and small hydraulic head prevented flow (Lionberger et al. 2004). Density differences and small hydraulic head prior to the 2002 breach at P3 usually prevented flow through an inverted siphon between P3 and Pond 4 (P4) (Lionberger et al. 2004). During salt production, the supernate was removed from the brine and stored in Pond 7 (P7), the bittern pond.

System hydrology changed in late 2002 after an unauthorized breach on P3 (529 ha) resulted in direct tidal flow in the breached pond and reduced salinities in P3 and P4 (382 ha), which received water flow directly from P3. In March 2006, the initiation of the Napa-Sonoma Marsh Restoration Project saw additional breaches constructed at P3, P4, and P5, opening a total of 1233 ha (36% of the total North Bay salt pond area) to tidal flow.

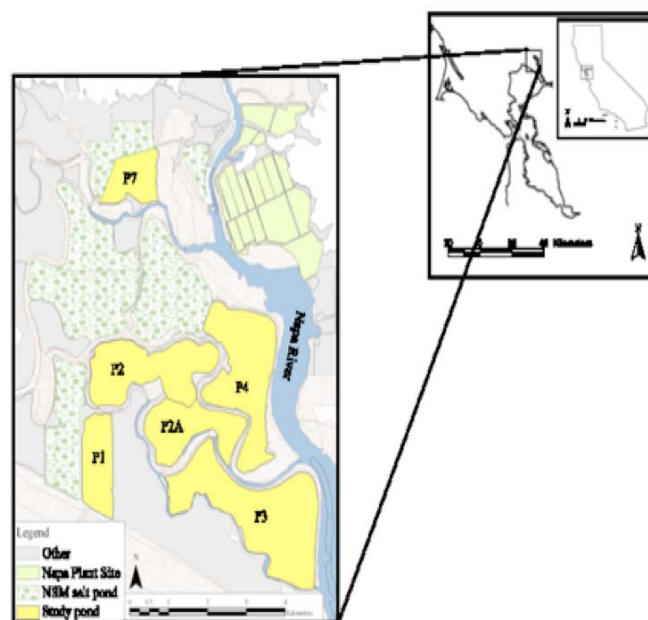


Figure 1—Former salt evaporation ponds, including 6 study ponds, in the Napa-Sonoma Marshes (NSM) Wildlife Area located 5 km northwest of Vallejo, California, USA on the northern edge of San Pablo Bay in the San Francisco Bay estuary.

METHODS

A subsample of six NSM salt ponds was selected for study between 1999 and 2008 to be representative of the salinity gradient in the salt pond system (Figure 1). We superimposed a 250 m x 250 m (6.25 ha) Universal Transverse Mercator (UTM) grid upon the sampled ponds to provide a framework for integrated sampling (Takekawa et al. 2006a). This grid system provided the basis for identifying bird locations in each pond (Matveev 1995; Posey et al. 1995). Ponds P1, P2, P3, P4, and P7 ranged in size from 123 ha to 529 ha and varied in mean salinity from 23 g l⁻¹ to 224 g l⁻¹ (Table 1), similar to the salinity range found in commercial salt production systems. Additionally, Pond 2A (P2A) was a 215 ha former salt pond that had been breached in 1995 and was mostly vegetated when the study began (Williams & Orr 2002).

Table 1—Mean winter salinity \pm SE at salt ponds of the Napa-Sonoma Marshes Wildlife Area, California, USA, prior to (1999–2002) and following (2003–2008) the breach at P3.

Pond	Salinity (g l ⁻¹) \pm SE	
	Winter 1999–2002	Winter 2003–2008
P1	18.5 \pm 3.1	17.6 \pm 1.8
P2	23.2 \pm 2.4	18.0 \pm 1.9
P3	40.4 \pm 1.9	10.5 \pm 1.5
P4	141.9 \pm 27.0	30.3 \pm 7.7
P7	223.7 \pm 18.0	219.6 \pm 20.9

Salinity

Salinity was measured monthly in P1, P2, P3, P4 and P7 from February 1999 until November 2001 and monthly from August 2003 through February 2008. Measurements were timed to occur within a week of bird surveys and locations were referenced to the 250 x 250 m UTM grid. Water levels were recorded from staff gages installed at a single location within each pond. Because pond salinities often exceeded the calibration capabilities of water quality meters, specific gravity was used for comparability to calculate salinity in all ponds and was measured with a hydrometer (Ertco, West Paterson, New Jersey) scaled for the appropriate range. These data were corrected for temperature and converted to salinity. Where pond salinity was less than 70 g l⁻¹, a Hydrolab Minisonde® (Hydrolab-Hach Company, Loveland, CO) was additionally used to measure specific conductance (internally converted to salinity with the 1978 Practical Salinity Scale) to check for consistency with concurrently collected specific gravity measurements.

Bird Surveys

Complete counts of the six ponds were conducted monthly from January 1999 to June 2001, bimonthly through August 2002, and monthly thereafter through February 2008. Observers conducted counts of species with binoculars and spotting scopes from vantage points on pond levees, and locations of waterbirds were placed within the grid cells of each pond to examine the spatial distribution of birds. More detailed location data were recorded to indicate when birds were on levees or islands rather than open water, and behavior was also recorded to indicate whether birds were foraging or roosting. Surveys were conducted during the day within 3 hours of the highest high tide when the largest number of waterbirds was roosting in the salt ponds. A low tide survey was additionally conducted on P3 in December 2007 for comparison with the high tide count conducted in the same month. Low tide access restrictions and the limited tide window made it possible only to count a portion of this large pond during this period, but the superimposed grid system allowed partial-pond comparisons between high and low tide counts.

Identified waterbirds were separated into guilds to examine differences among foraging groups rather than differences among species (Table 2). These foraging guilds included: 1) dabbling ducks—fed in the upper water column, e.g., *Anas clypeata* (northern shoveler); 2) diving ducks—fed in deeper water on benthic invertebrates, e.g., *Oxyura jamaicensis* (ruddy duck); 3) fish-eating birds—fish consumers, e.g., *Sterna forsteri* (Forster's tern); 4) herons—herons and egrets, e.g., *Ardea alba* (great egret); 5) small shorebirds—foraged in the top layer (< 3 cm) of sediments, e.g., *Calidris mauri* (western sandpiper); 6) medium shorebirds—reached deeper into the substratum than small shorebirds, e.g., *Limosa fedoa* (marbled godwit); and 7) gulls, e.g., *Larus californicus* (California gull).

Analyses

Differences in salt ponds were examined during the winter (Dec–Feb), spring (Mar–May), summer (June–Aug), and fall (Sep–Nov) seasons. Months were assigned to seasons to encompass bird migration chronology; these provided replicated measures for seasonal abundance comparisons between ponds. Because of higher and more consistent bird abundance during winter, winter bird abundances and salinity data were used for among-pond comparisons and for low and high tide comparisons. Bird densities were calculated to account for differences in pond area. Density differences between the winters prior to (1999–2002) and following (2003–2008) the P3 breach at different ponds were investigated on square-root transformed data using two-way analysis of variance (ANOVA) (SAS Institute 1990). Although ANOVA is reasonably robust to failed normality assumptions, we additionally performed the test on rank-transformed data to compare results when data did not meet the normality assumption (Zar 1999).

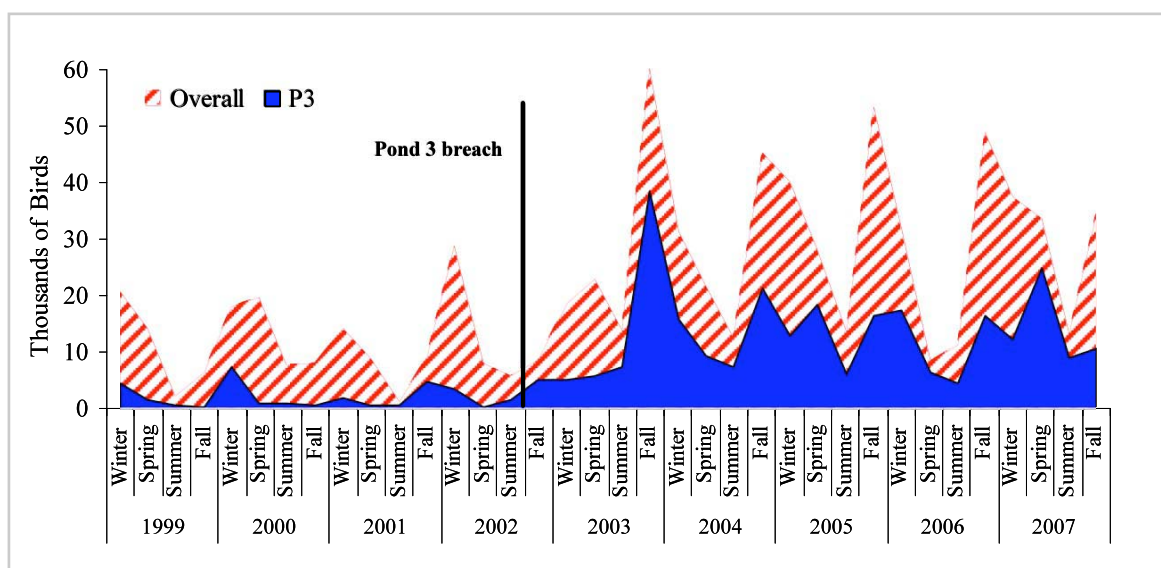


Figure 2—Combined seasonal bird abundances in study ponds P1, P2, P2A, P3, P4, and P7 from winter 1999 through fall 2007, Napa-Sonoma Marshes Wildlife Area, California, USA. Bird abundances in the breached pond, P3, are shown as a proportion of the total.

Because most bird species were migratory and exhibited high annual variability in overall abundance, counts were additionally standardized to total system abundance by calculating the proportional abundance at each salt pond (i.e., the sum of proportional abundances of a group of birds at all ponds totaled 100%). Changes in proportional abundance were interpreted as shifts in pond selection by groups of birds over time.

RESULTS

Total bird abundance increased after the breach of P3 (Figure 2). Highest numbers were observed during spring and fall migration periods and during winter, when overall bird numbers were on average 5.7 times higher than the preceding summer.

Pond salinity during winter was generally consistent, with standard deviation (SD) $< 8 \text{ g l}^{-1}$, but was more variable (SD $18\text{--}72 \text{ g l}^{-1}$) in the higher salinity ponds P4 and P7. Salinity declined at all salt ponds during the 5 years following the initial breach but this was most pronounced at P3, which decreased from 40 to 10 g l^{-1} , and at P4, which decreased from 142 to 30 g l^{-1} (Table 1).

Two-way ANOVA results on square-root transformed data suggested that overall winter bird densities differed between ponds ($F_{6,166} = 52.81$, $P < 0.0001$) and had a significant time (before vs. after breaching) effect as well ($F_{1,166} = 8.83$, $P = 0.0034$). However, data failed to meet the normality assumption (Shapiro-Wilk $W = 0.902$, $P < 0.0001$). Similar results were obtained when the test was performed on rank-transformed data (pond effect $F_{6,166} = 70.86$, $P < 0.0001$; time effect $F_{6,166} = 4.02$, $P = 0.0466$). The interaction between pond and time in

relation to breaching was not found to be significant when the ANOVA was performed on square-root transformed data ($F_{6,166} = 1.87$, $P = 0.0887$), but when the test was performed on rank-transformed data, there was a significant interaction effect ($F_{6,166} = 2.85$, $P = 0.0115$).

Winter densities increased 71% from $12.0 \text{ birds ha}^{-1}$ during 1999–2002 (pre-breach) to $20.6 \text{ birds ha}^{-1}$ during 2003–2008 (post-breach). Bird densities at individual ponds also increased during this period, except at P2A, which declined from $1.3 \text{ birds ha}^{-1}$ to $0.5 \text{ birds ha}^{-1}$, and P7, the bittern pond, which supported very few birds overall (Figure 3). The low salinity intake pond, P1, had the highest mean winter bird densities in the system both before ($21.2 \text{ birds ha}^{-1}$) and after ($34.7 \text{ birds ha}^{-1}$) the breach. P1 was followed by P4, which also had higher bird densities overall than both before ($20.7 \text{ birds ha}^{-1}$) and after ($27.6 \text{ birds ha}^{-1}$) the breach. Total bird abundance at P3 increased 178% after the breach, proportionally more than expected based on the 71% increase in overall system bird numbers. Before the breach, the density of birds at P3 was $8.3 \text{ birds ha}^{-1}$, only 70% of overall system density. After breaching, however, there were slightly more birds than would be expected if proportional abundance remained constant (23.0 vs. $20.6 \text{ birds ha}^{-1}$, or about 110%).

Pond 3 bird densities were lower than the rest of the system in the winters just prior to the breach, but following the breach, pond bird density increased to a level consistent with the other ponds. Pond 2A, the vegetated pond, had consistently low bird densities, generally $1\text{--}2 \text{ birds ha}^{-1}$, when compared to other ponds through all 10 years of the study (Figure 4).

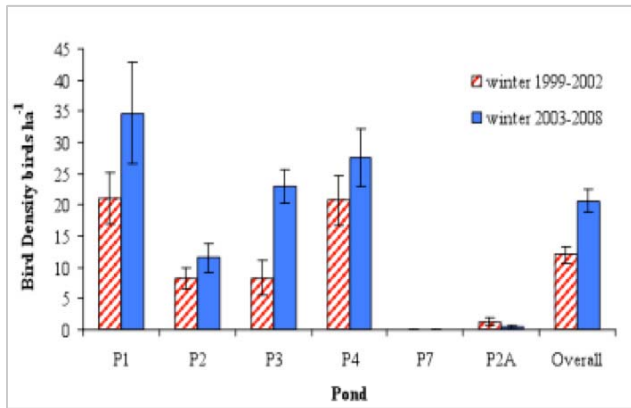


Figure 3—Overall bird density (mean \pm SE) at the Napa-Sonoma Marshes Wildlife Area, California, USA, during the winters prior to (1999–2002; $n = 9$) and following (2003–2008; $n = 17$) the breach at P3. Mean bird density at P7 was 0.

The highest observed bird densities at single surveys were at P3 and P4 during winters following the P3 breach and were over 124 birds ha⁻¹; most of these were small shorebirds. Although both small and medium shorebirds have increased proportionately more following the breach than other guilds in P3 during high tide surveys (Table 3), low tide abundances may be even higher. While dabbling and diving ducks declined at P3 during low tide, shorebirds more than doubled (Figure 5). Of these shorebirds, 98% were observed foraging, whereas only 57% were observed foraging during the high tide survey.

The high tide counts demonstrated that shorebird guilds were also highly responsive to system-level change. Prior to the breach during the winters of 1999–2002, 87% of all small shorebirds and 79% of all medium shorebirds were counted in P4 (Table 3). During the winters of 2003–2008, the proportion of all shorebirds on P4 declined to 32%, with subsequent increases on P3 for small shorebirds (1% to 46%) and medium shorebirds (2% to 37%). Diving ducks declined at P1 and P3 from a combined total of 57% of area diving ducks to 38% after the breach; this decline was reflected in an increase at P4 from 8% to 29% of area totals. Dabbling ducks also declined from 36% to 12% of area totals at P1 and increased from a shared total of 60% to 84% at P3 and P4. Forty-five percent of all fish-eating birds were counted at P3 before the breach, but this declined to 16% after breaching, while fish-eating birds increased from 0% at P4 before breaching to 22% afterwards. Similarly, herons declined at P2 from 56% to 20% and increased at P3 and P4 from 16% to 57%. Seventy-four percent of gulls were counted at P4 before the breach, but after the breach this number declined by half at P4 and increased by 30% at P2 and 11% at P3 (Table 3).

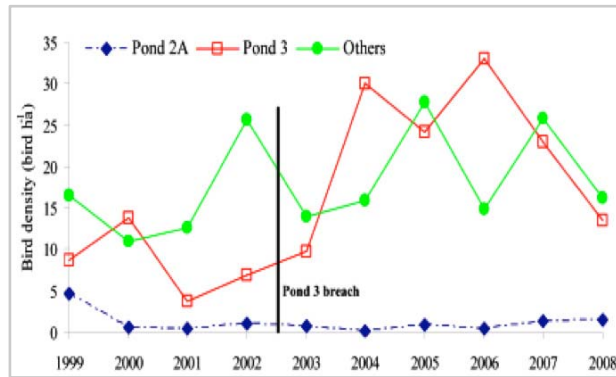


Figure 4—Mean winter bird density at P2A and P3, and combined average density at other study ponds (P1, P2, P4, and P7) at the Napa-Sonoma Marshes Wildlife Area, California, USA, from 1999 through 2008.

DISCUSSION

Waterbird Use of Salt Ponds

Salt ponds provide unique habitats that support large populations of migratory waterbirds (Takekawa et al. 2001, 2006a; Paracuellos et al. 2002; Warnock et al. 2002). Shorebirds in particular use salt ponds as important upland roosts during high tide, when adjacent mudflats are unavailable; insufficient availability of nearby roosts may constrain the ability of shorebirds to exploit mudflats as foraging habitats (Dias et al. 2006). Although large salt ponds are not natural features in SFB, their presence for the past 150 years (Ver Planck 1958) as well as their functional replacement of natural estuarine wetlands such as salt pannes has made them critical habitats for waterbirds. Salt ponds have provided these species with multiple advantages. The large expanses of water facilitated taking flight and predator avoidance, while the shallow, sheltered impoundments likely created a favorable microclimate for roosting (Warnock & Takekawa 1996). These conditions also favored foraging, and salt pond uplands have contributed significantly to shorebird foraging that have been reduced in natural estuarine habitats (Velasquez & Hockey 1991; Masero & Pérez-Hurtado 2001).

Velasquez (1992) noted that the abundance and species composition of macroinvertebrate prey was related to salinity, while the availability of prey to particular species of birds depended on depth. Lower salinity salt ponds support benthic invertebrates such as those used by shorebirds in shallow water and diving ducks in deeper water, and they are also favorable conditions for birds that consume fish, which generally cannot survive in salinities $> 80 \text{ g l}^{-1}$ (Takekawa et al. 2006a). However, hypersaline ponds may be particularly valuable for many shorebirds and other species that can forage on the dense populations of *Artemia* and *Ephydra* that thrive there. In mid-hypersaline

SFB ponds, *Artemia* represent an important food resource for species that exploit this prey, with biomass exceeding the combined macroinvertebrate biomass of other pond by several orders of magnitude (Takekawa et al. 2006a). *Ephydra* have likewise been found to be an important prey species, and are more likely fed on by many species of waterbirds than are *Artemia* (Anderson 1970).

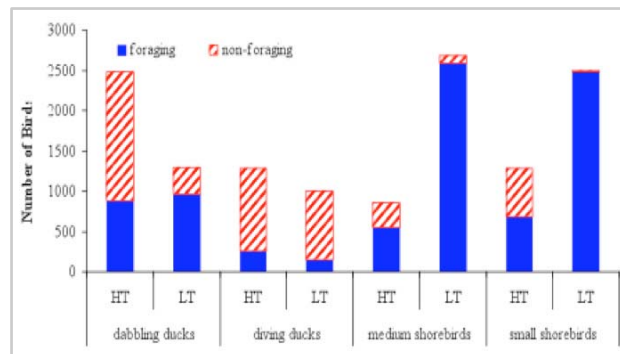


Figure 5—Winter 2008 bird abundance and activity (foraging or non-foraging) of dominant bird guilds at pond P3, Napa-Sonoma Marshes Wildlife Area, California, USA, during high (HT) and low tide (LT).

Bird use at the NSM largely conformed to expectations based on salinity and water depth. Prior to the P3 breach in summer 2002, highest winter bird densities were at P1 and P4. P1 was a mixohaline salt pond with variable water depth, and supported shorebirds at the shallow southern end and waterfowl at the deeper, northern end. Because of the low salinity, the pond supported benthic macroinvertebrates and many fish (Takekawa et al. 2006a), and was the only pond to support high proportions (> 10%) of every foraging guild examined. P4 was also somewhat variable in water depth, but it was a mid-hyperhaline pond and did not support fishes (Takekawa et al. 2006a). Although the pond supported about 10% of ducks in the system and many gulls, these species were primarily roosting at the pond and not foraging. The high bird densities at P4 were primarily due to shorebirds that were able to exploit the combination of variable, shallow water depth and dense populations of *Artemia* and *Ephydra* that were present at the pond during this period (Takekawa et al. 2006a). P2 and P3 had generally low bird density and similar composition. P2 was a mixohaline salt pond with consistent water depth and supported primarily diving ducks and fish-eating birds, which use deeper water, and herons, which forage along pond levees. P3 was a shallower, low-hyperhaline pond and supported nearly half of the dabbling ducks in the system as well as large proportions of diving ducks and fish-eating birds. P7, the bittern pond, was a high-hyperhaline pond and was too saline to support macroinvertebrates or fish, so it was not surprising that few birds were observed at the pond.

Changes in Pond Conditions Following Breaching

A key factor for restoring wetlands is to restore the hydrologic conditions that drive the structure and function of the wetland (Odum et al. 1995; Mitsch & Gosselink 2007). In tidal salt marshes, wetland hydrology is largely a function of the frequency and duration of tidal inundation, which is determined by the elevation of the site relative to tidal fluctuations (Montalto & Steenhuis 2004). In some salt ponds, such as P2A, restoration may be as simple as breaching levees according to hydrological patterns in the neighboring sloughs or the bay. Reintroduction of tidal action to a salt pond can encourage sedimentation and eventual vegetation establishment until the marsh reaches maturity. Initially, however, the expected effects of breaching are to reduce salinity to ambient conditions and to create tidally fluctuating water levels. As water depth and salinity are important parameters for determining habitat quality for birds, birds should respond immediately to these changes. Waterbirds are highly mobile and readily move between habitats in response to tidal fluctuations and changing foraging conditions (Burger et al. 1977).

Annual variation in bird numbers was high, but results of ANOVA tests on square-root and rank transformed bird density data suggested that bird densities increased following the breach. Additionally, a possible interaction effect between ponds and time relative to breaching suggested that some ponds may have changed more than others. This result suggests that changes in the system may have been related to changes at a few ponds rather than simply an increase in system bird numbers overall, and observed changes in physical characteristics and proportional bird distributions at P3 and P4 support this conclusion.

Immediately following the breach, P3 shifted from a low-hyperhaline pond to a mesohaline pond, the least saline pond in the system. Similarly, P4, which received inflow from P3, shifted from a mid- to high-hyperhaline pond to a low-hyperhaline pond. This reduction in salinity was likely responsible for some shifts in bird use, as diving ducks, fish-eating birds, and herons began to use P4 after the breach. Salinity and water level changes were most immediate in P3 because it was initially the only pond to receive direct tidal flow, and it became a dynamic habitat with continually shifting water levels. The addition of shallowly-inundated, open mudflat habitat at P3 and the concurrent loss of *Artemia* and *Ephydra* at P4 provoked a shift in some of the proportional distribution of shorebirds from P4 to P3 following the breach. Despite the redistribution of many birds to P3 and P4, the highest densities in the system remained at P1 following the breach, and the increased density at P4 was not inconsistent with the increase expected based on system-wide bird abundance increases in the years following the 2002 breach. What is notable is that P2 density increased very little. The

invariable bathymetry at P2 is likely responsible, as the pond is able to support a smaller subset of bird species than more variable ponds such as P1. Conversely, density at P3 increased much more than expected based on past proportional abundance, suggesting habitat quality improvements particularly for shorebirds.

Breached ponds provide unique low-tide foraging opportunities for shorebirds. Breached salt ponds are essentially temporary mudflats that may be inundated for different periods than mudflats in the SFB; thus, they may provide a longer foraging window for birds moving between habitats. Additionally, rapid early sedimentation, as has occurred at P3 (Takekawa et al. 2006b) may also make the pond favorable for rapid colonization by macroinvertebrates that can then be exploited by foraging shorebirds.

Waterbird Use of Restored Ponds

Breached salt ponds, while they may be heavily used by waterbirds, are a transitional habitat between two long-term states: managed salt ponds and mature tidal salt marsh. However, it is not always easy to predict how long the transition will last. P2A was > 80% vegetated 3 years after breaching (Goals Project 1999), but 6 years after its initial breach, adjacent P3 has < 5% vegetative cover (Athean & Takekawa, unpublished data). Williams & Orr (2002) noted that in addition to initial pond elevation, the primary constraints on marsh development in SFB were restricted tidal action, limited sediment supply, and high wind-wave energy. Tidal action was initially restricted at P3, as the pond was more than twice the size of P2A yet contained only a single breach (at first only 0.25 m, eventually around 30 m wide by 2006) before additional construction opened up the pond to full tidal action in 2006 (Takekawa et al. 2006b). It is possible that high wave energy could inhibit marsh development at P3, but although adjacent pond P2A had the highest wave power index of all sites examined by Williams & Orr (2002), it also had among the highest vegetation establishment rates. Sedimentation was significant in the years immediately following the breach (Takekawa et al. 2006b), so it is likely that sedimentation will continue and that vegetation establishment may be rapid once appropriate elevations are obtained.

Mature salt marsh supports some of the same bird species as salt ponds, but at much lower densities (Stralberg et al. 2005). Although P2A supported some dabbling ducks, overall waterbird densities were consistently low throughout the study period. P2A was the only pond that showed a decrease in overall bird density after the P3 breach despite a 71% increase in bird density overall. However, the species composition at P2A included marsh dependent birds not found at managed or recently breached

salt ponds, including endangered California clapper rails (*Rallus longirostris obsoletus*) and many marsh passerines.

CONCLUSIONS

Salt ponds, although not part of the natural SFB landscape, have become an important resource for waterbirds in the estuary. Converting salt ponds to tidal marsh will likely benefit some species at the expense of others. Most shorebirds prefer more open habitats rather than tidal marsh plain habitats (Warnock & Takekawa 1995). Development of coastal zones and interior valley wetlands have resulted in fewer areas available for migratory waterbirds in the flyway, and alternative wetlands may not exist outside of the SFB estuary to compensate for loss of waterbird habitats in the ecosystem.

An important consequence of this loss of habitat is that careful planning is needed to determine the number and configuration of ponds restored during a given timeframe to ensure that sufficient habitat is available for waterbirds. Although restoration is intended to benefit endangered tidal marsh species, the process of restoration may result in habitat loss for bird species that heavily use salt ponds while also remaining unsuitable for tidal marsh species until plants reach sufficient maturity to provide cover and forage. Early reintroduction of tidal action during salt pond restoration may initially result in a strong avian response to newly restored ponds, and consequently a negative response in managed ponds even if habitat quality in managed ponds remains constant. Adaptive monitoring of salt pond systems that include transitional, breached pond habitats will require careful interpretation rather than simple numerical evaluation.

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Table 2—Bird species and associated foraging guilds observed at the Napa-Sonoma Marshes Wildlife Area, California, USA, during the winters prior to (1999–2002) and following (2003–2008) the breach at P3.

Guild	Common Name	Scientific Name
Dabbling Ducks	American coot	<i>Fulica americana</i>
	American wigeon	<i>Anas americana</i>
	cinnamon teal	<i>Anas cyanoptera</i>
	Eurasian wigeon	<i>Anas penelope</i>
	gadwall	<i>Anas strepera</i>
	green-winged teal	<i>Anas crecca</i>
	mallard	<i>Anas platyrhynchos</i>
	northern pintail	<i>Anas acuta</i>
	northern shoveler	<i>Anas clypeata</i>
Diving Ducks	Barrow's goldeneye	<i>Bucephala islandica</i>
	bufflehead	<i>Bucephala albeola</i>
	canvasback	<i>Aythya valisineria</i>
	common goldeneye	<i>Bucephala clangula</i>
	greater scaup	<i>Aythya marila</i>
	lesser scaup	<i>Aythya affinis</i>
	long-tailed duck	<i>Clangula hyemalis</i>
	redhead	<i>Aythya americana</i>
	ruddy duck	<i>Oxyyura jamaicensis</i>
	scaup (greater, lesser)	<i>A. marila, A. affinis</i>
Eared Grebes	eared grebe	<i>Podiceps nigricollis</i>
Fisheaters	American white pelican	<i>Pelecanus erythrorhynchos</i>
	black tern	<i>Chlidonias niger</i>
	Brandt's cormorant	<i>Phalacrocorax penicillatus</i>
	Caspian tern	<i>Sterna caspia</i>
	Clark's grebe	<i>Aechmophorus clarkii</i>
	common merganser	<i>Mergus merganser</i>
	double-crested cormorant	<i>Phalacrocorax auritis</i>
	Forster's tern	<i>Sterna forsteri</i>
	horned grebe	<i>Podiceps auritus</i>
	least tern	<i>Sterna antillarum</i>
	pied-billed grebe	<i>Podilymbus podiceps</i>
	red-breasted merganser	<i>Mergus serrator</i>
Hérons	black-crowned night-heron	<i>Nycticorax nycticorax</i>
	great blue heron	<i>Ardea herodias</i>
	great egret	<i>Casmerodius albus</i>
	snowy egret	<i>Egretta thula</i>
Small Shorebirds	Baird's sandpiper	<i>Calidris bairdii</i>
	dowitcher (long, short-billed)	<i>Limnodromus scolopaceus, L. griseus</i>
	dunlin	<i>Calidris alpina</i>
	least sandpiper	<i>Calidris minutilla</i>
	semipalmated plover	<i>Charadrius semipalmatus</i>
	snowy plover	<i>Charadrius alexandrinus</i>
	spotted sandpiper	<i>Actitis macularia</i>
	western sandpiper	<i>Calidris mauri</i>
Medium Shorebirds	American avocet	<i>Recurvirostra americana</i>
	black-bellied plover	<i>Pluvialis squatarola</i>
	black-necked stilt	<i>Himantopus mexicanus</i>
	greater yellowlegs	<i>Tringa melanoleuca</i>
	killdeer	<i>Charadrius vociferous</i>
	long-billed curlew	<i>Numenius americanus</i>
	lesser yellowlegs	<i>Tringa flavipes</i>
	marbled godwit	<i>Limosa fedoa</i>
	stilt sandpiper	<i>Calidris himantopus</i>
	whimbrel	<i>Numenius phaeopus</i>
	willet	<i>Catoptrophorus semipalmatus</i>
Phalaropes	red-necked phalarope	<i>Phalaropus lobatus</i>
Gulls	Bonaparte's gull	<i>Larus philadelphia</i>
	California gull	<i>Larus californicus</i>
	glaucous-winged gull	<i>Larus glaucescens</i>
	herring gull	<i>Larus argentatus</i>
	mew gull	<i>Larus canus</i>
	ring-billed gull	<i>Larus delawarensis</i>
	Sabine's gull	<i>Xema sabini</i>

Table 3—The proportion of total birds within each foraging guild (mean \pm SE) that was observed at each study pond in the Napa-Sonoma Marshes Wildlife Area, California, USA, during the winters prior to (1999–2002) and following (2003–2008) the breach at P3.

	P1		P2		P3		P4		P7	
	99-02	03-08	99-02	03-08	99-02	03-08	99-02	03-08	99-02	03-08
Dabbling ducks	0.36 \pm 0.13	0.12 \pm 0.05	0.03 \pm 0.01	0.04 \pm 0.04	0.49 \pm 0.11	0.65 \pm 0.04	0.11 \pm 0.11	0.19 \pm 0.04	0.00 \pm 0.00	0.00 \pm 0.00
Diving ducks	0.21 \pm 0.02	0.15 \pm 0.03	0.34 \pm 0.09	0.33 \pm 0.07	0.36 \pm 0.09	0.23 \pm 0.03	0.08 \pm 0.04	0.29 \pm 0.06	0.00 \pm 0.00	0.00 \pm 0.00
Fish eaters	0.28 \pm 0.12	0.27 \pm 0.10	0.27 \pm 0.16	0.35 \pm 0.09	0.45 \pm 0.21	0.16 \pm 0.04	0.00 \pm 0.00	0.22 \pm 0.10	0.00 \pm 0.00	0.00 \pm 0.00
Gulls	0.17 \pm 0.12	0.12 \pm 0.04	0.01 \pm 0.00	0.31 \pm 0.16	0.08 \pm 0.05	0.19 \pm 0.08	0.74 \pm 0.16	0.37 \pm 0.15	0.00 \pm 0.00	0.01 \pm 0.01
Hérons	0.28 \pm 0.17	0.23 \pm 0.05	0.56 \pm 0.18	0.20 \pm 0.09	0.08 \pm 0.05	0.17 \pm 0.05	0.08 \pm 0.05	0.40 \pm 0.11	0.01 \pm 0.01	0.01 \pm 0.01
Medium shorebirds	0.18 \pm 0.08	0.31 \pm 0.10	0.00 \pm 0.00	0.00 \pm 0.00	0.02 \pm 0.01	0.37 \pm 0.06	0.79 \pm 0.08	0.32 \pm 0.09	0.00 \pm 0.00	0.00 \pm 0.00
Small shorebirds	0.12 \pm 0.06	0.14 \pm 0.09	0.01 \pm 0.01	0.08 \pm 0.08	0.01 \pm 0.01	0.46 \pm 0.12	0.87 \pm 0.06	0.32 \pm 0.14	0.00 \pm 0.00	0.00 \pm 0.00

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Limnology and Food Web Structure of a Large Terminal Ecosystem, Walker Lake (NV, USA)

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ABSTRACT

Walker Lake is a large, terminal, saline lake in the Western Great Basin of the United States. Diversions have greatly reduced river inflow, which has led to a decrease in lake volume by 75% since the 1880s. As a result there has been a concomitant increase in salinity levels and alteration to biotic community structure. This study provides a contemporary snapshot of the water quality, phytoplankton-zooplankton biomass, and the lake's food web structure. Water quality and zooplankton were sampled monthly (March to October 2007) from six locations at discrete depths. Nutrient concentrations were highly variable (ammonium levels - 0 to 30 ppb, nitrate - 0 to 12 ppb, total and dissolved phosphorus - 500 to 1000 ppb, and soluble reactive phosphorus - 400 to 600 ppb). The food web structure determined from stable isotope measurements (carbon and nitrogen) and stomach contents suggests benthic resources contributed greatly to fisheries energetics.

INTRODUCTION

Large, permanent lakes with high salinity occur infrequently in nature. Walker Lake is one of eight large, hypersaline lakes worldwide. It is one of many terminal lakes remnant of Lake Lahontan, a large lake from the Pleistocene era that began to desiccate roughly 10000 years ago (Benson 1978). Located in the rain shadow of the Sierra Nevada and Cascade ranges to the west and the Rocky Mountains to the east, evaporation rates in this area commonly exceed precipitation rates at lower elevations and are subjected to large annual and diel air temperature shifts (Hammer 1986).

Terminal lakes in semi arid regions are typically sensitive to changes in freshwater flow (Beutel 2001; Beutel et al. 2001). Many saline lakes are currently in decline due to reduction of inflow (Hammer 1986) and anthropogenic desiccation has occurred in numerous saline lakes worldwide (Hammer 1986; Williams 1993). Anthropogenic desiccation on Walker Lake, between 1882 and 1996, has decreased the lake volume by almost 75% and between 1979 and 2005 there has been a 99.9% reduction in river flow reaching the lake for agricultural uses. As a result of

these anthropogenic influences salinity has increased from 2.6 to 12.4 g/l (Beutel et al. 2001).

Although species diversity is low in the lake it did at one time support a robust fisheries. Prior to desiccation, Walker Lake supported a large population of Lahontan Cutthroat trout (*Oncorhynchus clarki henshawi*) and the forage fish, tui chub (*Gila bicolor*). However dam construction on the Walker River stopped spawning runs and in conjunction with other environmental factors the Walker Lake cutthroat trout strain is now extinct and is maintained by an intensive stocking program with non-Walker strains of Lahontan Cutthroat Trout (Elliot 1995). The benefits of increasing river flow will depend upon water quantity and quality, and timing of release. Fish are limited by high salinity and ion concentrations and if there is not an increase in flow to Walker Lake over the next ten years it may be unable to support a fishery in the future as the lake increases in salinity (Beutel et al. 2001). Increase to annual flow could off set the desiccation and increased levels of salinity in Walker Lake.

The goal of this study was to characterize the contemporary ecological condition in Walker Lake focusing on: i) documentation of seasonal changes in water quality and physical conditions ii) secondary consumers (zooplankton and benthic invertebrates) and iii) determine the predatory-prey interactions and food web structure.

MATERIALS AND METHODS

Study Area

Walker Lake is located in the desert region of west-central Nevada southeast of Reno, Nevada (N38 42.012, W118 42.948). With a surface elevation of 1234 m, it has an approximate area of 140 km² with a mean depth of 19.3 m. The Walker River discharge into the lake occurs on the north end and has two major branches, the East and West forks. The forks flow north roughly 200 km originating in the central southeastern Sierra's before turning south and towards the lake. Both forks contain reservoirs, Bridgeport on the upper East fork and Topaz on the West fork. Weber Reservoir is located on the main-stem 40 km north of the lake.

Water Quality

We sampled from six locations at regular intervals (4-6 weeks) between March 2007 and June 2008 in order to capture annual variability of water quality, zooplankton, and physical conditions affecting lower food web production. There are three main sampling locations running south to north and three more sampling locations near the inlet. Samples were collected at discrete depths (surface to depth at 3 m intervals) at the three main sampling locations, with surface grabs at the three inlet locations, to capture chemical processes during stratified and nonstratified periods along with fine scale physical processes.

A high precision, continuous water quality sampler (Seabird Technologies, model SBE 19) was calibrated for conditions at Walker Lake and used to measure temperature and dissolved oxygen at each of the six locations. Water quality constituents (total and dissolved phosphorus, soluble reactive phosphorus, ammonium, and nitrate) were analyzed from each discrete depth. Due to their saline nature, methods developed as part of the Clean Lakes Granting process for U.S. EPA for Nevada lakes were used to determine the different water quality constituents (Solórzano 1969; Liddicoat et al. 1975; Jones 1984; Reuter & Goldman 1990).

Primary and Secondary Consumers

Zooplankton was collected from three pelagic sampling stations during the same time as the limnological sampling. Zooplankton was collected using a vertical, zooplankton tow (153 μm mesh), from the bottom to the surface and preserved with sucrose–Lugol’s solution. In order to determine the current feeding behavior and energetics of zooplankton in Walker Lake a second zooplankton tow was taken at the same three sampling locations mentioned above. Live zooplankton from each of the dominant species (*Moina hutchinsoni*, *Leptodiptomus sicilis*, and *Hexarthra*) at the time of sampling, were pooled (10 samples; 50-100 individuals or each species) for stable isotope (carbon and nitrogen) analysis. Due to the patchy nature of zooplankton composition (Newton 2007) with the lake we assumed that zooplankton samples collected from our three locations were sufficient to determine biomass and abundance throughout the lake.

Research from other lakes suggests temporal and spatial changes of invertebrates by depth and over time due to temporal changes in water quality and algae production in pelagic and benthic habitats (Chandra et al. 2005; Vander Zanden et al. 2006). Recently, studies have suggested that

benthic invertebrate production is very important for fisheries energetics in Walker Lake (Chandra & Lawrence 2006). In order to capture changes across depth and inflow, benthic invertebrates were collected in the spring, summer, and fall using multiple (3 to 5) Petite Ponar grabs at 5 m intervals from the inlet of Walker Lake (1-5 m) to 30 m (deepest part of the lake). All samples were screened through a 500 μm mesh bucket for each location. Samples were immediately stored in 70% ethanol and identified to the lowest taxonomy possible. Invertebrates were measured for biomass and analyzed for carbon analysis to determine the source of energy uptake (benthic or pelagic algae).

Previous research suggests that alterations to water quality/lower food web production can alter ecosystem structure and fish growth (Vander Zanden et al. 2003; Chandra 2003; Chandra et al. 2005; Allen et al. 2006). In order to assess the contemporary food web structure in the lake prior to its restoration we measured stable isotope (carbon and nitrogen) from different fish species. Fish of different size classes were collected through coordination with state (Nevada Department of Wildlife) and federal (U.S. Fish and Wildlife service) biologists currently sampling the lake using 4 monofilament experimental gill nets (38 m x 1.8 m with mesh size starting at 1.27 cm and increasing by 0.64 cm until reaching 15.24 cm) set on the west side of the lake and creel census. Tui chub were identified for morphotype (benthic or pelagic) by counting gill rakers. Fish dorsal muscle tissues were collected from each fish for stable isotope analysis. Five Lahontan cutthroat trout were collected for analysis during creel surveys, while 48 tui chub were collected and utilized for stable isotope samples collected from gill netting. Tissue samples were dried at 70°C for 24 hours and ground to fine powder using a mortar and pestle. Samples were packed into tin capsules (8 x 5 mm), then carbon and nitrogen were analyzed using a continuous flow isotope ratio mass spectrometer (IRMS) (20-20, PDZ, Europa Science, Sandbach, United Kingdom). Samples were combusted to CO₂ and N₂ occurring at 1000°C in an inline elemental analyzer (PDZ Europa Science, ANCA-GSL). A Carbosieve G column (Supelco, Bellefonte, PA, USA) separated the gas before introduction to the IRMS. Standard gases (Pee Dee Belemnite for $\delta^{13}\text{C}$ and N₂ gas for $\delta^{15}\text{N}$) were injected directly into the IRMS before and after the sample peaks. Every twenty samples a replicate and standard was added to the analysis sequence to determine reproducibility and method variability.

Isotopic ratios were expressed as per mil (‰) notation. Mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (± 1 standard error, SE) for major taxonomic groups of the Walker Lake food web were calculated.

RESULTS

Water Quality

Temperature measurements indicated Walker Lake stratification periods during the summer starting in May and continuing through September with mixed conditions during other seasons. During December the lake becomes isothermic with an average temperature range in the winter between 6 and 11°C. Dissolved oxygen ranged from 5.3 mg/l at the surface in February to 9.5 mg/l in March (note: DO was not taken in July due to absence of the Seabird sampler during the sampling period). The hypolimnion became devoid of oxygen by the end of May (4 to 6°C bottom to surface) and remained anoxic throughout the summer and into early October (14 to 17°C bottom to surface).

Only the water quality data taken from our index site (deepest part of the lake) is presented here. Total phosphorus levels were generally very high ranging from 600 ppb in May around, 20 m, to 950 ppb in August. Phosphorus varied by depth during each sampling period indicating patchiness within the lake. Nitrogen concentrations were highly variable (ammonium 0-30 ppb, nitrate-nitrite 0-12 ppb) over time. During July nitrate levels increased to over 120 ppb at 20 m. August also showed an increase in nitrate levels between 18 m and 20 m. Average values for phosphorus and nitrogen are given in Table 1.

Primary and Secondary Consumers

The number of zooplankton was highly variable in space with the highest abundance occurring in the late spring and fall. The highest abundance of *Leptodiptomus* occurred in June (0.09 organisms/m³), at the northern end of the lake while *Moina* abundance was greatest in October (0.08 organisms/m³) at our southern sampling station (furthest from inflow). *Hexarthra* only occurred at two of the three locations in smaller numbers (< 0.01 organisms/m³ at each location) from the center of the lake in June to the southern end of the lake in July.

The major taxonomic groups identified from the benthic invertebrate sampling were oligochaeta, diptera, and odonata. Biomass and diversity was highest during June in the littoral zone (52 mg dry weight/m²), then gradually decreasing throughout the summer. Oligochaeta were only found during the June sampling period at 3 m of (12.9 mg dry weight/m²). Two odonata were identified in our

samples, *Coenagrion* and *Coniagrion*. *Coenagrion* was only found in the littoral zone and in our June (13.3 mg dry weight/m²) and August (12.7 mg dry weight/m²) sampling periods. *Zoniagrion* was only found at out 30 m sampling depth in June (9.5 mg dry weight/m²). However in our last sampling period in October *Zoniagrion* was found in the littoral and profundal zones at each depth sampled up to 18 m (12.9 mg dry weight/m²). The chironomidae, tanypodinae had the highest overall abundance during each sampling period and were also present at each depth. The highest abundance of tanypodinae was found in August at 4 m (13.5 mg dry weight/m²) and in October at 13 m (12.9 mg dry weight/m²).

Only two periphyton samples have been analyzed to date. Mean isotope values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were -22.0‰ and 4.4.0‰, respectively. Mean zooplankton isotope values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were -22.8‰ and 11.0‰, respectively. Mean benthic primary consumers isotopes for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were -19.9‰ and 9.4‰, respectively. However, there was a difference in the benthic invertebrate $\delta^{15}\text{N}$ signature in the littoral (8.3‰) and profundal zones (10.0‰) indicating an energy reliance difference. The only fish caught in the lake were tui chub (pelagic morphotype) and the top predator Lahontan cutthroat trout. Pelagic tui chub $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ concentrations were -20.5‰ and 12.6‰, respectively. Cutthroat trout $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ concentrations were -19.8‰ and 15.7‰, respectively (Figure 1).

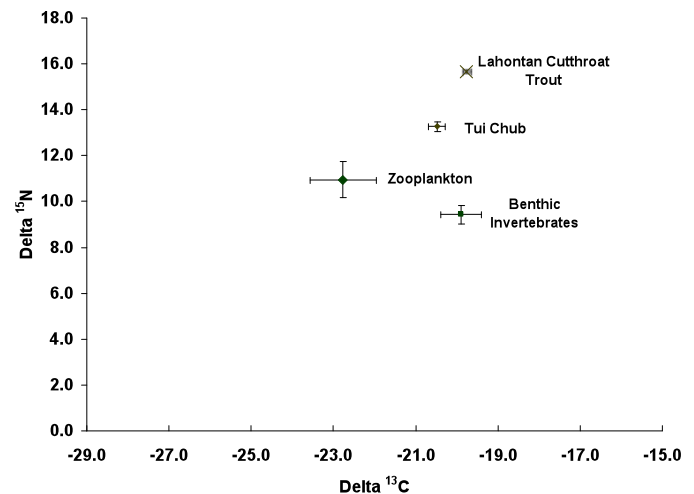


Figure 1—Stable isotope ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) food web diagram for the major taxa of Walker Lake, Nevada.

Table 1—Water quality constituents collected in 2007. All numbers are presented in ppb.

Constituents	March	May	June	August
Total Phosphorus	848.1 ± 17.2	822.9 ± 100.3	868.3 ± 51.9	901.6 ± 22.9
Dissolved Phosphorus	867.2 ± 14.4	688.8 ± 68.5	851.9 ± 42.4	747.9 ± 38.2
Soluble Reactive Phosphorus	469.6 ± 10.0	419.1 ± 26.1	434.0 ± 18.1	424.6 ± 29.0
Ammonium	12.3 ± 2.9	10.1 ± 11.8	22.4 ± 21.3	29.3 ± 52.2
Nitrate	5.3 ± 1.8	6.8 ± 1.7	27.1 ± 37.7	14.5 ± 17.0

DISCUSSION

Despite low water conditions and increased in salinity, Walker Lake exhibited monomictic conditions, stratifying during summer period. Typical of other lakes and similar to previous studies of Walker Lake more than a decade earlier, nutrient concentrations varied over time and depth. The lake continued to undergo anoxic conditions in the hypolimnion during summer resulting in the accumulation of ammonia. Internal loading processes do to sediment microbial activity and redox changes continue to be a strong contributor to the nitrogen budget of the lake. Similar finding have been shown for neighboring Pyramid Lake. Nitrogen loading also occurs from blue-green algae blooms (Beutel 2001). Phosphorus levels remained high throughout the study, similar to the findings of Beutel et al. (2001). The combination of nutrient data continued to suggest the lake is strongly N-limited even as water levels have declines in the lake.

Zooplankton varied spatially and temporally, typical for lake systems. Zooplankton species had different seasonal patterns and were found in highest abundances in shallow sites. The patchy nature of zooplankton composition within the lake is likely due to both biotic and abiotic factors. Biotic factors contributing to patchiness within Walker Lake may include diel vertical migration, competition, predation, and reproduction (Pinel-Alloul et al. 1988). It is well documented that zooplankton change positions in the water column on a daily bases for predator avoidance and to reduce competition and for reproduction (Horne & Goldman 1994; Wetzel 2001). Resource limitation in terms of food availability and quality in the seston likely played a large role in contributing to patchiness (Newton 2007). Furthermore, Walker Lake exhibits strong physical mixing and internal processes due to wind and weather changes throughout the season resulting in patchiness of both zooplankton and phytoplankton across the lake (Laurie Newton, unpublished data).

Benthic invertebrate's diversity was highest in June and decreased throughout the rest of the summer as the hypolimnetic water stratified and underwent periods of oxygen reduction. The littoral zone, 0 to 10 m, had the highest biomass found during late spring and fall. Odonata were almost entirely found in the littoral habitat, among macrovegetation and burrowing in surficial sediments. Odonata have high respiratory demands (Wetzel 2001) which would also explain why their biomass was highest in the spring and fall when oxygen levels were highest. Diptera are among the most important groups of aquatic insects and are commonly dominant components of benthic invertebrate communities. Because tanypodinae possess hemoglobin in their blood that functions at low levels of oxygen, they were found at all depths and throughout the seasons.

Only 2 fish species were caught in Walker Lake compared to 5 native fish taxa in neighboring Pyramid Lake. Only one tui chub (pelagic morphotype) was identified from the lake despite a large netting effort to collect fishes. It is believed that other fish species common to this region (Tahoe sucker, Lahontan redbreast shiners, speckled dace, tui chub-benthic morphotype) would exist in the lake given more freshwater circumstances. Stable isotope information suggests cutthroat trout, while maintained through hatchery processes is the top predator feeding on tui chubs and primary consumers. Pelagic tui chubs are the second dominant consumer feeding mostly on primary consumers. A continuous monitoring program by the state of Nevada's Department of Wildlife suggests limited recruitment of young of the year tui chub due to increasing saline condition and low freshwater flows entering the lake (Solberger, personal communication). It is unclear if this recruitment impacts cutthroat energetics and maintenance of their population however these fish comprise a significant amount of diet for cutthroat trout in neighboring Pyramid Lake (Chandra & Lawrence 2006).

Stable isotope carbon information also strongly suggests the Walker lakes fishery is supported by benthic production (Figure 1). Pelagic zooplankton is highly patchy in the lake environment and may not be available for fish consumption. Stomach analysis suggest both tui chub and cutthroat trout contain benthic invertebrate during all sampling periods (Umek & Chandra, unpublished data) with a dominance of Odonata in the stomachs during the early spring and fall creel surveys.

Walker Lake is currently under high saline and ion conditions. If there is not an increase in flow to Walker Lake over the next ten years it may be unable to support a trout fishery in the future as the lake increases in salinity (Beutel et al. 2001). An increase in annual flow could offset the desiccation and increased levels of salinity in Walker Lake. Furthermore changes in lake structure and food production in different habitat should be monitored simultaneously to determine impacts on fish production. These data should provide baseline data for a comparison study, if in the future, inflow from Walker River increases.

ACKNOWLEDGEMENTS

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The Effects of Increasing Total Dissolved Solids on the Walker Lake, Nevada, Fishery

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Pleistocene Lake Lahontan once occupied much of Nevada. Walker Lake, a desert terminal lake, is remnant of Lake Lahontan. It is fed by the Walker River, running from the east side of the Sierra Nevada through over 160 km of farming community before terminating at Walker Lake. Since the mid 1800s agricultural diversions have resulted in an approximately 45 m drop in lake elevation. Due to lack of flow and barriers created along the Walker River system, the historic Lahontan cutthroat trout (LCT) (*Oncorhynchus clarki henshawi*) have been unable to migrate and spawn upstream and maintain a self-sustaining population. The Nevada Department of Wildlife (NDOW) and the United States Fish and Wildlife Service (USFWS) historically stocked about 2.0×10^5 LCT and currently stock about 7.0×10^4 floy tagged LCT annually. However, increasingly toxic lake conditions are reducing survivability of the native LCT. High total dissolved solids (TDS, mostly bicarbonate, sodium, sulfate, and chloride) affect gill and kidney functions that impact survivorship and size of fishes. High TDS have resulted in unsuccessful acclimation of stocked

trout; fish die quickly when stocked directly from fresh water into Walker Lake. Fish planted in the river above the lake however, possibly self acclimate in the mixing zone. NDOW is currently working with the USFWS to examine survivorship of stocked LCT using tag recovery information. Figure 1 shows a decline in the ability of anglers to catch LCT since 1992, concurrent with an increase in TDS. While other native species once existed in Walker Lake, the LCT and Lahontan tui chub (*Gila bicolor pectinifer* and *G. bicolor obesa*) are the only species that have persisted in increased TDS. TDS has reached over 1.6×10^4 mg/l twice recently (in 2005 and 2007). Examinations of LCT survivorship, recruitment of tui chub, zooplankton composition and abundance, as well as intensive water quality analysis are currently part of an extensive study on Walker Lake. NDOW and USFWS hope to provide various management strategies to prolong the survival of fishes in Walker Lake while others work on requiring water rights for the lake.

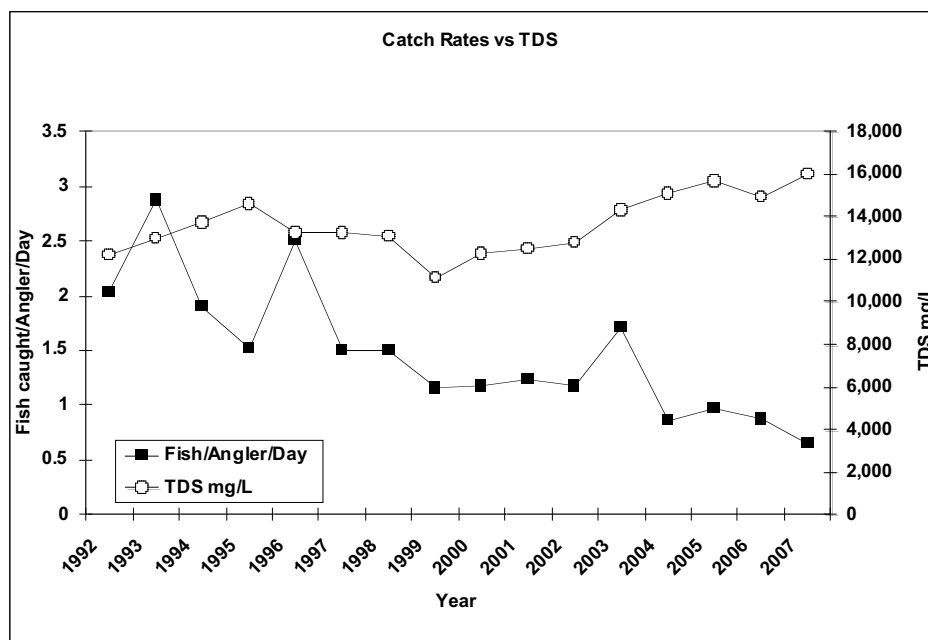


Figure 1—Catch rates from roving creel surveys show a decline in fish caught since 1992 as LCT respond to increasing TDS. Catch rates are also affected by fish planting numbers being reduced by more than half of historical numbers.

Distribution of Major Anions and Trace Elements in the Unsaturated Zone at Franklin Lake Playa, California, USA

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ABSTRACT

The composition of surficial salts formed near dry and drying saline lakes are partly the product of processes active in the unsaturated zone between the ground surface and the water table. These processes were investigated by determining the abundance of water-extractable solutes in sediment from the ground surface to the water table (~2.8 m) beneath Franklin Lake playa, California. Accumulation of solutes in the sediment is attributed to evaporation of aqueous solutions transported upward from the water table through the capillary fringe to an evaporation front that is currently 20-30 cm below the ground surface. Salts in the sediment from 0 to 20 cm depth are depleted in chloride and enriched in carbonate relative to deeper samples. Chloride depletion is most likely a product of selective dissolution by vertical recharge. The entire unsaturated zone contains greater amounts of carbonate than expected for evaporation of ground water and is best explained by preferential dissolution of more soluble salts in recharging precipitation but may also reflect the assimilation of CO₂ as carbonate minerals precipitate. Variations in the concentrations of arsenic, molybdenum, tungsten, and uranium in the water-soluble fraction of the sediment are complex and suggest unique geochemical controls on the abundance of each element. The distribution of these trace element abundances indicates that arsenic is the element most likely to accumulate in near-surface salts.

INTRODUCTION

Surficial sediments exposed on the margins of saline lakes and other arid and semi-arid settings accumulate salts through evaporation of shallow ground water (Salama et al. 1999; Reynolds et al. 2007; Elmore et al. 2008). These salts limit agriculture, are implicated as a major contribution to dust emissions, and are an important component of salinization of surface water in endorheic basins (Salama et al. 1999; Reynolds et al. 2007; Abuduwaili et al. 2008; Elmore et al. 2008). In addition to the detrimental effects of salinization, salts formed on some evaporative surfaces accumulate bioavailable toxic trace elements that can be dispersed in atmospheric dusts (Reheis 1997). Although much is understood of the processes of salt accumulation,

knowledge about controls on the major and trace element contents of the salts is incomplete. More complete descriptions of processes that affect the metal contents of salt crusts are necessary for understanding the implications to arid and semi-arid landscapes and their inhabitants.

Several studies have examined the processes responsible for salinization of soil as a result of transport of water and dissolved salts through the capillary fringe to an evaporative surface (Qayyum & Kemper 1962; Hassan & Ghaibeh 1977; Shimojima et al. 1996; Rose et al. 2005; Gowing et al. 2006). White, efflorescent salts coating sediment surfaces in arid and semi-arid settings are a visible reminder of these processes. Evaporative concentration also occurs in the subsurface at the depth of transition from liquid water to vapor-dominated transport—the zone of evaporation or evaporation front (Rose et al. 2005).

Although the physical aspect of salt accumulation has been described, relatively few investigations have explored chemical processes that might affect the distribution and accumulation of major salt components. One study in Spain showed that the relative abundances of chloride, sulfate, and carbonate species vary with depth in saline soil profiles, with chloride content generally increasing near the ground surface (Vizcayno et al. 1995). Szabolcs & Lesztak (1969) experimentally evaluated the distribution of sodium salts of chloride, sulfate, and carbonate and found that sodium chloride and sodium sulfate accumulated near the evaporation front whereas sodium carbonate inhibited the rise of water in the capillary fringe through changes in mechanical properties of the substrate.

Goldstein et al. (2007) reported that the chemical composition of soluble salts extracted from surface sediment in the center of Franklin Lake playa, California, could not be explained by simple evaporation of ground water found 2 to 3 m below the ground surface. This result contrasts with findings elsewhere in the drainage basin, where surface salts are compositionally similar to water in the immediately underlying saturated sediment.

A possible cause of the inconsistency is the separation of dissolved species by processes within the unsaturated zone underlying Franklin Lake playa. This paper describes the varied concentration of soluble major anions and selected trace elements within the unsaturated sediment beneath Franklin Lake playa and speculates on processes responsible for their accumulation at varying depths.

THE SETTING

Franklin Lake playa is located in Inyo County, California, approximately 60 km west of Las Vegas, Nevada. The playa covers an area of about 14 km² within the Mojave Desert and is at 600 m elevation (Czarnecki 1997). Temperatures range from -5 to 45°C, annual precipitation is typically 8 to 10 cm yr⁻¹, and pan evaporation is estimated to be 250 cm yr⁻¹ (Winograd & Thordarson 1975). Czarnecki (1997) determined that the playa has an average annual evaporation rate of 0.16 cm day⁻¹ through loss of water vapor from the unsaturated zone. The surface of Franklin Lake playa is covered with a soft puffy crust near the center, with salt pans and hard crusts on the margins. Fluvial channels of the Amargosa River and Carson Slough cover the western area of the playa, and every few years they carry surface water in response to heavy rain events. Nonetheless, surface water has not been observed on the soft puffy surface during the span of recent investigations (Czarnecki 1997; Reynolds et al., this volume). The Franklin Lake playa has characteristics of a bypass playa (Motts 1970) by which ground water recharges sediment beneath the playa but also flows through sediment beneath the Amargosa River and continues to the south.

METHODS

Sample Collection

Sediment samples were collected in April 2007 from an area of the playa characterized by relatively soft puffy crusts that lack visible accumulations of salt (Figure 1). Vegetation is absent on this part of the playa, with the closest plants found only in shallow channels 300 m east of the sample site. A soil pit was excavated to a depth of 95 cm, and a hand auger was used to collect samples from the base of the soil pit to 281 cm. The near-surface pit samples were collected at 0 to 1 cm, 1 to 5 cm, and 5 to 10 cm. The remaining samples from the soil pit were collected at 10 cm intervals. The augered samples were collected in depth intervals that varied from 6 to 16 cm thick (Table 1). Immediately upon excavation of a depth interval, approximately 20 g of the sample was placed into a pre-weighed scintillation vial that was then sealed with a lid and electrical tape. A corresponding bulk sample was

placed in a plastic bag for grain-size, mineralogic, and water-extraction analyses.

Ground-water composition was determined in 2006 on samples drawn from two shallow wells (GS-15 and GS-18; Figure 1). Well GS-15 is 300 m north of the sediment sample site, and GS-18 is 700 m to the south. The wells were installed in 1982 by Czarnecki (1997) to depths of 7 and 8 m, respectively. Water depths below ground surface at the time of sampling were 2.6 m for GS-15 and 3.2 m at GS-18; these water levels are similar to values reported in 1983–1984 (Czarnecki 1990). Wells were pumped dry, and then within 5 hours were sampled using a peristaltic pump. Water was filtered to < 0.2 µm with a polyethersulfone syringe filter, and the resulting filtrate was collected in HDPE bottles. Cation sample bottles were cleaned in the laboratory with nitric acid and deionized water; anion sample bottles were soaked in deionized water prior to field work. All bottles were rinsed with sample water prior to filling. Cation and trace element samples were acidified with high-purity nitric acid to pH < 2. Anion samples were stored with ice or refrigerated until analyzed.

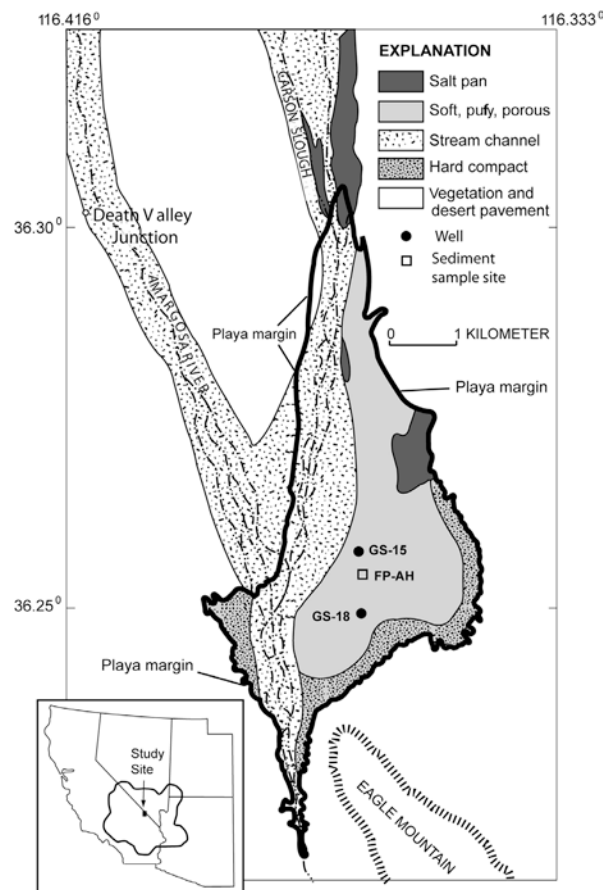


Figure 1—Map of the distribution of surficial materials and locations of well and sediment sample sites within the Franklin Lake playa. Index map depicts the western United States with an outline of the Mojave Desert.

Table 1—Chemical composition of water-extract solutions prepared by reacting 1 gram of dried sediment from Franklin Lake playa with 30 ml of de-ionized water for one hour. (Alkalinity is expressed as CaCO₃; Na, sodium; As, arsenic; Mo, molybdenum; W, tungsten; U, uranium; ---d, duplicate).

Depth (cm)	Alkalinity mg l ⁻¹	Chloride mg l ⁻¹	Sulfate mg l ⁻¹	Na mg l ⁻¹	As mg l ⁻¹	Mo mg l ⁻¹	W mg l ⁻¹	U mg l ⁻¹
0-1	317	1.6	159	252	38	<2	0.6	<0.1
0-1d	357	14.2	171	238	46	<2	1.7	<0.1
0-5	693	3.8	312	506	46	<2	1.1	0.3
5-10	633	0.5	364	494	65	<2	1.8	0.4
10-20	977	162	631	880	260	30	8.4	9.8
20-30	1000	468	607	1050	850	140	14.0	100
20-30d	1047	510	655	1040	890	142	15.4	104
30-40	617	524	426	693	790	134	11.3	57
40-50	510	450	333	636	730	127	9.6	37
50-60	467	420	297	665	700	129	9.4	28
50-60d	470	409	289	669	700	127	9.4	28
60-70	427	402	292	607	750	141	10.2	26
70-80	390	426	311	610	740	144	8.7	24
80-90	347	314	246	497	630	116	7.2	19
90-95	353	372	290	527	690	125	9.1	20
95-101	308	283	236	466	660	121	6.7	13.3
101-124	353	263	229	485	660	121	7.8	15.5
124-137	373	230	200	463	460	112	4.9	12.6
124-137d	360	233	203	432	430	107	4.5	11.4
137-145	232	151	131	316	240	70	5.9	7.5
145-159	161	116	91	213	140	46	6.9	4.2
159-163	139	87	70	168	100	36	9.5	3.1
163-169	150	108	83	173	119	41	13.9	3.4
169-177	151	121	87	218	119	49	15.3	4.1
169-177d	150	128	93	216	125	50	16.1	4.0
177-184	140	89	61	107	68	29	14.2	2.6
184-193	168	181	113	157	152	66	7.7	6.7
184-193d	170	170	109	155	146	65	7.6	6.5
193-203	177	211	134	175	176	79	4.5	7.9
203-217	179	217	139	180	169	80	8.9	8.4
217-228	115	88	58	93	81	33	14.5	3.3
217-228d	104	100	63	92	114	43	24.1	4.6
228-237	125	108	71	107	95	36	7.3	3.7
237-248	129	114	78	113	103	44	11.6	3.8
248-258	136	164	105	136	111	57	11.4	4.4
258-266	201	170	126	177	141	67	7.9	8.0
266-281	149	211	130	161	132	80	7.2	7.2

ANALYSES

Water content, particle size, mineralogy, and the chemical composition of water extracts were determined on the sediment samples. Gravimetric water content was determined by difference between the weight of sediment samples in the scintillation vials on arrival to the laboratory and after drying for 1 week at 50°C and 110°C. After drying

at 110°C the samples were placed in a chamber at 100% humidity at 22°C ± 2 until the weekly weight gain was less than 0.2% (approximately 6 weeks). Particle size was determined on weighed air-dried splits of the bulk samples that were treated to remove organic matter (30% H₂O₂) and carbonate minerals (15% HCl). Samples were sieved to obtain the > 2 mm and < 2 mm size fractions. The > 2 mm size fraction was weighed and reported as gravel. The

< 2 mm size fraction was suspended in water amended with sodium hexametaphosphate and analyzed with a Malvern particle-size laser diffraction analyzer. Bulk mineralogy for all samples was determined by X-ray diffraction (XRD) on the ground < 2 μm fraction. Clay mineralogy was determined by XRD of oriented mounts of the < 2 μm fraction.

Water-soluble constituents of the sediment were determined by shaking 1 gram of dried, homogenized sediment with 30 ml of 18 mega-Ohm water for 1 hour. The samples were then filtered using a 0.2 μm filter and subsequently centrifuged to remove particles larger than 50 nm. Specific conductance and pH were measured on the resulting solution with calibrated meters. The solutions were analyzed in USGS laboratories to determine cation, anion and trace metal concentrations according to the procedures for ion chromatography (IC), inductively coupled plasma atomic emission spectroscopy (ICP-AES), and inductively coupled plasma mass spectrometry (ICP-MS) described in Taggart (2002). Total alkalinity as CaCO_3 was determined by titration of a 10 ml aliquot of the solution with 0.16, or 1.6 N sulfuric acid. Replicate extractions of separate sample aliquots were reproducible generally within 10 relative percent for all analytical methods (Table 1), indicating that the 1 gram sample was representative.

Water samples were analyzed at the time of collection and later at USGS laboratories. Specific conductance, temperature, and pH were determined in the field using calibrated meters; total alkalinity as CaCO_3 also was determined in the field by titration of a 50 ml unfiltered aliquot with 1.6 N sulfuric acid. Cation, anion, and trace metal concentrations were determined by the same procedures used for analysis of water-extract solutions.

Geochemical Modeling

The saturation states of salt minerals in the water extracts and groundwater samples were evaluated with geochemical modeling. PHREEQC v. 2.14.2 (Parkhurst & Appelo 1999) calculated the saturation states using the Pitzer thermodynamic database.

RESULTS

Sediment Characteristics

Sediment at the sample site consists of 137 cm of reddish silt overlying a unit composed mostly of sand and gravel (Figure 2). The upper 20 cm of reddish silt has low field moisture content, is hard, and exhibits a blocky structure. Below this depth, the silty sediment is visibly moist and has a loose, granular texture. Below 137 cm, the pale gray sand

and gravel mixture has greater apparent water content and a very loose structure. The contact of the two units is gradational as indicated by the change in median grain size (Figure 2).

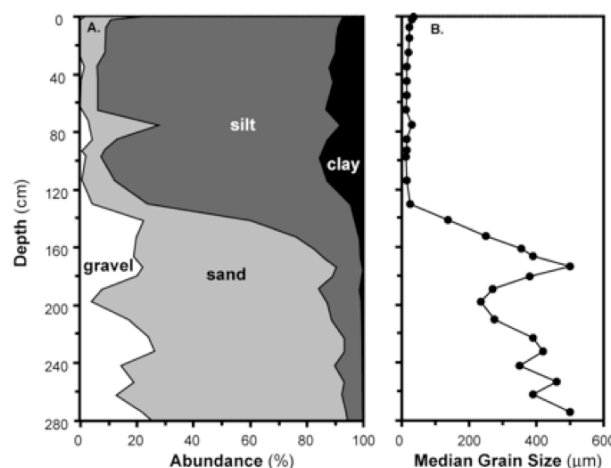


Figure 2—A) Abundance of gravel, sand, silt, and clay, and B) median grain size of Franklin Lake playa sediment samples collected from the ground surface to a depth of 281 cm. Samples were collected continuously in intervals of varied thickness (Table 1). Values are plotted at the mid-points of sampled intervals.

The sediment consists mainly of quartz and calcite. Other minerals common to most samples include plagioclase, analcime, and halite. Muscovite, dolomite, and barite were identified in only a few of the samples. The < 2 μm fraction of the sediment is composed of smectite (aluminum- and magnesium-rich varieties), illite, illite-smectite, and traces of kaolinite. The mineralogy is consistent with detritus eroded from rock and sediment units in the drainage basin (Winograd & Thordarson 1975; Khoury et al. 1982) except for halite, which formed by evaporation at the sample site and as a result of drying in the laboratory.

The water content of the sediment based on weight loss upon heating to 110°C ranged from 4 to 20 weight percent (Figure 3a). This water likely includes ‘free’ water, water adsorbed to mineral surfaces, water within the interlayers of expansive clays, and water of crystallization in some hydrated salts. The amount of water lost varied with depth as a function of particle size and position relative to the ground surface and the water table. The lowest water content was measured in the near-surface samples, with similarly low concentrations reported for the coarse-grained sediment between 120 and 180 cm and 220 to 250 cm depth. The high water content between 10 and 120 cm is consistent with the greater matric potential of fine particles in this depth range. The increased water content in the 180 to 210 cm interval is attributed to the proximity of the saturated zone and the smaller median grain size of this interval relative to adjacent sediment. The high water content of the deepest sample is attributed to underlying

water-saturated sediment. Figure 3b presents the amount of water adsorbed by the dried sample relative to the gravimetric water content. The ratio indicates that sediment shallower than 20 cm was undersaturated with respect to water vapor at the time of sample collection. Sediment from 20 to 50 cm depth has a ratio that is slightly greater than one. Deeper sediment is characterized by increasing ratios with increasing depth, which is interpreted to indicate the presence of greater amounts of *in situ* liquid water as the saturated zone is approached.

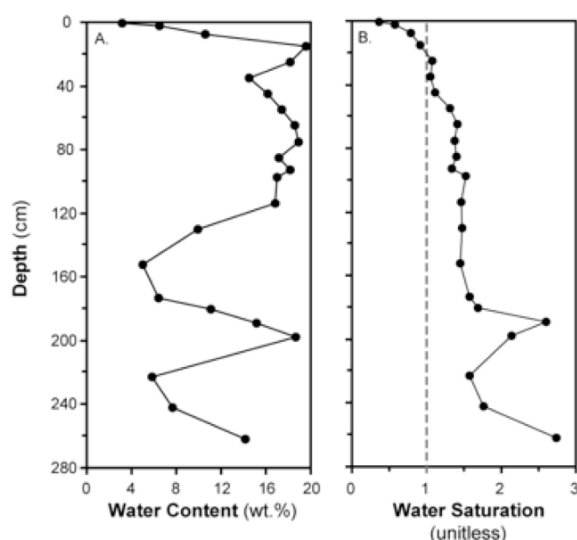


Figure 3—A) Gravimetric water content of Franklin Lake playa sediment samples as determined by weight loss after drying at 110°C. B) The water saturation of the sediment samples as determined by the ratio of the gravimetric water content (110°C) divided by the weight gain of the dried sediment exposed to 100% humidity for 6 weeks. Water content was not determined for all samples analyzed for water-soluble extracts. Samples were collected continuously in intervals of varied thickness. Values are plotted at the mid-points of sampled intervals.

Ground Water Composition

Select compositional parameters measured on water samples from wells GS-15 and GS-18 are presented in Table 2. The ground water is characterized by elevated pH (> 9.4), specific conductance ($> 90 \text{ mS cm}^{-1}$), and total alkalinity ($> 9000 \text{ mg l}^{-1}$ as CaCO_3). Sodium is the dominant cation, with major amounts of chloride, sulfate, and carbonate species present. Calculations with PHREEQC determined that $> 95\%$ of the alkalinity was attributable to carbonate species. Arsenic (As), molybdenum (Mo), tungsten (W), and uranium (U) are commonly considered as trace constituents in ground water but are present in high concentrations beneath Franklin Lake playa. If the ground water was evaporated, the resulting anhydrous residue would be expected to contain from 160 to 200 ppm As, 120 to 190 ppm Mo, 3 to 4 ppm W, and 40 to 55 ppm U. The relatively high content of

dissolved nitrate and absence of dissolved iron and manganese (Table 2) are interpreted to indicate that the water has a high oxidation potential. Evaluation of the saturation state of minerals in this water with PHREEQC determined that all sodium salts are undersaturated. Comparison of our water analyses with data of McKinley et al. (1991) found that major ion concentrations have increased by approximately 50% since 1983.

Table 2—Chemical composition of ground water sampled from wells in the Franklin Lake playa near the sediment sample site.

Parameter	GS-15	GS-18
Specific Conductance (mS cm^{-1})	90.30	99.50
pH	9.49	9.65
Sodium (mg l^{-1})	28000	37000
Potassium (mg l^{-1})	1170.00	1080.00
Calcium (mg l^{-1})	<1.00	<1.00
Magnesium (mg l^{-1})	<1.00	<1.00
Fluoride (mg l^{-1})	26.00	6.00
Chloride (mg l^{-1})	26000	28500
Nitrate (mg l^{-1})	500	1100.00
Sulfate (mg l^{-1})	16800	23500
Alkalinity as CaCO_3 (mg l^{-1})	9360.00	15510
Iron (mg l^{-1})	<0.01	<0.01
Manganese (mg l^{-1})	<0.01	<0.01
Arsenic (mg l^{-1})	15.50	15.80
Boron (mg l^{-1})	84.00	157.00
Molybdenum (mg l^{-1})	14.60	12.10
Uranium (mg l^{-1})	4.30	4.00
Tungsten (mg l^{-1})	0.33	0.30
Total Dissolved Solids (mg l^{-1})	78200	100800

Water Extraction

Concentrations of selected dissolved species in the extract solutions are presented in Table 1. The water-soluble weight fractions of the dried samples based on summing the dissolved species range from 0.009 to 0.10 (Figure 4). Specific conductance of the extract solutions varied from 700 to 4300 $\mu\text{S cm}^{-1}$ and correlated with total salt extracted ($r = 0.98$). Soluble content increases from the ground surface to the 20-30 cm-depth interval and then decreases to approximately 160 cm; below this depth the water-soluble fraction varies in a narrow range. The change in the slope corresponds approximately to the change in grain size (Figures 2, 4). The extract solutions had pH values ranging from 10.5 to 9.5.

Variations in the soluble components of the sediment were assessed by normalizing the element abundance data to the relative amount of salt extracted. The concentration of a

dissolved constituent in the extract solution (Table 1) was normalized to the amount of water used, the dried sediment weight, and the total mass of salt extracted. The result of this calculation estimated the concentration of the selected constituent in an evaporated residue of anhydrous salt.

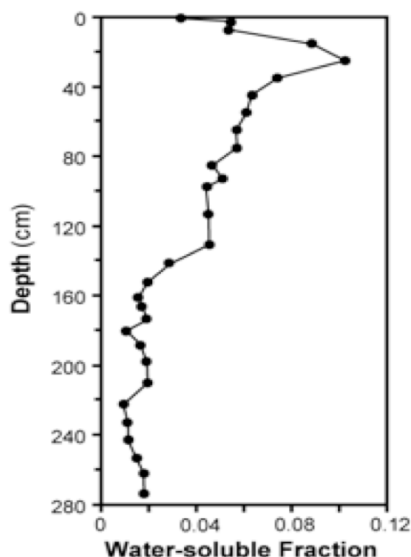


Figure 4—Mass fraction of water-soluble substances extracted from the Franklin Lake playa sediment samples by reaction with distilled water for 1 hour. Mass was calculated as the sum of dissolved major ions. Samples were collected continuously in intervals of varied thickness. Values are plotted at the mid-points of sampled intervals.

The water-extract solutions contained large amounts of sodium, chloride, sulfate, and carbonate species (Table 1). Their abundance on a bulk-sediment basis varied with depth in parallel to the total water-soluble fraction of the sediment (Figure 4; Table 1). In addition, the relative abundances of chloride, sulfate, and total carbonate (as estimated from alkalinity) vary with depth. These abundances are plotted as normalized, equivalent values to facilitate direct comparison (Figure 5). Nitrate was not considered in this evaluation because its fraction of the total anions is relatively low (0.02 to 0.05). Samples shallower than 20 cm are notable for the high abundance of carbonate and low content of chloride relative to the deeper samples. This relation is somewhat reversed from 30 to 100 cm, where chloride exceeds carbonate. Between 100 and 190 cm chloride and carbonate contents are subequal. Below 190 cm, carbonate and chloride vary inversely. The relative abundance of sulfate is greatest in the upper 20 cm; below 20 cm sulfate decreases gradually to the bottom of the sampled interval. The relative abundance of chloride, sulfate, and carbonate in the water extracts deviate from the amounts expected based on groundwater composition. The average equivalent proportion of chloride, sulfate, and

carbonate in ground water sampled from GS-15 and GS-18 are 0.54, 0.29, and 0.17, respectively (Figure 5). Similar proportions of anions were obtained for water samples collected in 1983 (McKinley et al. 1991).

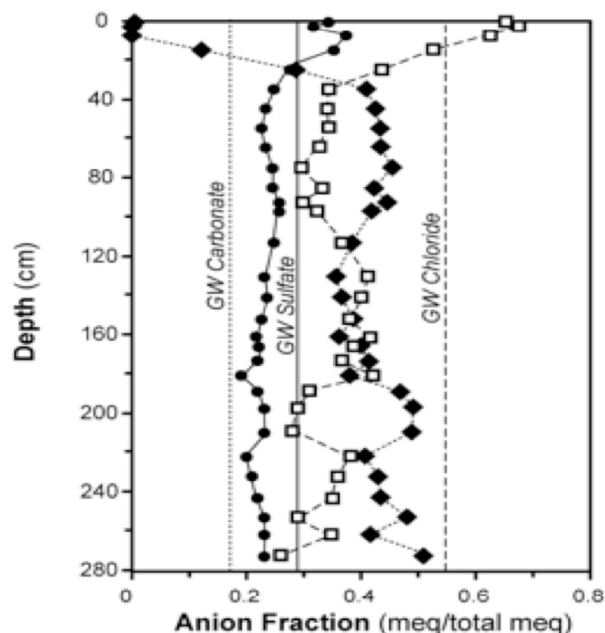


Figure 5—The relative abundance of water-soluble carbonate, sulfate and chloride extracted from the Franklin Lake playa sediment. Values were calculated by dividing the equivalents of each ion by the sum of anion equivalents. (♦ - chloride; ● - sulfate; □ - carbonate; vertical lines indicate proportions of anions in ground water (GW; Table 1)). Samples were collected continuously in intervals of varied thickness. Values are plotted at the mid-points of sampled intervals.

The amounts of anions and sodium extracted (Table 1) were combined with the gravimetric water content (Figure 3) to evaluate the saturation state of soluble salts in the sediment. These calculations were performed using PHREEQC and assuming that all water lost by heating is available as liquid water. Calculations with PHREEQC determined that samples from 0 to 40 cm depth are variably oversaturated with phases such as trona ($\text{Na}_3\text{H}(\text{CO}_3)_2 \cdot 2\text{H}_2\text{O}$), burkeite ($\text{Na}_6\text{CO}_3(\text{SO}_4)_2$), mirabilite ($\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$), glaserite ($\text{NaK}_3(\text{SO}_4)_2$), nahcolite (NaHCO_3), halite (NaCl), and natron ($\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$). Oversaturation predicts that some solid salts are components of the sediment as sampled. Samples between 40 and 177 cm are at saturation or slightly undersaturated with phases such as trona, mirabilite, and nahcolite. Two samples in this interval, 124 to 137 cm and 159 to 163 cm, were oversaturated with trona and burkeite. Below 177 cm, water contained in the sediment was undersaturated with all salts, indicating that the extracted ions were dissolved in residual water in the lowest 1 m of sediment.

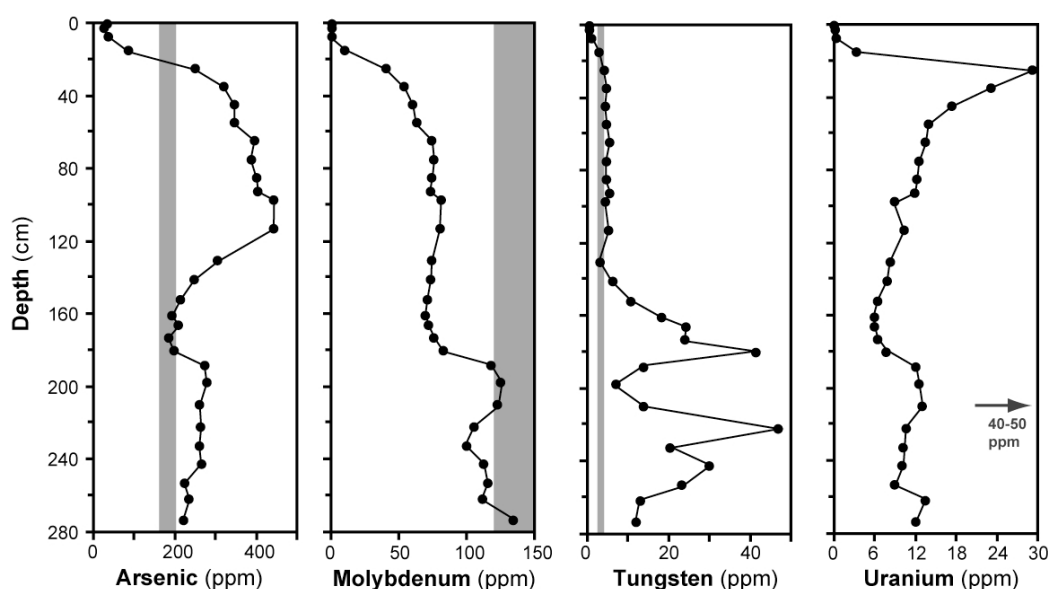


Figure 6—Concentration of water-soluble arsenic, molybdenum, tungsten, and uranium extracted from the Franklin Lake playa sediment expressed as amount in the water-soluble fraction of each sample. Vertical gray bars indicate concentrations expected by evaporation of ground water compositions (Table 1) except for uranium, which is indicated by a numeric range. Samples were collected continuously in intervals of varied thickness. Values are plotted at the mid-points of sampled intervals.

The concentrations of As, Mo, W, and U in the water soluble fraction vary with depth (Table 2; Figure 6). All four elements have relatively low abundances in the shallowest samples, with variations below 20 cm that are unique to each element. Arsenic content increases systematically from the ground surface to a maximum near 110 cm. From 120 to 190 cm, the As concentration decreases through the interval characterized by increasing grain size (Figure 2). Arsenic contents in samples below 190 cm are relatively constant. The upper 180 cm of the Mo profile is similar to that of As, although the concentration decrease between 120 and 190 cm is more subtle. Below 190 cm Mo content increases to approach values expected for evaporated ground water. Tungsten has a concentration profile similar to As and Mo in the upper 130 cm of sediment. Beginning at 140 cm depth, the W concentration is substantially greater than that measured in the shallow sediment, and two maxima of 40 to 50 ppm W are apparent. Uranium concentrations are below detection in the shallowest samples and reach an abrupt maximum of 29 ppm in the 20 to 30 cm sampled interval. Below this depth, uranium content declines systematically to 6 ppm at 160 cm, and then varies between 9 and 14 ppm through the 160 to 280 cm interval.

Chemical analyses detected high contents of silica (186 mg l^{-1}) and iron (12 mg l^{-1}) in a few of the water extract solutions. The concentrations of SiO_2 and Fe are attributed to colloids smaller than 50 nm. Suspended particles were expected given the high pH and Na content

of the extract solutions and clay content of the sediment. Colloids can interfere with resolving dissolved elements from those bound to colloids. Nevertheless, the soluble elements considered in this analysis have sufficiently high concentrations in the extract solutions and chemical characteristics such that colloids are unlikely to account for a significant portion of the measured amounts.

DISCUSSION

The gross distribution of soluble components in the unsaturated zone beneath Franklin Lake playa is generally similar to results of previous studies that examined salt accumulation in evaporative environments where the evaporation front is below the ground surface (Hassan & Ghaibeh 1977; Gowing et al. 2006). These studies demonstrated that the depth of salt deposition is dependent on the position of the water table, grain size of the substrate, ground-water salinity, and evaporation rate. Maximum salt content of the Franklin Lake playa sediment at 20 to 30 cm depth is attributed to the transport of salt upward through the capillary fringe to the evaporation front (Figure 3). The depth of the evaporation front at 20 cm is consistent with the position expected in silty loam overlying a water table at a depth of approximately 2.5 m (Gowing et al. 2006). Salt in the 0 to 20 cm deep samples may reflect accumulation as a previous, higher water table receded, possible diffusion upward subsequent to rainfall or flood events, or accumulation from limited amounts of liquid water that continue to move upward. The latter explanation

is consistent with the observed increase in salt contents above an evaporation front during column experiments (Hassan & Ghaibeh 1977).

The relative abundance of water-soluble anions in the upper 30 cm of sediment changes rapidly with increasing depth (Figure 5). The processes responsible for the increase of chloride and decrease of sulfate and carbonate are uncertain. Ground water in the past may have been compositionally distinct, with lower chloride and greater carbonate contents than modern ground water. The anion composition of this upper sediment may also be modified by atmospheric input, although this seems unlikely given the mass of salt in the underlying ground water. The normalized anion equivalent fractions of recent precipitation in Death Valley, 50 km to the northwest, is 0.34 nitrate, 0.09 chloride, 0.17 sulfate, and 0.4 carbonate (NADP 2008). In contrast, the surface sediment at Franklin Lake playa contains less than 0.05 nitrate, and chloride (Figure 5) is depleted relative to these precipitation values. Loss of nitrate is plausible due to denitrification and other processes (Walvoord et al. 2003), but chloride is expected to be conservative. The low relative chloride content in the unsaturated zone requires either selective removal of chloride or preferential enrichment of sulfate and carbonate. Similar depletion of chloride relative to ground water is recognized in efflorescent salts formed from alkaline water in Spain (Vizcayno et al. 1995). Selective dissolution (Shimajima et al. 1996) of chloride salts may favor elution from the surface sediment during infrequent vertical recharge events or from water condensed when the ground surface is colder than the subsurface (Fritton et al. 1967; Nassar & Horton 1989). Consistent with selective elution of chloride salts, the solubility of sodium chloride is markedly greater than that of sodium sulfate and sodium carbonate phases. Chloride may also be lost by preferential transport in dust emissions from the playa surface, although our observations suggest that chloride-rich salt crusts are hard and resistant to wind erosion. No mechanism for enhanced transport of carbonate and sulfate relative to chloride through the capillary fringe has been recognized, though the inverse behavior has been observed (Szabolcs & Lesztak 1969; Shimajima et al. 1996).

Considering the long history of Franklin Lake playa, the amount of salt in the unsaturated zone is remarkably small, which may further support the downward transport of some salts. Applying the 0.16 cm day^{-1} evaporation rate of Czarnecki (1997) to ground water in GS-18 would result in deposition of $5.8 \text{ g of salt cm}^{-2} \text{ yr}^{-1}$ if the water mass were evaporated to dryness. Considering a 2.8 m column of sediment with an estimated bulk density of 1.8 g cm^{-3} , the measured mass of the soluble fraction of the sediment could be acquired in less than 3 years. Two explanations are

offered to account for the apparent lack of salt. Vertical recharge events may remove soluble phases from the unsaturated zone and transfer them to ground water. Vertical recharge through the sediment is likely considering that water does not pond on the puffy surface following heavy rain events, despite the anticipated deflocculation of expansive clays in the sodium-rich and high pH environment (Szabolcs & Lesztak 1969). An alternative explanation is that soluble constituents accumulate more slowly than the vapor transfer indicated by Czarnecki (1997). This would require the upward vertical movement of water without the transfer and storage of dissolved salts. Either hypothesis might account for the 50% increase in total dissolved solids of the ground water in the 20 years between our analyses and those presented in McKinley et al. (1991).

Relative anion abundances vary within a narrow range in samples below 30 cm. Sulfate declines slightly with increasing depth, whereas the fractions of chloride and carbonate vary inversely. The increased chloride content between 30 and 100 cm is consistent with downward movement of chloride from the upper 20 cm with vertical recharge that was insufficient to reach the water table. Of greater interest is the contrast in relative abundance of anions in the soluble fraction and the underlying ground water. Chloride is lower than expected, whereas sulfate is slightly lower and carbonate is much greater. The compositional contrast could reflect stratification such that ground water at the water table near 2.6 to 3.2 m below ground surface would be different from that intersected by the approximately 7 m deep screened intervals of the sampled wells (Czarnecki 1997). Preferential elution of chloride and lesser amounts of sulfate due to greater solubility may also account for the apparent deficiency of those ions. The concentration profile in Figure 5 could reflect the superposition of sulfate and chloride that accumulated on residual sodium carbonate subsequent to a recent recharge event. Based on weather records presented by Reynolds et al. (this volume), 7.37 cm of rain fell in the area two months prior to collection of the sediment samples.

A third, more speculative explanation for the relative contrasting anion content between the unsaturated zone and subjacent ground water is assimilation of carbon dioxide from pore gases in the unsaturated zones. PHREEQC was used to simulate geochemical reactions as ground water from GS-15 and GS-18 evaporates during rise through the capillary fringe. Simulations reacted ground water with calcite and a pore gas having a partial pressure of CO_2 from atmospheric to 0.01 atmosphere ($\text{pCO}_2 = -3.5$ to -2). This range of pCO_2 is consistent with measured values in the unsaturated zone elsewhere along the Amargosa River

(Walvoord et al. 2005). Evaporation was simulated to proceed to a loss of 95% of contained water, and mirabilite, burkeite, halite, nahcolite and trona were permitted to precipitate. In an environment where CO_2 content is equal air, there is a net loss of CO_2 from the resulting salts and residual brines into the gas phase (Figure 7). This result is consistent with the general similarity of the anion composition of salts formed at the ground surface directly overlying water-saturated sediment with the composition of associated ground water. In contrast, at a pCO_2 of -2, the amount of CO_2 in the salts and residual brines increased by 47%. The amount of added CO_2 is insufficient to account for the carbonate excess. Greater amounts of CO_2 may be provided by higher pCO_2 or reactions not considered in this evaluation.

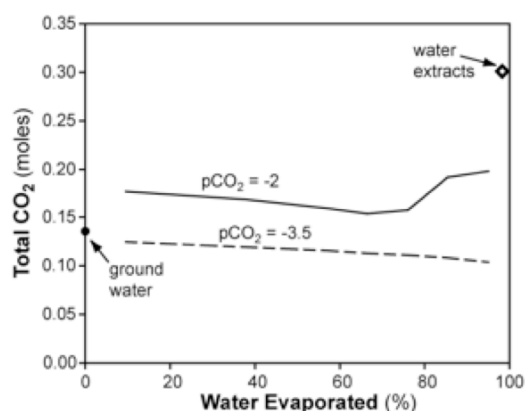


Figure 7—Total moles of carbon dioxide contained in residual water and precipitated salts relative to the amount of water lost during evaporation of one liter of ground water. Results were calculated using PHREEQC assuming partial pressures of carbon dioxide of 10^{-2} and $10^{-3.5}$ and allowing mirabilite, burkeite, halite, nahcolite and trona to precipitate. Although the pCO_2 of -2 increases the total carbonate species, the amount of increase is inadequate to explain the typical water-extractable carbonate content of the sediment.

The amount and composition of salt stored in the unsaturated zone has implications for the evolution of groundwater composition and surface water, if present. Preferential retention of sulfate and carbonate salts relative to chloride in the upper portion of the unsaturated zone could account for the enrichment of chloride in ground water beneath the playa. During an evaluation of the water composition beneath the Franklin Lake playa, Root (2000) simulated the mixing and evaporation of probable recharge waters in the basin and found that the concentration of chloride exceeded the amount accounted for by simple evaporation. An unrecognized chloride-rich recharge component was proposed to account for the discrepancy (Root 2000). Alternatively, selective elution of chloride from the unsaturated zone into the ground water during recharge events also could account for the apparent enrichment.

The concentration profiles for As, Mo, W, and U in the soluble fraction of the sediments (Figure 6) supports differentiation based on chemical characteristics of each element. Unfortunately, available thermodynamic data are insufficient for accurate simulation of adsorption, ion pairing, and solubility of these metals in high-pH, saline solutions. Nonetheless, general aspects of the concentration profiles are understandable from the chemical characteristics of the metal species. At the $\text{pH} > 9$ and oxidizing conditions of the ground water and unsaturated zone, As, Mo, and W are expected to occur dominantly as negatively charged arsenate (HAsO_4^{2-}), molybdate (MoO_4^{2-}), and tungstate (WO_4^{2-}) ions (Nordstrom & Archer 2003; Seiler et al. 2005). The parallel concentration profiles of these elements in the upper 120 cm of the sediment are consistent with their shared characteristic as divalent anions. The lower concentrations in the upper 30 cm may reflect depletion by dissolution and transport in vertical recharge, similar to chloride. Although the profiles are similar relative to the concentrations predicted from ground water (Table 1), As is enriched, Mo is depleted, and W is similar. Below 120 cm, where salt content is attributed to residual brine rather than solid salts, As, Mo, and W have different concentration profile characteristics. Arsenic decreases and Mo increases, as both approach concentrations expected for evaporated ground water. In contrast, W contents are substantially greater than predicted. A simplistic interpretation is that W and Mo favor the aqueous phase, whereas As is accumulated in precipitated salts below the depth of maximum salt accumulation. The As-containing salts must have relatively low solubility to avoid flushing in the recharge water that eluted chloride and possibly Mo. All three element profiles show decreasing concentration upward as the evaporation front is approached. The differential behavior of the three elements may reflect differences in transport by upward-moving liquid water, solubility in vertical recharge, or possibly metal extraction from, or binding to, sediments by reaction with residual brines.

In contrast to the As, Mo, and W anions, U is expected to be present as the uranyl cation (UO_2^{2+}) in ground water and the unsaturated zone beneath Franklin Lake playa. Based on the composition of the salts and water, dissolved uranyl ion is predicted to be strongly complexed by two or three carbonate ions (Langmuir 1978). The accumulation of uranium at 20 cm below the ground surface coincident with the maxima of salt accumulation implies preferential transport to the evaporation front. Nonetheless, the concentration of uranium in the water-soluble extractions is markedly lower than the 40 to 50 ppm predicted for evaporation of ground water. This may indicate a strong preference for retention of uranyl ion in ground water rather than accumulation in forming salts during transport to the

evaporation front, or preferential elution with downward recharge. The latter is unlikely to solely account for the lack of U because the abrupt enrichment of that element is not matched by any other soluble species.

The unsaturated zone beneath Franklin Lake playa contains significant amounts of salt and readily soluble As, Mo, W, and U. Climate change, disturbance of the subsurface, and anthropogenic modification of the water balance could cause redistribution of significant amounts of these constituents. Their distribution in arid and semi-arid basins is poorly documented, and the processes that affect their retention and accumulation are incompletely known. Nevertheless, wind erosion at these types of settings produces saline mineral-rich dust that may contain toxic metals (Reheis 1997). Agricultural development, water diversions, and evaluation of air quality therefore should include storage of salt and trace elements in the subsurface as part of any environmental assessment. Disturbances of these settings can potentially exacerbate wind erosion and dust emission, resulting in wider dispersion of the salts and trace elements.

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Dust Emission at Franklin Lake Playa, Mojave Desert (USA): Response to Meteorological and Hydrologic Changes 2005–2008

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ABSTRACT

Playa type, size, and setting; playa hydrology; and surface-sediment characteristics are important controls on the type and amount of atmospheric dust emitted from playas. Soft, evaporite-rich sediment develops on the surfaces of some Mojave Desert (USA) playas (wet playas), where the water table is shallow (< 4 m). These areas are sources of atmospheric dust because of continuous or episodic replenishment of wind-erodible salts and disruption of the ground surface during salt formation by evaporation of ground water. Dust emission at Franklin Lake playa was monitored between March 2005 and April 2008. The dust record, based on day-time remote digital camera images captured during high wind, and compared with a nearby precipitation record, shows that aridity suppresses dust emission. High frequency of dust generation appears to be associated with relatively wet periods, identified as either heavy precipitation events or sustained regional precipitation over a few months. Several factors may act separately or in combination to account for this relation. Dust emission may respond rapidly to heavy precipitation when the dissolution of hard, wind-resistant evaporite-mineral crusts is followed by the development of soft surfaces with thin, newly formed crusts that are vulnerable to wind erosion and (or) the production of loose aggregates of evaporite minerals that are quickly removed by even moderate winds. Dust loading may also increase when relatively high regional precipitation leads to decreasing depth to the water table, thereby increasing rates of vapor discharge, development of evaporite minerals, and temporary softening of playa surfaces. The seasonality of wind strength was not a major factor in dust-storm frequency at the playa. The lack of major dust emissions related to flood-derived sediment at Franklin Lake playa contrasts with some dry-lake systems elsewhere that may produce large amounts of dust from flood sediments. Flood sediments do not commonly accumulate on the surface of Franklin Lake playa because through-going drainage prevents frequent inundation and deposition of widespread flood sediment.

INTRODUCTION

Dry lakes have been recognized as important sources of atmospheric dust (e.g., Goudie 1978, 1983; Young & Evans 1986; Shaw & Thomas 1989; Gill 1996; Prospero et al. 2002; Goudie & Middleton 2006; Washington et al. 2006). Concerns about the effects of dust on global and regional climate, air quality, human health, and ecosystems currently generate interest in the conditions responsible for dust emission from them (e.g., Gill 1996; Reheis 1997; Prospero et al. 2002; Bryant 2003; Washington et al. 2003; Goudie & Middleton 2006; Bryant et al. 2007; Reynolds et al. 2007). Bryant et al. (2007) summarized general associations between dust from dry-lakes and hydrologic change at the scale of basins and noted the need to document processes of dust emission and their underlying climatic controls at single dust sources, because large variability in these emissions remain difficult to explain (e.g., Mahowald et al. 2003; Luo et al. 2004; Zender & Kwon 2005).

At the outset, it is important to consider the types and sizes of dry lakes or playas (Shaw & Thomas 1989) along with their climatic and geologic settings, as factors that influence dust emission from them. Our study focuses on Franklin Lake playa (FLP) in the central Mojave Desert, USA (Figure 1), a small (about 14 km² in area), wet playa that intermittently produces dust plumes. Flood events on this playa are rare and limited to through-going channels and parts of the playa. In its small size, this playa differs from much larger ephemeral lakes elsewhere that can be globally significant dust sources (see Prospero et al. 2002; Goudie & Middleton 2006; Bryant 2003; Bryant et al. 2007). Nevertheless, the capacity to produce evaporite mineral dust at FLP (Reynolds et al. 2007) bears resemblance to significant dust producers, such as Owens (dry) Lake (California), parts of the desiccated Aral Sea, and many other dry saline lakes (Gill 1996).

Descriptions of playas, and the distinction between a wet and dry playa, are provided in several articles (e.g., Shaw & Thomas 1989; Smoot & Lowenstein 1991; Rosen 1994; Gill 1996). Wet playas are characterized by shallow depth to the saturated zone, typically less than about 4 m, thereby allowing vapor discharge through the surface. Evaporation of high-TDS ground water in wet-playas produces

saline minerals at the surface and within sediment between the surface and ground water table (the capillary fringe zone). Wet playas may thus be closely related to saline lakes, representing conditions that span alternating states of lake expansion and drying to long-term desiccation. Surfaces of wet playas are commonly dynamic, changing over very short time spans (on the order of weeks to months) between soft and hard, having relatively thick (approximately > 5 mm) crusts. When soft and dry in the upper few cm, wet-playa surfaces may be vulnerable to wind erosion and dust emission (Saint-Amand et al. 1986; Gill & Gillette 1991; Cahill et al. 1996; Gill et al. 2002). In contrast, dry playas are characterized by greater depths to the saturated zone (> 4 m depth) and lack of evaporation of ground water at the surface, all of which lead to hard, stable surfaces of clastic sediment. Typically, dry playas do not produce much dust unless disturbed.

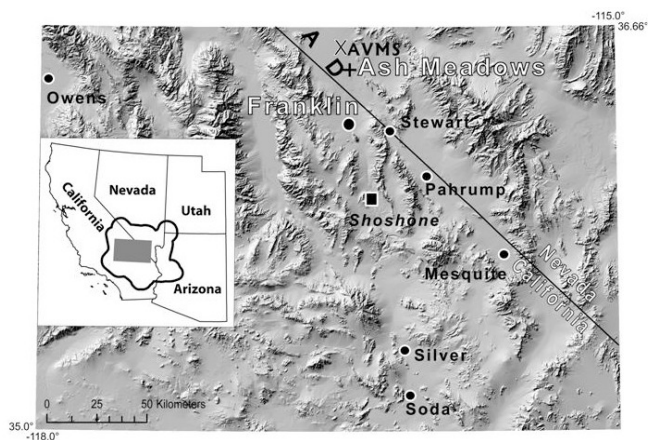


Figure 1—Location map of part of the Mojave Desert, which is indicated by the heavy line in the inset map. The map shows playas (filled circles), town (filled square), Amargosa Valley meteorological station (AVMS, denoted by X), and Ash Meadows (+). AD denote Amargosa Desert.

Many studies have characterized natural conditions of wind erosion at playas with respect to crust type, crust thickness, water content, and wind shear stress. In one set of approaches, wind erosion of natural surfaces is measured using wind tunnels or eolian sediment collectors with monitored meteorological conditions (e.g., Gillette et al. 1980, 1982, 2001; Reid et al. 1994; Cahill et al. 1996). Wind-tunnels and penetrometer tests have also been applied to samples collected in the field or prepared in the laboratory to simulate natural conditions, including varying concentrations of different salts (Nickling & Ecclestone 1981; Nickling 1984; Rice et al. 1996; Argaman et al. 2006).

Despite detailed understanding about eolian particle movement at playa surfaces, there are still gaps in understanding the many factors that promote or retard dust emission. This paper builds on previous studies, especially at nearby Owens (dry) Lake (Figure 1; Saint-Amand et al. 1986; Reid et al. 1994; Cahill et al. 1996; Gillette et al. 2001; Gill et al. 2002) and at FLP (Czarnecki 1990, 1997), to address some of the conditions that influence dust emission from FLP and their underlying causes.

Earlier work indicated that, at any one time, FLP supports many different types of surfaces varying greatly in mineral-crust properties (Czarnecki 1997; Reynolds et al. 2007). Moreover, some areas of the playa change rapidly in these properties, at times having high dust-emission potential and at other times low potential. Observations from our work (Reynolds et al. 2007) suggested important hydrologic influences on dust emission, leading to a hypothesis that relatively wet conditions, caused by high local or regional rainfall, promoted dust emission and that long-term dry conditions impeded emission. To test this hypothesis, we chose to examine the possible influences of precipitation and ground-water-depth levels on the frequency of dust emission. Precipitation and perhaps local flooding of the playa surface might enhance dust emissivity by providing new dust sources, such as clastic flood-sediment, or by temporarily removing saline-mineral crusts, thereby rendering the surface vulnerable to wind erosion before or as mineral crusts begin to re-form. A higher potentiometric surface might additionally promote development of wind-erodible saline-mineral fluff. We also considered variations in seasonal wind strength and temperature as factors that might influence dust emission (cf. Saint-Amand et al. 1986; Reid et al. 1994; Cahill et al. 1996).

SETTING

Franklin Lake playa is centered at 36.252° N, 116.375° W in the Mojave Desert of southeastern California (Figure 1). The playa covers 14.2 km² at about 610 m above mean sea level (Figure 2). The Mojave Desert receives most of its precipitation (50-125 mm annually) from winter frontal storms from the Pacific Ocean (Hastings & Turner 1965). Nevertheless, the frequency, seasonality, and amount of precipitation in the low-elevation parts of the Mojave Desert may vary considerably partly related to ENSO (El Nino-Southern Oscillation) states, monsoon strength, as well as tracks of winter frontal and tropical low-pressure systems.

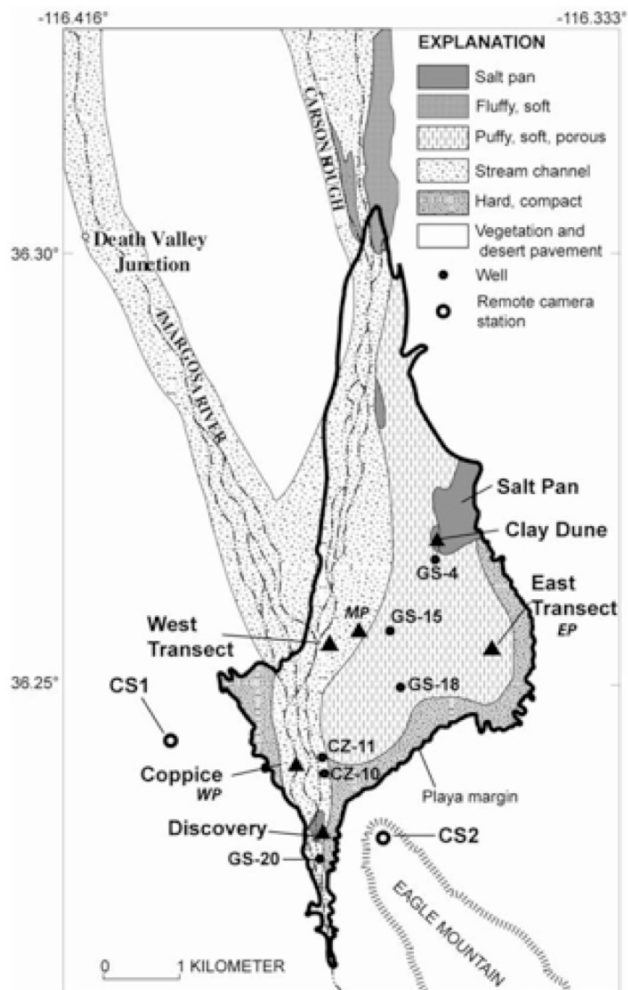


Figure 2—Distribution of surfaces at Franklin Lake playa, locations of camera sites (CS1 and CS2) and wells, and locations discussed in the text. Modified from figures 3 and 4 in Czarnecki (1997). Triangles mark the locations of study sites for erosion and crustal properties discussed in Reynolds et al. (2007): WP, west-playa at Coppice site; MP, middle-playa; EP, east-playa at East Transect site.

At FLP, as at other wet playas, the evaporative loss of large quantities of water may produce “fluffy” sediment composed of loose aggregates of evaporite minerals with a high pore-space volume (Czarnecki 1997; Reynolds et al. 2007). Some surfaces consist of more compact, but still friable, “puffy” sediment (Czarnecki 1997), having fewer evaporite minerals as a result of lower rates of evaporation and (or) lower salinity of evaporating ground water. On hot days at FLP, evaporite minerals crystallize in the capillary fringe zone within minutes of exposure of wet sediment to air. Thick saline-mineral crusts form periodically at FLP within areas that produce fluffy and puffy surfaces at other times. Common evaporite minerals at FLP include halite (NaCl), trona ($\text{Na}_3\text{H}(\text{CO}_3)_2 \cdot 2\text{H}_2\text{O}$), thenardite (Na_2SO_4), and burkeite ($\text{Na}_6\text{CO}_3(\text{SO}_4)_2$) (Breit et al., this volume); mirabilite ($\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$) is probably ephemerally present. These minerals are derived primarily from ground-

water solutes originating in ground water discharged into the basin and interaction of ground water with sediments in the valley-fill aquifer (Winograd & Thordarson 1975; Breit et al., this volume). Following heavy rain, local dissolution of evaporite minerals may also transfer solutes to the valley-fill aquifer. Reynolds et al. (2007) described surfaces at FLP and their temporal changes over two years (May 2003–May 2005) with respect to types of mineral crusts and their rheological properties that describe varying degrees of hardness.

Ground water flows southward from the Amargosa Desert to FLP (Figure 1), as described by the shallow ground-water potentiometric surface in the valley-fill aquifer (plate 1 in Winograd & Thordarson 1975). This flow is focused along the ephemeral Amargosa River and Carson Slough channels, thereby delivering solutes from throughout the valley to FLP. Ground-water salinities are relatively low in the confined flow of the valley-fill aquifer in the northern part of FLP, with TDS values of about 1000 to 5000 mg l^{-1} (Walker & Eakin 1963). Ground water from areas closer to the Amargosa River and Carson Slough, which provide most surface-water flow to FLP, have TDS levels ranging from 6000–20000 mg l^{-1} . In contrast, ground water samples from 11 sampling sites on the playa reveal enormous variations in salinity (McKinley et al. 1991), with highest salinities (TDS reaching about 70000–80000 mg l^{-1}) beneath parts of the playa to the east of the channels of the Amargosa River and Carson Slough (Figure 2). The elevated TDS in ground water at FLP reflects ground-water evaporation, with highest salinities produced in the ground water having relatively long residence times (Czarnecki, personal comm. 2006). Breit et al. (this volume) describe water-extractable solutes in the upper 2.8 m of sediment at central FLP, including depth concentrations of the trace elements, arsenic, molybdenum, tungsten, and uranium.

Unlike several other several playas (e.g., Silver Lake; Stewart Lake) in the region, FLP does not become a lake during times of heavy precipitation and runoff, although bank overflow may result in short-lived sheet flows. For example, FLP remained free of standing water during periods of heavy precipitation (238 mm near Pahrump Playa; <http://www.wrcc.dri.edu/>) between September 2004 and March 2005, as revealed by monitoring using MODIS and Landsat satellite images.

Dust emission from FLP has been documented previously (Czarnecki 1997) and is reported by many other observers, including nearby residents. The parts of FLP with fluffy sediments frequently emit dust under moderate winds; anecdotal information and infrequent on-site observations suggested that puffy, thinly crusted sediments are susceptible to dust emission in winds higher than about 22 m per second.

METHODS

The frequency of dust emission was determined by examining images taken by remote digital cameras triggered by wind speed at the camera site. The camera system is based on one described by Tigges et al. (2001). One camera operated between 10 March 2005 and 04 November 2006 at a height of 4 m on an abandoned telegraph pole about 200 m west of the western margin of FLP at an elevation of 610 m AMSL. Two cameras operated at a higher elevation (695 m AMSL) on a ridge near the south end of the playa between 05 November 2006 and 07 April 2008. The images acquired from the ridge cameras provided increased areal coverage of the playa, enhancing the ability to locate dust-source areas. The cameras began to acquire images when wind speed, which was sampled every 2 seconds, exceeded 4.9 m s^{-1} as a 10 minute average. Images were acquired every 10 minutes at and above the triggering wind speed.

“Dust days” were identified by examining > 14400 images downloaded from the remote cameras. A dust day was tabulated when considered significant and sustained on the basis of a series of images that showed multiple dust emissions. Cyclonic dust devils or other minor, shorter-lived events were not counted. The number of dust days reported here represents an undercount of dust emissions, primarily because nighttime dust events could not be detected. Nighttime dust emission is documented in another part of the Mojave Desert where sensors, which detect particle movement caused by wind, reveal nearly equal frequency of day and nighttime dust emission (F. Urban, written comm. 2008). Another source of undercounting is related to location of the cameras on the ridge. It is likely that this location was to some unknown degree sheltered from southerly winds and thereby that some dust plumes went unrecorded. Despite these uncertainties, the dust-day counts provide a measure of the frequency of dust emission from FLP that can be related to changing environmental conditions at FLP.

Precipitation, wind, and temperature records were obtained from the meteorological station at Amargosa Valley, Nevada (Amargosa Valley Meteorological Station-AVMS; http://www.wrcc.dri.edu/cgi-in/cemp_stations.pl?stn=amar&prod=5). This station is located at 36.5692 deg. N, 116.4589 deg. W and at 740 m elevation, 27 km north of FLP. We also obtained a partial record of wind at and near FLP (July 2005 to April 2008), using a cup anemometer at each camera station (used to trigger the cameras) and a cup anemometer at West Transect site on the playa (Figure 2).

Two separate sensor stations (Figure 2; see also Reynolds et al. 2007) were placed on the playa floor for the purpose of measuring wind speed and direction, as well as soil-

moisture content using dielectric sensors placed at depths of 10, 20, and 30 cm. Hourly records began in May 2005 at Discovery site and in July 2005 at the West Transect site. Gaps in the data records occurred during March 2006 and from mid-April to mid-May 2006. The readings are recorded as a raw voltage output and have not been calibrated for the soil type to produce a true volumetric measure; however, the raw voltage readings produce a time-series record of infiltration and thus precipitation events.

Over an 18 month period from November 2006 through April 2008, we monitored ground-water depth from thirteen pre-established piezometers and wells using a steel tape, with 7-10 total measurements at each site.

RESULTS

Imaging

Over the 37 month period of record, camera images detected 71 days of dust emission. The distribution of these dust days over the sample period is uneven (Figure 3), with 33 dust days recorded within a seven-month period of the autumn of 2005 and the winter-spring of 2005/2006 and 18 more during a six-month period of autumn 2007 into spring 2008. In contrast, the period of autumn 2006 through winter-spring 2007 (spanning nearly nine months) was marked by a total of only four observed dust days. Eight dust days were noted during March 2005, the first month of camera operation, but this dusty period was followed by only one detected dust day (in July 2005) until November 2005.

Precipitation at Amargosa Valley and Soil Moisture at Franklin Lake Playa

Precipitation at the Amargosa Valley meteorological station (AVMS) was highly variable over the period of record (Figure 3). Within the 48 month period, 13 months had more than 1 cm rainfall, and 24 months had less than 0.2 mm, of which 14 had no rain. The most prolonged wet spell was from October 2004 through February 2005, with rainfall more than 2 cm per month for five consecutive months that culminated in 6.73 cm of rain during February 2005. July through September 2005 brought four consecutive months of rainfall between 0.94 and 2.45 cm per month. Another four consecutive months of rain occurred between January and April 2006, with highest amount during March (2.54 cm). A 15 month interval from May 2006 through August 2007 marked the driest period of this record. This dry spell was broken by 7.37 cm of rain during September 2007, the highest monthly amount of the record and resulting in flooding of the Amargosa River through FLP.

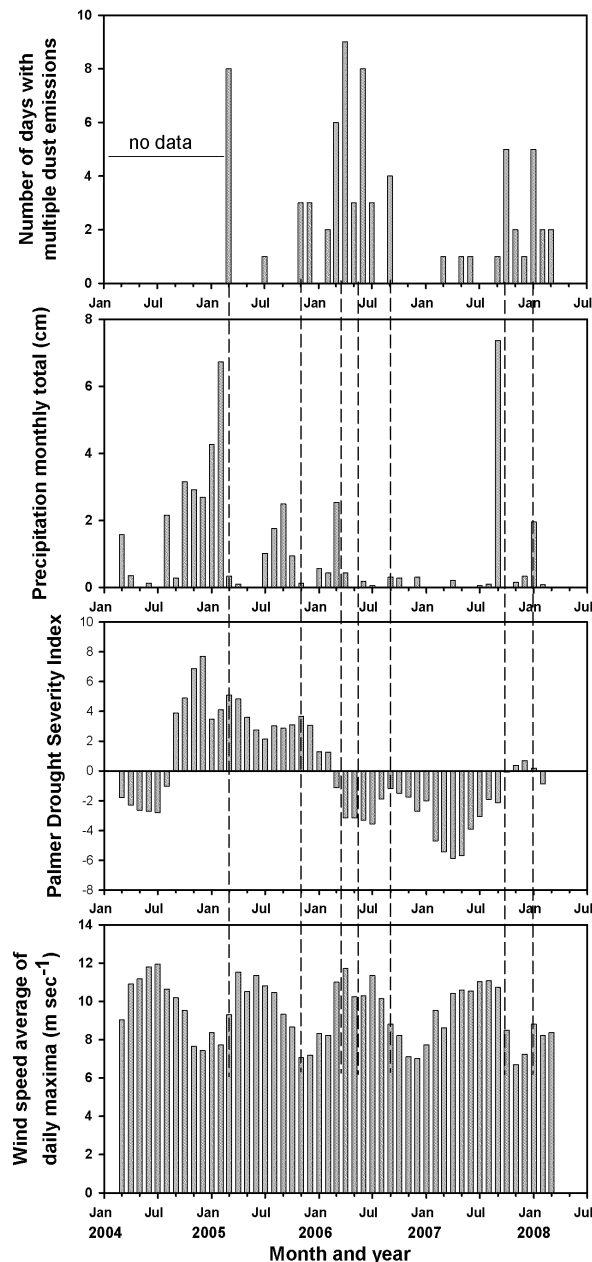


Figure 3—Plots of monthly dust days at Franklin Lake playa from examination of digital images taken by automatically triggered cameras beginning March 2005 (described in text); meteorological data (precipitation, wind) from the Amargosa Valley meteorological station (AVMS); and a modified Palmer drought index (unitless values) for the region of southern Nevada (26-04). Vertical dashed lines indicate most of the months during which a high number of dust days were detected.

Most large rainfall events recorded at AVMS are expressed by sharp changes in soil moisture at FLP. For these associated changes, we conclude that rainfall also occurred at FLP. This association is observed for all rain events > 2 mm at AVMS. These comparisons and soil moisture data at FLP indicates that rainfall at Amargosa Valley is a reliable proxy for the major rain events at FLP. As expected, some smaller rain events at Amargosa Valley are

not expressed in soil moisture at FLP, highlighting the spatial variability of some smaller rain events.

Periods of sustained rainfall or drought are reflected in the Palmer Drought Index for southern Nevada (PDI; Nevada region 4; <ftp://ftp.cpc.ncep.noaa.gov/hdocs/temp2/>), thereby providing a general record of moisture for the study site. The PDI is a hydrological drought index used to assess the severity of dry or wet spells on the basis of the principles of a balance between moisture supply and demand (see Palmer 1965; PDI values < -1 reflect drought, < -3 , severe drought). Over the period of record, the study region alternated among an initially dry period to an 18 month-long wet period, a return to a 19 month-long drought, and most recently an abrupt positive shift to near-zero PDI values since the heavy rainfall of September 2007 (Figure 3).

Because soil moisture measurements likely reflect both water content and soil conductivity influenced by solid or dissolved salts, it is difficult to assess directly the effect of precipitation on water content in near-surface sediments. Nevertheless, the very high precipitation during September 2007 apparently led to long-term wetting of the uppermost capillary fringe zone. The soil moisture reading doubled and remained at this relatively high level for seven months (through April 2008) when data were last collected. This long-term condition coincides with a shift in PDI from negative to slightly positive.

Comparison among the number of dust days, precipitation, and PDI shows several long-term (multi-month) associations. For example, the very dry interval between August 2006 and late September 2007 was a period of only four dust days (Figure 3). The highest frequency of dust days (November 2005–July 2006) occurred during and immediately following frequent rainfall (precipitation during nine of 10 months between July 2005 and April 2006). Within this interval of frequent dust emission, PDI values shifted from relatively moist to dry conditions across February–March 2006. Dust-emission frequency additionally corresponds with positive or neutral PDI (after September 2007). The results further illustrate some apparent short-term responses of dust emission to heavy precipitation. Frequent emission in March 2005 followed heavy rainfall in February, as well as several preceding months having rain. Dusty conditions in October 2007 followed exceptionally high precipitation in late September, and similarly, dust emission in January 2008 followed heavy rain just a few days earlier.

Wind

Wind strength in the area is seasonal with uniformly weakest winds during November and December; relatively strong winds typically occur from March through September (Figure 3). To assess regional winds during the 71 observed dust days at FLP, we examined wind conditions from AVMS data between March 2005 and April 2008 (the period of imaging record). For these 71 days (4.6% of the record), the maximum winds ranged 6.4–22.3 m s^{-1} , and averaged 12.8 m s^{-1} (standard deviation, 3.1 m s^{-1}). By contrast, 264 days (17.1% of the record) had maximum winds in excess of 12.8 m s^{-1} . At AVMS, daily maximum winds ranged 2.9–23.3 m s^{-1} , and the average of the maximum wind each month was 16.8 m s^{-1} (standard deviation, 2.2 m s^{-1}). Moreover, the 12 month period with few dust days had six months during which the average daily maximum wind speed exceeded 10.0 m s^{-1} at AVMS. At FLP daily maximum winds during the 71 dust days ranged 10.5–26.6 m s^{-1} , averaging 17.9 m s^{-1} (standard deviation, 3.9 m s^{-1}).

Depth to Ground Water

The depth to ground water at FLP, and hence the thickness of the capillary fringe zone, is temporally variable in places and seemingly invariant in others. Water levels that were measured in piezometers by Czarnecki (1990) during a 16–24 month period (1983–1985) showed very little variation for wells in highly transmissive sediments and large variation, sometimes gradual and sometimes abrupt, for wells in less permeable sediments. Our results between November 2006 (or February 2007 for some wells) and April 2008 show a range of behavior that includes rises (to nearly 40 cm) and declines (by as much as ~80 cm) in water levels in most wells, and little change in other wells. These measurements spanned the driest interval until the heavy precipitation of September 2007. Several wells (GS-15, CZ10, CZ11, GS-4, GS-18) had water-level declines during the dry spell that might be related to regional aridity indicated by PDI values (Figure 4). Most wells (11 of 13) showed increase in water-table elevations at some time after September 2007, and seven of 13 wells had increases in the first measurement (in November) made after September 2007.

Air Temperature

As with other North American desert settings, seasonal air temperature ranges greatly. Monthly average temperatures for July exceed 30°C, whereas winter-month averages typically ranged from 5 to 8°C. Temperature might influence dust emission in several ways. Firstly, very high summer temperatures, combined with sustained low

humidity, would stabilize salt crusts to suppress dust generation (see Saint-Amand et al. 1986; Reid et al. 1994). Secondly, daytime temperatures of autumn and spring might promote evaporation and evapotranspiration, where salt grass is common, to produce evaporite minerals at and near the surface. Thirdly, temperature might control the presence and absence of mirabilite ($\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$), the stability of which is favored at relatively low temperature (at higher temperatures thenardite is favored). Mirabilite commonly forms acicular crystals that protrude slightly above the surface, preferentially exposing it to wind. We found no correspondence between temperature and dust emission on a monthly basis, except, perhaps, for the absence of detected dust days during August.

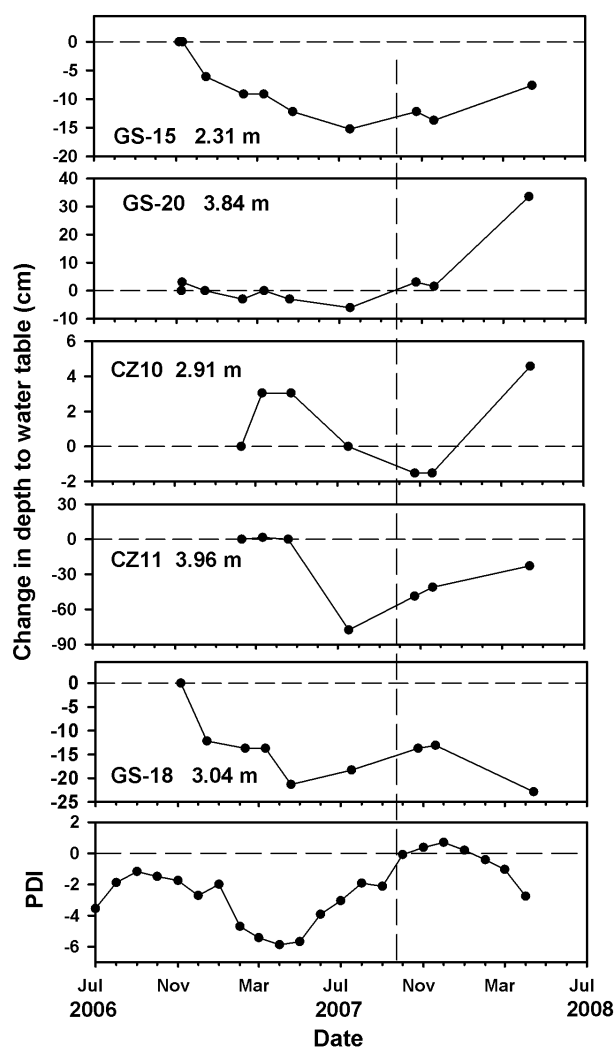


Figure 4—Plots showing changes in depth to the top of the water table beneath Franklin Lake playa over time for wells shown in Figure 2. Total well depth is given in meters after the well-site designation. Positive values indicate rises in the elevation of the water table, relative to the initial measurement in this study; negative values indicate drops in the elevation of the water table. Bottom plot shows monthly Palmer drought index (PDI; unitless values) from southern Nevada region 26-04. Vertical dashed line marks the time of the heavy rainfall during September 2007.

DISCUSSION

No single factor or condition can explain the pattern of dust emission during the study. Investigations at similar settings have demonstrated that, in addition to wind strength, properties of mineral crusts strongly control wind-erosion vulnerability of surfaces at wet playas. In the following discussion, we first consider the role of wind in observed dust emission. We do not describe here crustal properties (crustal hardness, thickness, and mineralogy; and surface moisture) but instead look beyond such factors to the temporal relations among dust emission and regional moisture balance (precipitation, ground-water levels) for clues to underlying controls on surface conditions and dust emission.

Observations at FLP and AVMS imply that wind is not a dominant control on observed dust days. The study area commonly has winds in excess of those required for eolian activity; however, many of the windiest days were not accompanied by dust emission, and emission is sometimes observed on days of only moderate wind. Most days (77%) in the area between March 2005 and April 2008 had sufficient maximum winds ($> 6.7 \text{ m s}^{-1}$) to generate aeolian activity, but significant dust emission was observed on only about 5% of the days during imaging. Assuming that the camera system at FLP detected about half of the dust events, estimated dust emission at FLP still falls far short of the frequency and amounts expected if the surface were vulnerable to wind erosion much of the time. Nevertheless, plots of the number of dust days against monthly means for daily maximum wind speed and the average monthly wind speeds appear to indicate a very weak positive correlation between monthly dust days and monthly averages of daily maximum wind speed (Figure 5).

The results reveal an association between moisture and dust emission. For this general association, however, no single moisture condition can account for the observed wind-erosion history. At one end of the spectrum of conditions, long-term and severe aridity apparently created surface conditions that suppressed dust emission. In this respect, an important feature of the dust-emission record at FLP is the paucity of dust days from late September 2006 to late September 2007 within the very dry interval between May 2006 and late September 2007 (Figure 3). At the other end of the spectrum, periods of dust emission occurred during and as long as several months after prolonged intervals of precipitation. For example, the period having the highest frequency of dust days (November 2005 to September 2006) overlapped and immediately trailed an 18 month

interval of positive PDI (Figure 3). Infrequently, dust emission closely followed high precipitation events, sometimes within a few days or weeks.

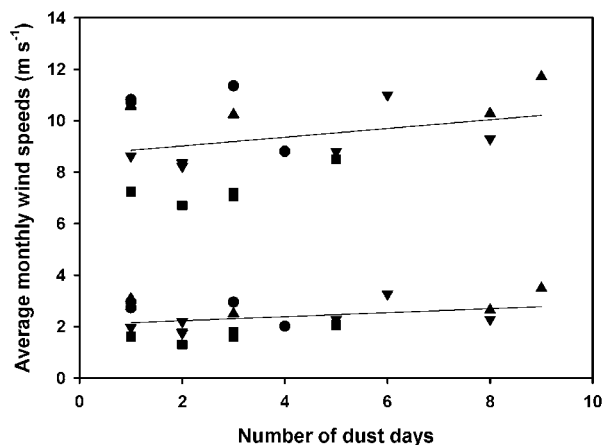


Figure 5—Plots of number of dust days against monthly wind speeds (upper plot, average of the daily maximum wind speed; lower plot, average monthly wind speed). Inverted triangles denote data for 2005; triangles for 2006; circles for 2007; squares for 2008. The regression line in the upper plot gives $r^2 = 0.08$ and in the lower plot gives $r^2 = 0.11$.

The infrequent rapid response of dust emission to precipitation may result from at least two conditions. Firstly, rainfall directly onto playa surfaces may initiate the development of soft surfaces vulnerable to wind erosion and (or) the production of loose aggregates of evaporite minerals that are easily entrained in wind. We surmise that heavy rainfall can initiate the following chain of events: (a) dissolution of saline-mineral crust, (b) movement of salts into the capillary fringe zone, (c) evaporation, and (d) rapid concentration of salts at and near the surface that are vulnerable to wind entrainment. Direct, on-site observations of the first and last links in this chain (Reynolds et al. 2007) lead us to conclude that heavy rainfall can sometimes promote dust emission shortly afterward by these mechanisms. This mode of dust generation would require sufficient rainfall to remove pre-existing evaporite-mineral crusts that previously resisted wind erosion.

Secondly, flooding might introduce fresh, fine-grained sediment into the playa area. Dust derived from flood sediments in dry lakes has been documented elsewhere. As examples, Bryant (2003) and Bryant et al. (2007) demonstrated that individual inundation events had both a rapid and lingering effect on dust emissions. Inundation suppressed dust emission but afterward had the effect of promoting dust plumes through emission of easily deflated flood sediment.

During the study period, FLP was not inundated, and, thus, the situation at this playa differs from those at large dry lakes in southern Africa that undergo occasional inundation (Bryant 2003; Bryant et al. 2007). Any flood sediment at FLP, therefore, would have been confined to channels or areas of brief overland flow that are primarily associated with the Amargosa River in the western and southern parts of the playa (Figure 2). Dust seen in the digital images can help determine the importance of flood-sediment vis-à-vis evaporite-mineral sources on the basis of dust-emission locations, whether from fluvial channels or from commonly salt-covered areas. In addition, the color of the dust would indicate whether it is derived from evaporite minerals, in which case the dust would be white, or from clastic sediment, in which case the dust would have a distinct brown or tan color by contrast. For these reasons, we examined images of dust emissions in March 2005 and October 2007 that followed heavy rainfall. In some of the March 2005 images, evaporite-mineral dust was observed likely emanating from the northeastern playa-Carson Slough area. Other March 2005 images appear to show dust composed of both evaporite and clastic minerals, but the locations of their sources could not be discerned. The October 2007 images reveal dominantly white dust, consistent with airborne evaporite minerals (Figure 6). If clastic mineral dust from channels is present, it is obscured by widespread, dense evaporite-mineral dust. Flood-derived sediment is likely a part of the dust load from FLP, but we did not see clear evidence for it during this study.



Figure 6—Digital image of Franklin Lake playa looking north from camera site 2 (CS2, Figure 2) showing widespread evaporite mineral dust. Image was taken on October 5, 2007, 13 days after heavy precipitation on September 21–22. On October 5, winds on the playa averaged 14.3 m s^{-1} , with a maximum of 20.6 m s^{-1} .

We lack evidence to document the fundamental controls on dust emission that occurred during and after prolonged intervals of precipitation, such as whether rising water-table depths increased wind-erosion by softening surfaces or

increasing rates of vapor discharge. Water-well measurements indicate that heavy rainfall in and near the playa locally raised the potentiometric surface, perhaps caused by increased recharge and ground-water flow. The capillary fringe zone showed greatest thinning (relatively high water tables) in wells following the heavy rainfall of September 2007, illustrating that ground water under some parts of the playa can respond quickly (on the order of a few months) to heavy regional precipitation (Figure 4).

An indirect way to estimate possible effects of long-term moisture on dust emission is by comparing the monthly number of observed dust days to monthly PDI. For this exercise, we examined values for the same months and also with PDI shifted forward in time by one to seven months. These shifts were intended to account for time lags between soil moisture (PDI) and potential responses in ground water and then playa-surface conditions. Comparing the results for the same months, 25 dust days occurred during times of positive PDI, but most dust days (46, of 71 dust days observed during this study) occurred during months of positive PDI when it was shifted ahead by five and six months (Figure 7).

Despite lack of detailed and specific documentation about how the timing and amount of precipitation affect playa surfaces, higher frequency of dust emission appears to be related to relatively moist conditions over periods of months. This association may be related to higher moisture content in the capillary fringe zone leading to higher rates of vapor discharge and perhaps development of isolated evaporite minerals near the surface, all of which in combination may expand and soften playa surfaces. As evaporite-mineral crystals form, their mass physically displaces rock-derived clastic minerals, expanding the volume of sediment. Retention of water from direct precipitation and decreasing depth to the saturated zone are two factors that plausibly contribute to: (1) the development of soft surfaces, (2) the ephemeral development of very thin mineral crusts as well as the occasional production of loosely aggregated salt minerals at the surface, and (3) consequent dust emission with winds of varying strengths.

Our observations that changing surfaces at FLP correspond to varying hydrological conditions (Reynolds et al. 2007) are similar to observations made over many decades at wet playas and drying saline lakes in the Mojave Desert and elsewhere (e.g., Thompson 1929; Stone 1956; Neal 1965, 1972; Neal & Motts 1967; Saint-Amand et al. 1986). Recent dust emission at FLP, however, has apparently differed from the timing of dust events at Owens (dry) Lake that exhibited seasonality during the 1980s and 1990s (e.g., Saint-Amand et al. 1986; Reid et al. 1994; Cahill et al. 1996; Gillette et al. 2001; Gill et al. 2002). At Owens (dry)

Lake, efflorescent salt crusts vulnerable to wind erosion developed during late winter and spring following wintertime conditions of relatively high precipitation and low temperature. Hard, stable crust resistant to breakup by sand saltation dominated during late spring and summer under conditions of low humidity and high temperatures. This general cycle of saline crustal change can be discerned at FLP, but conditions for dust emission were possible nearly every month because rainfall and high wind have occurred over much of some years during the study period. August was the only month without significant dust emission at FLP between March 2005 and April 2008.

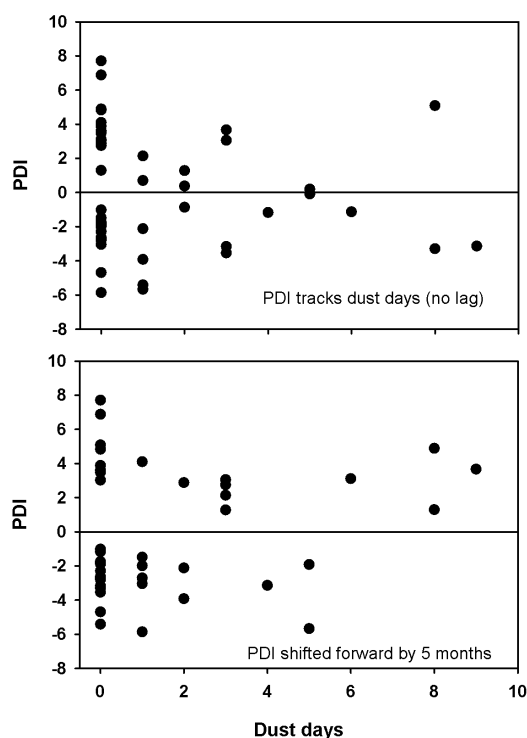


Figure 7—Number of monthly dust days plotted against monthly Palmer drought index (PDI) from southern Nevada region 26-04. In upper diagram, PDI tracks dust days for the same months. In lower diagram, PDI data (unitless values) are shifted forward in time by five months.

Dust emission in the Mojave Desert has generally occurred during the late winter and spring, as recorded by visibility data during the 1980s to the early 1990s from widely separated meteorological stations (Brazel & Nickling 1987; Bach, et al. 1996). High variations in interannual dust emission have been associated with antecedent precipitation (Brazel & Nickling 1987; Bach et al. 1996), with more dust during dry periods and less dust following heavy antecedent precipitation that encourages plant growth. This relation likely reflects the condition that sparsely vegetated landscapes dominate the Mojave Desert (see Wallace et al. 2008). Recent monitoring of dust emission from sparsely

vegetated sites in the Mojave Desert reveal the importance of annual plant growth to suppression of dust emission after wet autumn, winter, or early spring (Urban et al. 2007). Wet playas, such as FLP, have a different, apparently opposite, response to precipitation. As subordinate dust sources in the Mojave Desert, wet playas in the past have not greatly affected regional dust patterns as identified from visibility analyses. Nevertheless, recent studies have detected increased wet-playa dust emission as a result of periodic heavy precipitation. By examining relations among El Niño-Southern Oscillation (ENSO) events, dust sources, and dust composition, Okin & Reheis (2002) and Reheis (2006) found that the fluxes of soluble-salt dust, and its amounts relative to silt-clay dust, increased at sites close to wet playas during regionally wet El Niño events.

CONCLUSIONS

Understanding controls on dust emission from dryland basins with dry lakes and playas remains a challenge. Comparison among recent investigations shows that different basins can have variable dust frequencies determined by responses to seasonal and inter-annual climate, sizes of basins, features of their drainage systems, and aspects of their land uses. Such variability has implications for the development of regional and global climate models, and for distinguishing between natural and anthropogenic causes of dust flux at local, regional, and global scales (Bryant et al. 2007).

Conditions at FLP are similar in some important respects to those at Owens (dry) Lake, California (e.g., Reid et al. 1994; Cahill et al. 1996) and parts of the margins of the Aral Sea that generate “white dust” (see Goudie & Middleton 2006). Conditions at FLP further appear to be similar to conditions at many other settings that emit evaporite-mineral dust, judging from the color of dust as well as the points of origins of the plumes on the basis of satellite images.

For other large and complex dust-producing areas (Etosha Pan, Namibia; the Makgadikgadi Pans, Botswana, as examples), Bryant (2003) and Bryant et al. (2007) demonstrated that atypically large inundation events had both a rapid and lingering effect on increasing dust emissions. Inundation initially suppressed dust emission but afterward promoted emission of easily deflated flood sediment. Overall, unusually heavy flooding changed the timing of dust emission by suppressing dust generation longer than typical into dry seasons and delaying peak emissions during the following dry season.

Franklin Lake playa contrasts with some globally significant dry-lake dust sources in basin size and in its incapacity to accumulate large amounts of flood sediment. Nevertheless, FLP provides an example of a dust-producing playa where a large proportion of the total dust load is composed of evaporite minerals. At FLP, sustained aridity impedes dust emission, probably by development of salt-crusts that resist wind erosion. Local and regional moisture, either through rapid effects of direct rainfall on the playa surface or longer-term (months) effects of decreasing depth to the ground-water table, seems to promote dust emission at almost any time of year by creating relatively soft surfaces vulnerable to wind erosion.

ACKNOWLEDGMENTS

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Recovery of the Threatened Lahontan Cutthroat Trout in a Desert Terminal Lake

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Walker Lake is the terminus of the Walker River watershed and southernmost arm of the Pleistocene Lake Lahontan. Due primarily to upstream diversion, Walker Lake's volume has declined considerably, resulting in a current total dissolved solid measurement of near 16 g/l. Lacustrine populations of Lahontan cutthroat trout (LCT), native to Walker Lake, have been extirpated from most of their lake habitats. The primary goal of this effort is to improve LCT survival in Walker Lake and study the lake ecosystem's response to changing total dissolved solids (TDS) levels. To improve our understanding of the fishery, we have been conducting research to determine LCT survival, population abundance and distribution, and refinement of stocking techniques to increase immediate stocking survival. Netting and creel surveys indicate that some post-stocking survival occurs and that the average growth rate is 18 mm/month for stocked fish. The most effective means of improving stocking survival is allowing LCT to self-acclimate to the high TDS in Walker Lake by stocking them in the river/lake confluence. We sampled with trap and trammel nets and angling in 2006, 2007 and 2008, and captured 9, 164 and 73 LCT, respectively, although our most intensive sampling occurred during 2008. To date our most effective recapture method of tagged fish has been through the creel surveys. This method's biggest drawback is that these fish are not returned to the population, thereby ending our ability to determine long-term survival of LCT. Future efforts of this study are to increase the number of fish returned to the population by anglers, increase the number of fish captured by net and troll sampling, and determine the influence of TDS on the long-term survival of LCT in Walker Lake.

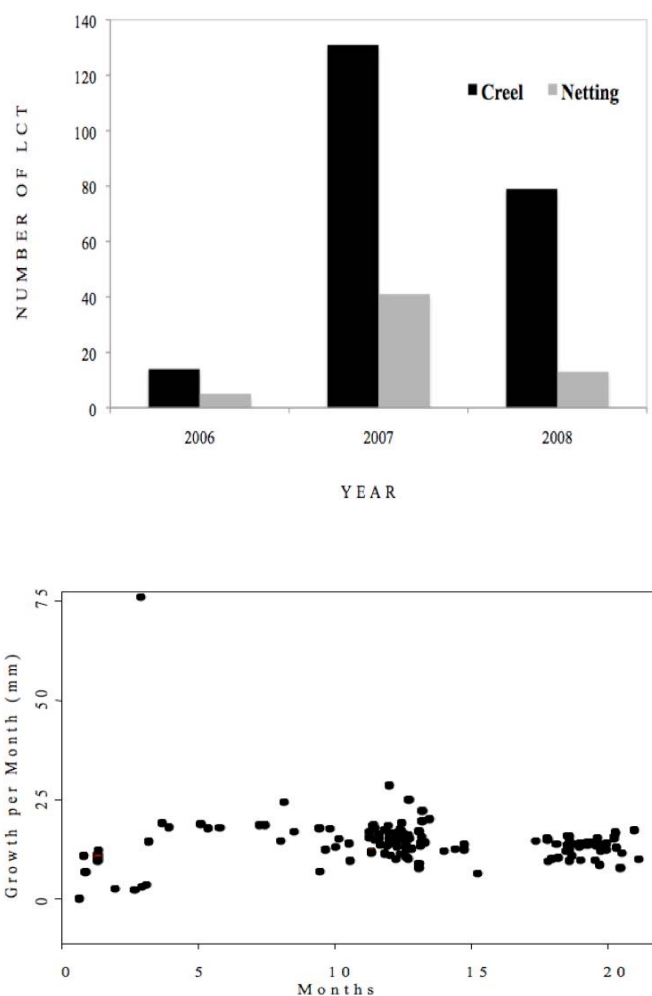


Figure 1—The number of Lahontan cutthroat trout (LCT) caught by creel and netting (top) from 2006 through 2008 and their growth in millimeters (bottom).

Salt Flat Basin's Contribution to Regional Dust Production and Potential Influence on Dry Deposition in the Guadalupe Mountains (Texas, USA)

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Saline lake and playa basins are amongst the most potent sources of mineral aerosols (dust) on Earth. The mineralogy and particle size of wind-erodible surface sediments in such basins are major controls of the nature of aerosols mobilized into remote areas downwind. This investigation evaluates the characteristics of dust emitted from the Salt Flat Basin of far western Texas (USA) (Figure 1). The Salt Flat Basin contains several ephemeral lakes and playas which, when dry, are major dust emitters. Aeolian sediment traps of different designs were deployed from mid-2005 through mid-2007 at 0.5, 1 and 1.5 m above the ground at three sites in the basin, two of which had been identified from satellite imagery as dust sources, and also at the crest of the Guadalupe/Delaware Mountains to the east (site of a National Park and an aerosol sampler in the IMPROVE nationwide air monitoring network) (Figure 1). Dust samples were collected twice a year (spring and fall accumulation). The particle size and chemistry of samples were evaluated by laser granulometry, proton-induced X-ray emission, ion chromatography, and X-ray diffraction. Dust flux in the Salt Flat Basin was highly variable with season and location, ranging from $10 \text{ g m}^{-2} \text{ yr}^{-1}$ (summer 2005) to

$150 \text{ g m}^{-2} \text{ yr}^{-1}$ (spring 2007), with an overall average of $50 \text{ g m}^{-2} \text{ yr}^{-1}$ for 32 total samples collected, consistent with seasonal dust storm frequency and similar to what has been measured at other locations in the southwestern USA (Table 1). Dust emitted from the Salt Flat Basin is rich in fine silt and clay-sized particles, potentially contributing to long-distance ($> 100 \text{ km}$) transport, especially during the spring windy season. The chemical composition of the dust was consistent with the known composition of the playa surfaces and surrounding lithology, but showed a geochemical fractionation with height and mode of transport (emission vs. deposition). Saltating particles near the ground were comprised primarily of gypsum and other sulfate and potash minerals, while suspended and falling dust was comprised primarily of silicate minerals including kaolinite and potassium feldspar. For the duration of the study the Salt Flat Basin did not appear to be a major source of aerosols at the Guadalupe Mountains IMPROVE site (GUMO), suggesting that GUMO is a valid regional sampling site not unduly influenced by dust emissions from the Salt Flat Basin located $\sim 20 \text{ km}$ upwind.

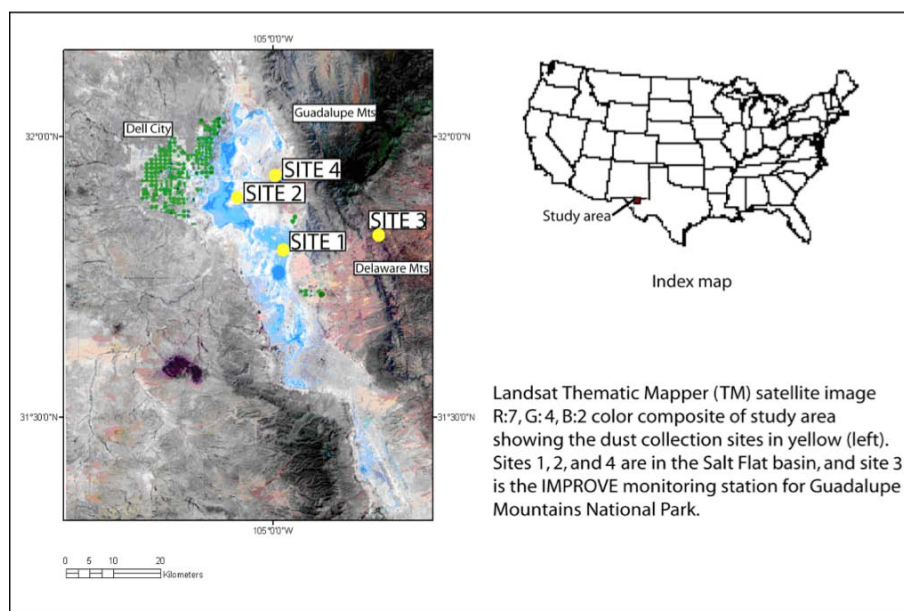


Figure 1—Location of Salt Flat basin and sampling sites.

Table 1—Dust flux measured at various locations (after Perez, A.E. 2008. Application of integrated remote sensing and GIS technologies to geoenvironmental issues in far west Texas and southern New Mexico, Ph.D. dissertation, University of Texas at El Paso, and Crabtree, G.W. 2004. Dustfall on the southern high plains of Texas. M.Sc. thesis, Texas Tech University).

Location	Dust Flux ($\text{g m}^{-2} \text{yr}^{-1}$)	Trap Height (m)
Salt Flat Basin, Texas (this study)	50 (range 10-150)	1.5
Southern California	4.3-33.9	2.0
Central Arizona	48.0	3-5
Las Cruces, New Mexico	7.6-28.1	0.9
Texas Panhandle	13.0-27.0	1.4
Edwards Plateau, Texas	10.8-13.7	1.5
Bighorn Basin, Wyoming	4.6-12.3	2.0
Great Plains, USA	20.2-90.8	0.6
Negev Desert, Israel	156.0-240.0	0-5
Israel (Mediterranean coast)	57.0-102.0	2.0
Israel (semiarid)	89.0-217.0	2.0
Lubbock, Texas	102.0	10.0
Big Spring, Texas	77.0	10.0
Lake Lahontan, Nevada	81.0	0.0

Geochemistry and Potential Playa Sources of the January 7, 2008 Southwestern New Mexico “Milky Rain”

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On the afternoon of January 7, 2008, an approximately 1300 km² region of Grant County, New Mexico, USA (Figure 1A), was subject to an unusual rainfall of milky-white color that left a hard, solid residue on surfaces (Figure 1D). Concerned residents, who collected samples from cisterns, rain gauges, and roof runoff, contacted area universities and state agencies to arrange for chemical analyses. Initial speculation of the source targeted everything from local mine tailings to Pacific Rim volcanic ash. Scientific evaluation of wind back trajectories, MODIS satellite images, and photographic observations indicated a possible regional source mechanism: wind erosion and transport of whitish dust from Willcox Playa in southeastern Arizona, USA (Figure 1B), followed by downwind entrainment into clouds, nucleation and wet deposition in localized rain showers downwind. Other rare “milky rain” events worldwide were traced to rainout of dust from saline lake basins (Resane, T.H. et al. 2004, South African Journal of Science 100: 483-487), and plumes of Willcox Playa dust have been previously observed to have been advected above the region as aerosols. Milky rain pH was 7.20 compared with the

regional average of 5.66. Electrical conductivity ranged from 46.5 to 115 $\mu\text{S}/\text{cm}$ as opposed to an average of 4.3 $\mu\text{S}/\text{cm}$ for the region. Analysis of major ions indicated elevated levels of calcium, magnesium, sodium, potassium, sulfate and chloride, as would be expected in playa-type deposits. A National Atmospheric Deposition Program site at the Gila Cliff Dwellings determined the calcium concentration of the rain (5.8 mg/l) to be within the top 1.0% of all data gathered by the network in a typical 5 year period. Upon analysis, the New Mexico Environment Department concluded that the event samples had similar chemistry to surface water samples obtained from the Lordsburg Playas in the early 1990s and reassured the public regarding its possible (non-) toxicity; the Lordsburg Playas (a known dust source along the same trajectory between the affected region and Willcox Playa) may have also contributed to this event. SEM analysis of water filter residue (insolubles and precipitates) from the event showed particles $\leq 1 \mu\text{m}$ in diameter (Figure 1C) with an elemental composition (Si, Al, Ca, and Mg) consistent with aluminosilicate and evaporite minerals.

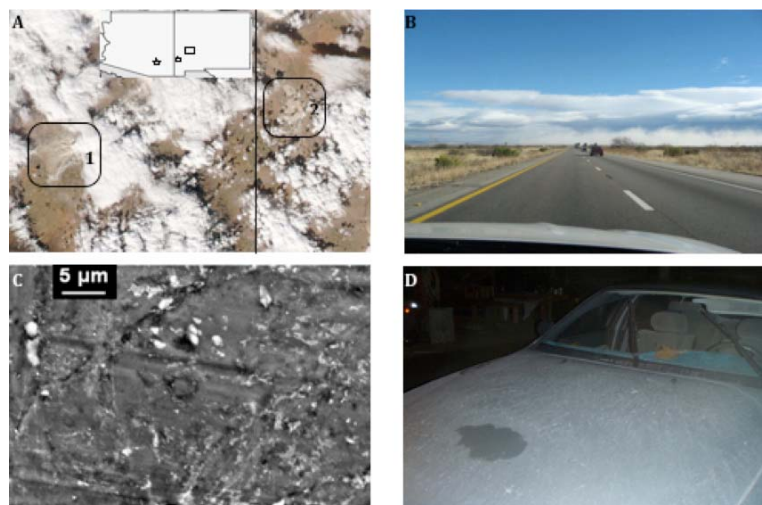


Figure 1—A: MODIS satellite image of the event showing plumes of dust rising off the Willcox Playa (1) and location of Lordsburg Playas. Inset map shows approximate location of Willcox location (star), Lordsburg Playas (circle), and area affected by milky rain (square) (2). B: Dust plumes moving right to left (south to north) across Interstate Highway 10. The image was coincidentally taken on day of event. Willcox Playa is just off image to right. C: Scanning electron micrograph of filter residue. D: Image of dried residue left on car hood (Photo courtesy of Alyson Siwik).

Planktonic Bloom-Forming *Nodularia* in the Saline Lake Alchichica, Mexico

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ABSTRACT

Lake Alchichica displays a characteristic cyanobacterial bloom associated with the onset of the stratification period (April-June). It mostly consists of the nitrogen-fixing cyanobacterium *Nodularia spumigena*. Filaments of *Nodularia* were straight, usually with an evident colorless, transparent sheath, with gas vesicles and without akinetes. The bloom developed in waters with a conductivity (K_{25}) of 13.47 to 14.14 mS cm⁻¹ (total dissolved solids around 8.7-9.2 g l⁻¹). Water column temperature was between 14.1 and 21.2°C, the pH fluctuated between 8.8 and 10.0, and dissolved oxygen between 0 and 9.3 mg l⁻¹. The annual mean concentration of N-NO₃ was highest in 2001 (1.0 µM) and lowest in 2000 (0.4 µM). For P-PO₄ the highest mean value was measured in 2002 (0.8 µM) with the lowest value in 1999 (0.4 µM). We monitored the bloom throughout four annual cycles (1999–2002). The intensity of growth and extent of the presence of *N. spumigena* in Lake Alchichica differed among the years. The highest mean density was observed in 2001 (1.36×10^{11} cells m⁻²), while the lowest values were found in 2000 (1.54×10^{10} cells m⁻²). Mean values for 1999 and 2002 were 2.08×10^{10} cells m⁻² and 2.29×10^{10} cells m⁻², respectively. *N. spumigena* is found regularly during a three-month period, but peak concentrations are reached only for a few weeks. In calm weather and following solar heating, the *Nodularia* filaments floated to the lake surface, causing the bloom to become apparent. The intensity of growth was correlated with the annual concentration of N-NO₃, indicating the role of the cyanobacteria as a source of new nitrogen to the lake.

INTRODUCTION

Blooms of the nitrogen fixing cyanobacteria *Nodularia spumigena* Mertens have been reported around the world. They are common in the Baltic Sea (Komárek et al. 1993; Musial & Pliński 2003), in the North Sea and in brackish Emsmere (Nehring 1993), and in numerous estuaries and bays along the southern and western coast of Australia and New Zealand (Musial & Pliński 2003). Blooms have also been reported for North and South America: in Great Salt Lake (Felix & Rushforth 1979), in brackish coastal waters

and inland lakes and ponds in British Columbia, Canada (Nordin & Stein 1980), in Pyramid and Walker lakes in Nevada, USA (Cooper & Koch 1984; Galat et al. 1990), in Brazil (Werner & Rosa 1992), and in a shallow coastal lagoon in Uruguay (Pérez et al. 1999). Cyanobacterial blooms cannot be explained simply by the biological features of the species. The phenomenon is induced by many environmental factors which play an important limiting and controlling role. Among these factors Musial & Pliński (2003) and Mazur Marzec et al. (2006) mention phosphorus and nitrogen supply, as well as the N:P ratio, surface water temperature, light availability, calm weather, thermal stratification, and salinity.

The occurrence of *Nodularia* blooms in the hyposaline Lake Alchichica, Mexico, has previously been observed for several years, associated to the onset of the stratification period (April-June). Arredondo Figueroa et al. (1983) and Alcocer et al. (2000) reported *Nodularia* blooms in Lake Alchichica in the 1980s and 1990s (Oliva et al. 2001). Similar *Nodularia* blooms also develop in Lake Atexcac (Macek et al. 1994; Tavera & Komárek 1996), a nearby lake that shares ecological and morphological features with Lake Alchichica. According to personal observations and recorded data, the intensity and extent of the presence of *Nodularia* appeared to differ among the years. In the present paper we discuss the spatial and temporal dynamics of *Nodularia* during four annual cycles (1999–2002) in Lake Alchichica. The main physical and chemical variables are also presented.

AREA OF STUDY

Lake Alchichica is a deep crater lake located in the state of Puebla (19° 24' N and 97° 24' W), Central Mexico (Figure 1). The annual air temperature in the area fluctuates between 5.5 and 30°C with a mean value of 14.4°C. Having an arid climate, with annual precipitation of less than 500 mm and an annual evaporation rate of 1590 mm (Adame et al. 2008), this high altitude plateau (≥ 2300 m above sea level) named Los Llanos de San Juan can be described as a “cool desert”.



Figure 1—Lake Alchichica, Puebla, Mexico.

The surface area of Lake Alchichica is 2.3 km² (diameter 1733 m). It has a volume of 9.42×10^7 m³, a maximum depth of 62 m and a mean depth of 40.9 m (Filonov et al. 2006). The lake is warm monomictic (Alcocer et al. 2000). Mixing takes place from the end of December or the beginning of January until the onset of the stratification period by the end of April or beginning of May. A well-developed thermocline is present from June-July until October-November. After November, the thermocline deepens and becomes weaker until its breakup in late December or early January. Besides the spring bloom of *Nodularia*, Lake Alchichica displays a winter diatom bloom coinciding with the mixing period.

Lake Alchichica is a unique hyposaline (8.5 ± 0.2 g l⁻¹; Na-Mg and Cl-HCO₃ dominated) and alkaline (pH = 9.0 ± 0.1) aquatic system (Vilaclara et al. 1993), characterized by several endemic species and unique features such as tufa towers. The endemic biota described include the atherinid fish *Poblana alchichica* (De Buen 1945), the ambystomatid salamander *Ambystoma taylori* (Brandon et al. 1981), the isopod *Caecidotea williamsi* (Escobar Briones & Alcocer 2002), and more recently the centric diatom *Cyclotella alchichicana* (Oliva et al. 2006).

MATERIALS AND METHODS

Sampling took place monthly at the central and deepest part of the lake during 1999–2002. Mid-day *in situ* profiles of temperature, dissolved oxygen, pH and conductivity (K₂₅) were obtained with a calibrated Hydrolab® DS3/SVR3 multiparameter water-quality data logger and logging system (discrete readings every meter). Ten water samples (depth 2, 5, 10, 15, 20, 25, 30, 40, 50 and 60 m) for phytoplankton analysis were obtained with a 5 l Niskin-type water sampler. Two 500 ml sub-samples from each sampling depth were fixed, one with 4% formaldehyde and

the other with Lugol's solution (1%). Cyanobacteria were counted in a 50 ml settling chamber with a Zeiss inverted microscope following the Utermöhl method (APHA 1985; Wetzel & Likens 2000). The length and width of *Nodularia* filaments, vegetative cells and heterocytes were measured with an eyepiece micrometer. A hundred independent measurements were made for each cell type. The length of the filament measured was then divided by the length of the cells. This procedure was repeated several times with different filaments and an arithmetic mean was established (APHA et al. 1985). Integrated values for the water column were obtained using linear interpolation, and the total abundance of *Nodularia* was expressed per unit area (cells m⁻²) rather than per volume units since the phytoplankton rarely homogeneously distributed (Payne 1986).

Another set of samples was collected from the same depths for nutrient analyses. These samples were maintained in the cold (4°C) and in darkness until analysis. Nitrate and phosphate (soluble reactive phosphorus) analysis followed standard methods (Strickland & Parsons 1972), adapted by Kirkwood (1994) for a Skalar Sanplus segmented flow autoanalyzer system.



Figure 2—*Nodularia spumigena* straight filaments showing heterocytes. Scale bar = 10 µm.

RESULTS

Nodularia in Lake Alchichica showed the following morphological traits: filaments were straight and composed of discoid vegetative cells 7–12 µm wide (mean 8.1 µm) and 2.7–3.6 µm long (mean 3.4 µm), with gas vesicles. Filaments usually had an evident colorless, transparent sheath. Heterocytes were 6.1–10.9 µm wide (mean 10 µm) and 4.5–6.4 µm long (mean 5.6 µm). They were present after every 12 to 16 vegetative cells (mean 13 cells) (Figure 2). Akinetes were never observed. According to its

morphological features and comparison with other descriptions (Nordin & Stein 1980; Pérez et al. 1999), our species corresponds to *Nodularia spumigena*. It is important to point out the existence of several morphotypes associated with local environmental conditions (Musial & Plinski 2003; Mazur Marzec et al. 2006). A definitive identification of *N. spumigena* requires studies that include ultrastructural and molecular biology considerations (Albertano et al. 1996; Hayes & Barker 1997). Such studies are still pending on the *Nodularia* of Lake Alchichica.

The K_{25} of Lake Alchichica varied from 13.47 to 14.14 mS cm^{-1} (around 8.7-9.2 g l^{-1}) during the study period, confirming its hyposaline nature. The water column temperature ranged between 14.1 and 21.2°C, the pH fluctuated between 8.8 and 10.0, and dissolved oxygen between 0 and 9.3 mg l^{-1} during the four years. Anoxic conditions were present in the deeper layers during the stratification period. Table 1 presents the N-NO_3^- annual mean concentration, being highest in 2001 (1.0 μM) and lowest in 2000 (0.4 μM). For P-PO_4 the highest mean value was measured in 2002 (0.8 μM) and the lowest value in 1999 (0.4 μM). *N. spumigena* blooms in Lake Alchichica became apparent when calm weather and solar heating were present and filaments floated to the lake surface (Figure 3).

Table 1—Annual range and mean concentration values of N-NO_3^- and P-PO_4^{3-} in Lake Alchichica, 1999–2002.

Year	1999	2000	2001	2002
$\text{N-NO}_3^- (\mu\text{M})$				
Range	0.04-4.2	0.01-1.9	0.06-5.2	0.01-4.8
Annual mean value	0.7	0.4	1.0	0.5
$\text{P-PO}_4 (\mu\text{M})$				
Range	0.01-3.2	0.05-3.6	0.2-4.0	0.12-4.2
Annual mean value	0.4	0.6	0.7	0.8

N. spumigena was the dominant species of the phytoplankton assemblage within the cyanobacteria bloom in all four years. The intensity of growth and extent of the presence of *N. spumigena* in Lake Alchichica differed among years (Figure 4). In 2001 the highest mean integrated column density was found ($1.36 \times 10^{11} \text{ cells m}^{-2}$), while 2000 had the lowest values ($1.54 \times 10^{10} \text{ cells m}^{-2}$). Mean values for 1999 and 2002 were 2.02×10^{10} and $2.29 \times 10^{10} \text{ cells m}^{-2}$, respectively. The maximum single-depth density was $1.05 \times 10^5 \text{ cells ml}^{-1}$ (surface water, May

30th 2001). The species was found along a four-month period (April to July) with peak concentrations lasting a few weeks only. In 1999 *Nodularia* was present for longer (7 months), including the mixing phase of the lake, when a diatom bloom is usually present. In 2002 the presence of *Nodularia* began in May and lasted until September.

We tried to establish a criterion to assess the presence of a bloom. The average value ($4.64 \times 10^{10} \text{ cells m}^{-2}$), using all data when *Nodularia* was present, was used as a criterion for bloom conditions. Values above this average were considered as bloom. According to this criterion, blooms were present in June 1999, in May and June 2001 and in June 2002, but not in 2000 (Figure 4).



Figure 3—Accumulation of filaments during the *N. spumigena* bloom.

DISCUSSION

N. spumigena trichomes exist in different forms. In the Baltic Sea, *N. spumigena* displays three different forms: straight, coiled and spirally coiled (Komárek et al. 1993). In contrast, the population from Lake Alchichica is always straight, similar to other North and South American *N. spumigena* populations such as those in brackish coastal waters and inland lakes of British Columbia, Canada (Nordin & Stein 1980), in Walker and Pyramid Lakes, Nevada (Cooper & Koch 1984; Galat et al. 1990), in Great Salt Lake, Utah (Stephens 1990), in Rio Grande do Sul, Brasil (Werner & Rosa 1992), and in Castillos Lagoon, Uruguay (Pérez et al. 1999).

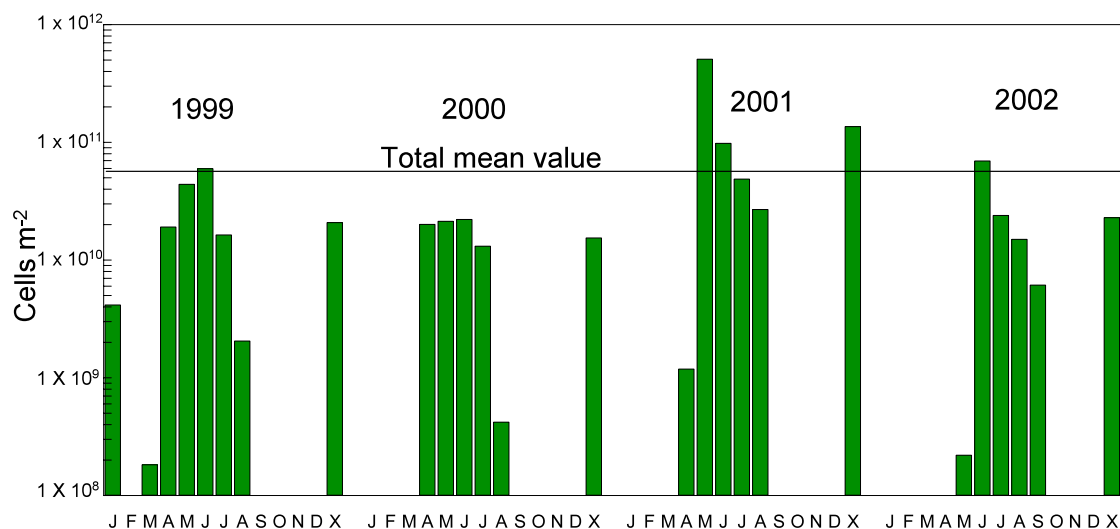


Figure 4—Interannual variation of densities of *N. spumigena* integrated over the water column in Lake Alchichica, 1999–2002. X = annual mean value.

Analyses of the 16S rRNA nucleotide sequence by Lehtimäki et al. (2000) indicated a great similarity between genotypes of the genus *Nodularia*. Although this gene is conserved and cannot therefore be used as a tool in the taxonomic identification of *Nodularia* species, there was a notable difference in the gene sequence between toxic and non-toxic *Nodularia* species. Laamanen et al. (2001) and Lyra et al. (2005) concluded that in the Baltic Sea there are only three species of *Nodularia*: one planktonic and toxin producing, with gas vesicles, which fits the description of *N. spumigena*, and two benthic, non-nodularin-producing species, without gas vesicles, namely *N. sphaerocarpa* and *N. harveyana*. The Lake Alchichica population is planktonic and contains gas vesicles; however, there is currently no evidence that this species produces toxins like *N. spumigena* from the Baltic Sea. Levels of nodularin will be tested in the future.

Some blooms of *N. spumigena* have been related to eutrophication processes (Hallegraeff 1993), although Pérez et al. (1999) mentioned that cyanobacterial blooms may be natural events and are not necessarily a consequence of eutrophication. The presence of *Nodularia* blooms in Lake Alchichica agrees with the last point of view, since this is an oligotrophic lake.

There are several factors that influence the growth and the extent of development of *N. spumigena* blooms in water bodies, the most important being salinity, temperature and light availability (Hamel & Huber 1985). *N. spumigena* requires salt concentrations between 5 and 20 g l⁻¹ to be present. Maximum growth is achieved at salt concentrations from 5 to 10 g l⁻¹, especially when sulfate and sodium are dominant (Nordin & Stein 1980). Salinity in Lake Alchichica is in the range between 8.5 and 9 g l⁻¹, and its water contains high concentrations of sulfate and sodium (Vilaclara et al. 1993); both characteristics appear adequate

for *Nodularia* growth requirements. High water temperatures promote *Nodularia* blooms in saline and brackish waters (Lehtimäki 2000). According to Nordin & Stein (1980), best growth is achieved between 25 and 30°C. The highest temperatures in Lake Alchichica surface waters range between 18 and 21°C, below the optimum range. Nordin & Stein (1980) indicate pH values above 10.0 to be the best for *Nodularia* growth; Lake Alchichica's pH is close to this optimum value. Light availability is also an important factor for *Nodularia* to develop. From April to June, while *Nodularia* usually reaches its maximum densities, solar radiation showed the highest values (up to 1200 W m⁻²). In addition, Lake Alchichica is a transparent lake with a photic zone depth between 13 and 38 m (Adame et al. 2008). A short period that combines high solar radiation and calm weather appears to favor accumulation of the cyanobacteria at the lake surface, making presence of *Nodularia* clearly visible. Cloudy and windy weather prevents the cyanobacteria from accumulating at the water surface (Lehtimäki 2000).

Nitrogen and phosphorus concentrations and the N/P ratio are also important factors. Scarcity of nitrogen promotes development of nitrogen fixing cyanobacteria such as *Nodularia* (Paerl 1996). Due to its nitrogen-fixation capacity, *Nodularia* has a competitive advantage when N is the limiting nutrient for phytoplankton growth. The low dissolved inorganic nitrogen: soluble reactive phosphorus ratios (1.2–12.9, mean 6.71) found in Lake Alchichica by Adame et al. (2008) clearly suggests nitrogen to be the nutrient that most likely limits phytoplankton growth.

Several investigations have shown that low inorganic N concentrations precede blooms of nitrogen fixing cyanobacteria, while a decrease in phosphate levels has been observed in conjunction with blooms of *N. spumigena* (Cooper & Koch 1984). High concentrations of ammonium,

nitrate, and organic nutrients are released into the water during cyanobacterial decay (Engström-Öst et al. 2002). In Lake Alchichica, the highest N-NO₃ concentrations were coupled with the highest *N. spumigena* growth (2001), and the lowest NO₃⁻ concentrations with the lowest densities (2000).

It is difficult to compare *N. spumigena* densities from different lakes, since values are given in different units (filaments ml⁻¹ or cells ml⁻¹) and are not calculated on an area basis (i.e., cells m⁻²). Maximum densities are commonly found at or close to the surface (around a depth of one meter) but in our case *N. spumigena* was usually found in the upper 10 m and some times throughout the entire water column (60 m). Lake Alchichica peak concentrations (1.05 x 10⁵ cells ml⁻¹) are much higher than those reported from Walker Lake (3.4 x 10⁴ cells ml⁻¹) by Cooper & Koch (1984) and from Pyramid Lake (3200 cells ml⁻¹) by Galat et al. (1981). Felix & Rushforth (1979) mentioned maximum densities of one thousand filaments per milliliter in Great Salt Lake. According to Paerl (1988), densities above 10⁴ to 10⁶ cells per milliliter of a single species could indicate the presence of a bloom. Following this criterion, *N. spumigena* bloomed in Lake Alchichica only in May 2001 with values between 4.1 x 10⁴ and 1.1 x 10⁵ cells ml⁻¹ were observed in the top five meters. The rest of the time, densities were always below 10⁴ cells per milliliter.

N. spumigena appears to be an important source of nitrogen for Lake Alchichica. Falcón et al. (2002) measured acetylene reduction rates of up to 78.9 ± 12.1 μmol m⁻² h⁻¹ during the 2001 bloom. As mentioned previously, N-NO₃ concentrations after the 2001 bloom were the highest of the whole period, while the minimum N-NO₃ concentrations coincided with the lowest *N. spumigena* densities in 2000. This observation provides indirect support for the relevance of this species as a source of new nitrogen to the lake, as has been observed in other saline lakes (Hamilton Galat & Galat 1983). Nitrogen appears to be incorporated in the lake throughout the *Nodularia* bloom, and the nitrogen is released as ammonia during its mineralization by heterotrophic bacteria. Other modes of incorporation such as foraging are considered to be of low importance due to the fact that this species is poorly used as food for zooplankton (Hamilton Galat & Galat 1983).

In conclusion, while the water temperature is below its optimum range, the salinity, pH and light conditions explain the presence of *N. spumigena* in Lake Alchichica. The concentrations of nitrogen and phosphorus and their ratio determine the peak growth of *N. spumigena* during the onset of the early stratification. The intensity of *N. spumigena* growth appears to be related with the post-peak N-NO₃ concentration, indicating the role of the cyanobacteria as a source of new nitrogen to the lake through N₂ fixation and subsequent mineralization.

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Algal and Cyanobacterial Saline Biofilms of the Grande Coastal Lagoon, Lima, Peru

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ABSTRACT

Tropical coastal wetland ecosystems are widely distributed in arid regions. The Grande coastal lagoon in Peru's central plain is shallow, eutrophic and alkaline, exposed to the annual hydrological regime with flooding and desiccation periods, when a salt crust is formed. The brackish to hypersaline habitats showed salinity gradients from 2-90 ppt (NaCl) to saturation, pH values from 7.0 to 10.5, temperatures from 18 to 31°C, phosphate concentrations from 0.5 to 50 mg l⁻¹ and nitrate up to 0.88 mg l⁻¹. Dominance of halophilic biofilms of benthic cyanobacteria followed by diatoms and the submerged macrophytes *Chara hornemannii* and *Ruppia maritima* during the clear water state supported the alternative stable states for this lagoon. A cohesive slimy layer formed mainly of the cyanobacteria *Chroococcus dispersus*, *C. turgidus*, *Aphanothece stagnina*, *Oscillatoria tenuis*, *Lyngbya martensiana*, *L. diguetii*, *Phormidium valderianum* associated with *C. hornemannii*, *Rhizoclonium hieroglyphicum* resting cells, *Aphanizomenon flos-aquae* akinetes, and *Tetraselmis contracta* cysts. Cyanobacterial biofilms flourished on the dried lagoon bed below the dicotyledonous halophytes *Salicornia fruticosa*, *Sesuvium portulacastrum* and *Baccopa monnieri*. The adaptive strategies included a biomass allocation (extracellular matrix formation) and complex reproductive processes for successful colonization.

INTRODUCTION

Extreme environments such as saline and hypersaline lagoons are hostile to most forms of life; however, they harbor significant populations of microorganisms. Their colonization by primary producers (algae and cyanobacteria) demonstrates that such organisms can adapt to extreme ecological niches. Both eukaryotes and prokaryotes have evolved a broad variety of adaptations, including the accumulation of osmolytes, to cope with osmotic and ionic stress (Ben-Amotz & Avron 1983; Javor 1989; Kirst 1995; Oren 2000).

In shallow water ecosystems, the dynamics of the sediments influence water quality, and the nutrient exchange rates across the sediment-water interface are regulated by chemical equilibria. Sediments can be colonized by biofilms: complex biotic systems known as microbial mats, microphytobenthos or periphytic mats. They represent unique systems in which microorganism assemblages

coexist at an interface, with a distinct macromolecular matrix typically attached to a surface in which complex food webs occur (Davey & O'Toole 2000; Larson & Passy 2005; De Vicente et al. 2006). Photosynthetic activity by benthic microalgae is the primary source of fixed carbon in shallow aquatic ecosystems. Microalgae are abundant in many soft-sediment aquatic habitats (estuaries, shallow subtidal seas), and they can contribute up to 50% of the total autotrophic production in some ecosystems (Underwood & Kromkamp 1999). Microphytobenthos may represent up to 50% of the microalgae present in the water column after its resuspension during low tide in coastal ecosystems (De Jonge & van Beusekom 1992). The activity of benthic attached algae in wetlands may rival or even surpass primary productivity rates of aquatic macrophytes (Cronk & Mitsch 1994; Poulickova et al. 2008).

Coastal wetland ecosystems are widely distributed in arid regions along the Peruvian coast in western South America. These coastal saline wetlands have rarely been studied, and the few published accounts of the photosynthetic organisms are mainly descriptive. Presence of the cyanobacteria *Aphanothece halophytica*, *Pleurocapsa entophysaloides* and *Microcoleus chthonoplastes* has been reported and some data have been obtained based on enrichment culture studies, yielding isolates such as *Dunaliella viridis*, *D. salina* and *Tetraselmis contracta* (Montoya & Golubic 1991; Montoya & Olivera 1993; Aguilar 1998). The Grande coastal lagoon forms part of the biological corridor of the South American Pacific coast. Because of the important functions of this shallow lagoon ecosystem, we here examined the biofilm community structure and its species composition (algae and cyanobacteria), as well as their spatial and temporal variability related to their succession along the salinity gradient until the lagoon dries out with formation of a saline crust.

MATERIALS AND METHODS

The Grande coastal lagoon is located in the central coastal region of Peru in the department of Lima, Cañete province, in the Chilca district (12°33'14.96"–12° 33'21.70" S, 76°42'42.38"–76°42'50.49" W), approximately 69.5-70 km south of Lima, along the Pan-American highway.

Algae were collected and the physical - chemical parameters (pH, salinity, temperature, phosphate, nitrate) of the lagoon were monitored intermittently between 2001 and 2007. Biofilm collections included the sampling of patches of microphytobenthos from the mud flats. Portions of benthic mats and endolithic submerged growth were removed from the substrate with a scalpel, and microalgal cells, both bound to sediment particles and free in the sediments, were recovered. Biofilms were examined *in vivo* and the settled algal material was removed. In some cases cells were detached by breaking the mucopolysaccharide layers within the assemblage. Subsamples were fixed and preserved in 5% formaldehyde and in Lugol's solution.

Identification of algae and cyanobacteria was performed using the following manuals: for Cyanophyta/Cyanobacteria, have been consulted Geitler (1932), Anagnostidis & Komárek (1988), and Komárek & Anagnostidis (1986, 1989); for diatoms, Patrick & Reimer (1975), Round et al. (1996), and Krammer & Lange-Bertalot (1997); for chlorophytes, Lerche (1937), Silva (1982), Poole & Raven (1997), and Round (1984); for the prasinophyte, Butcher (1959) and Hori et al. (1982).

Salinity (NaCl) was measured using an American Optical T/C salinometer. The pH was recorded with indicator paper (5.5-14.0). Phosphate (as orthophosphate ions in mg l^{-1}) was measured using Merck analytical strips, and nitrates were quantified (mg l^{-1}) by the La Motte nitrate test kit (model NCR). Morphospecies were identified and morphometric data, microphotography sequences of the vegetative and reproductive stages were obtained.

RESULTS

The Study Area

The tropical Peruvian desert extends as a narrow coastal band along the western edge of the Andean Cordillera and is interrupted by coastal lagoons and occasional rivers. The Grande coastal lagoon, located at the northern part of the Puerto Viejo wetlands, reaches up to 50 m above sea level. This inland eutrophic brackish - saline shallow lagoon has an area of approximately 60 km^2 and a maximum depth of 3 m. The salinity gradients were between 2-90 ppt (NaCl) up to saturation, the pH range was from 7.0 to 10.5, the water temperature ranged from 18 to 31°C , phosphate concentrations were from 0.5 to 50 mg l^{-1} , and nitrate was up to 0.88 mg l^{-1} .

The southern end of the lagoon is partially surrounded and sheltered by the La Bruja Hills, where the Andes coastal slope intercepts the winds. The wind force pushed the soil dust particles into the water column and together with the

aeolian dust (mineral and salts) contributed to the bioavailable nutrients at the lagoon surface and in the water bed. The lagoon sediment consisted of mudflats composed of particles that include silt, clay, sand, decaying vegetation, and animal and microbial particulates. Soft sediments were present on the surface as saline loam soil structure with mineral and organic colloids. The colloidal organic substance possesses valuable retentive properties to algae attachment, providing the substratum necessary for the formation of their assemblages. In some cases, water disturbance induced by wind action removed the sediment surface and caused resuspension of the benthos. This phenomenon is linked to light attenuation in the unconsolidated sediments; however, regeneration of the unique benthic algal communities was observed.

The Grande coastal lagoon with its athalassohaline waters has been exposed to unstable conditions throughout the last years. It is subjected to an annual fluctuating hydrological regime, with flooding and desiccation periods and formation of a saline crust, during which halophilic cyanobacteria and microalgae thrive. It is periodically flooded by subterranean seepage according to the water table levels (aquifers) of the Chilca river, followed by prolonged intensive evaporation with drastic reduction of the water column and with permanent and periodical formation of pools with hypersaline conditions, mainly in summer and early fall (January through April). The flooding period starts about mid-fall (early May) through 4 to 5 months up to mid-spring (late October through early November). It is followed by a desiccation period with emersion of the sediment with microphytobenthos biofilms, increase of the lacustrine fringe, and a sequence of increasingly saline brines where evaporite minerals precipitate from the brines (superficial and interstitial). Progressive efflorescence of salts (crystallization and precipitation of salts) is displayed on the exposed soft surface sediments with the development of whitish saline crusts. Deposited precipitates are mainly chlorides (halite), carbonates (calcite, aragonite, natron), sulfates (gypsum, mirabilite, anhydrite and magnesium sulfate), and potash. The hydrological input from underground is usually fresh water, causing dissolution of salt on the saline soil. This saline intrusion causes a renewed increase in salinity of the water column, leading to considerable fluctuations in salinity.

The regime shifts in the Grande lagoon due to drastic water level changes are mainly related to seepage inflow and weather conditions. The shallow lagoon provided evidence of alternating stable states with a regime shift from a clear to a turbid state according to the annual hydrological regime. Water level decreased in summer, and a salinity increase (2-90 ppt) was induced with a shift from a clear to

a turbid state. The eutrophic conditions in the lagoon during the warmer season (January through March) promoted the turbid state with phytoplanktonic water blooms of *Aphanizomenon flos-aquae*, *Chroococcus dispersus*, *Merismopedia glauca*, *Rhabdoderma minima*, *Euglena chlorophoenicea*, *Prorocentrum cassubicum* and *Tetraselmis contracta*. The sediment disturbance during the flooding period also increased the concentration of suspended particles (turbid water), attenuating the light available to macrophytes and benthic cyanobacteria and microalgae. Nevertheless, permanent pools of turbid waters within the lagoon harbor submerged macrophytes such as the stone-wort *Chara hornemannii* and the seagrass *Ruppia maritima* which remain present as perennial sciophytes, as well as microphytobenthic biofilm communities. During the clear water state, submerged macrophytes patches were observed, densely colonized by young stands of *C. hornemannii* after the flooding period and early fall (April) right after water blooms collapsed. During the desiccation period, the upper part of the dominant charophyte stands became whitish, and later they showed a seasonal collapse (die-off) in most of the dried sediment lagoon.

Algal and Cyanobacterial Biofilm Colonization

As a consequence of the regime shifts in the Grande lagoon, the submerged macrophytes were able to coexist with algae and cyanobacterial mats within the photic zone up to 5 mm thick benthic biofilms. Their presence was evident during the clear water state with salinity gradients from 2 to 41 ppt, the highest salt concentration that enabled macrophyte growth in this lagoon.

The multispecies biofilm structure composition (algae and cyanobacteria) included 38 salt-tolerant species, distributed in the following taxa: Cyanophyceae (21), Bacillariophyceae (10), Chlorophyceae (5), Prasinophyceae (1) and Dinophyceae (1). The benthic biofilm communities were mainly formed by the cyanophytes *Chroococcus turgidus*, *C. dispersus*, *C. giganteus*, *Aphanothece stagnina*, *Aphanocapsa pulchra*, *Gomphosphaeria aponina*, *Johannesbaptistia pellucida*, *Oscillatoria limnetica*, *O. tenuis*, *Spirulina subsalsa*, *Phormidium valderianum*, *Lyngbya martensiana*, *L. diguetii*, *L. aestuarii*, *Microcoleus chthonoplastes*, *Calothrix crustacea*, *Nodularia spumigena*, *N. harveyana*, *Aphanizomenon flos-aquae*, *Gloeotrichia natans*, *Pleurocapsa entophysaloides*, the diatoms *Campylodiscus americanus*, *Cyclotella meneghiniana*, *Cylindrotheca closterium*, *Synedra ulna*, *Amphora coffeaeformis*, *Pleurosigma strigosum*, *Entomoneis* sp., *Nitzschia* sp., *Navicula* sp., *Mastogloia* sp., the chlorophytes *Dunaliella viridis*, *Oocystis eremosphaeria*, *Enteromorpha intestinalis*, *Oedogonium pringsheimii*, *Rhizoclonium hieroglyphicum*, the prasinophyte *Tetraselmis contracta* and the dinophyte *Prorocentrum cassubicum*.

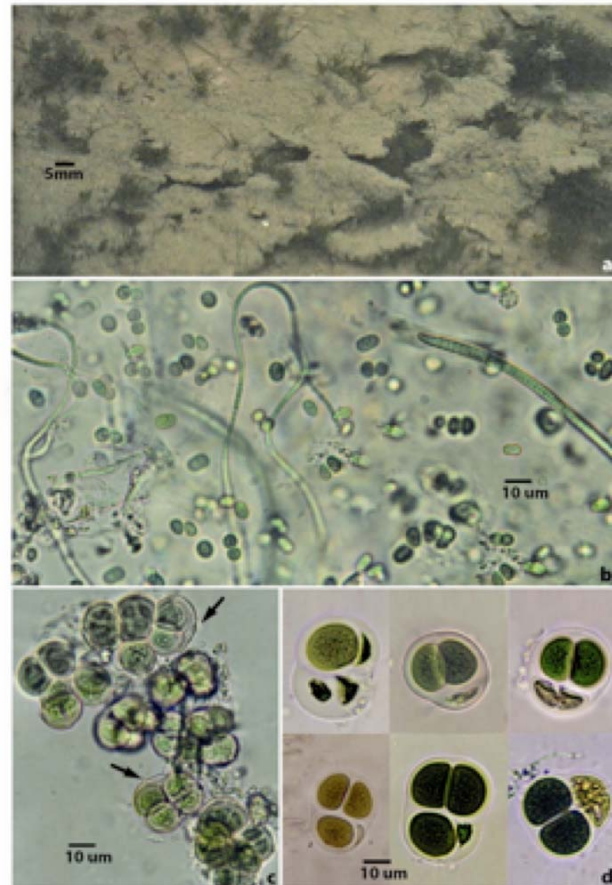


Figure 1—(a), Algae and cyanobacterial benthic cohesive biofilms forming undulated layers, partially lifted from the bottom of the Grande lagoon and associated with macrophyte young stands of *Chara hornemannii* and *Ruppia maritima*; (b), Biofilm species composition of a mixed community with cyanobacterial cells and colonies of *Chroococcus dispersus* associated with filaments of *Lyngbya diguetii* and *Oscillatoria tenuis* within the mucilaginous matrix; (c), Aggregated capsulated colonies of *C. turgidus* with stratified sheath from a biofilm on the air-exposed sediment surface; (d), Partially reactivated capsulated colonies of *C. turgidus* after a period of drying, with some damaged cells.

A multispecies biofilm with complex and aggregated microphytobenthos communities colonized the loamy mud sediment surfaces. Cohesive laminate or flocculent patterns were also influenced by the adhesive and adsorption properties of the mucilaginous material released mainly by sheathed cyanobacteria: *Lyngbya martensiana*, *L. aestuarii*, *L. diguetii*, *Microcoleus chthonoplastes*, *Aphanothece stagnina*, *Aphanocapsa pulchra*, *Gomphosphaeria aponina*, *Pleurocapsa entophysaloides*, *Nodularia spumigena* and *Chroococcus turgidus*.

The spatial arrangement of the biofilm showed a cohesive slime layer embedding the cyanobacterial communities with variation in thickness and compactness (Figure 1a). Cyanobacterial cells and colonies (microcolony cluster size) typified the less compact biofilms, mainly composed of a mixture of *Chroococcus dispersus*, *Lyngbya diguetii* and *Oscillatoria tenuis* (Figure 1b). Compacted biofilms formed

mainly as evaporation and gradual desiccation occurred, and they were composed of loosely or tightly packed filaments of the oscillatoriacean *Lyngbya martensiana*, *Johannesbaptistia pellucida*, and the heterocystous cyanobacteria *Nodularia spumigena*, *Calothrix crustacea*, and the green algae *Enteromorpha intestinalis* and *Rhizoclonium hieroglyphicum*.

The microphytobenthos was subjected to variability in flooding and recurrent drought effects in situ as gradually water depletion increased. Its heterogeneity and complexity was related to adaptive strategies of the algae and cyanobacteria such as the alternation of transitional life forms (planktic and benthic). The planktonic species *Chroococcus turgidus*, *C. dispersus*, *Aphanocapsa pulchra*, *Gomphosphaeria aponina*, *Tetraselmis contracta*, *Prorocentrum cassubicum*, *Aphanizomenon flos-aquae*, *Nodularia spumigena* and *Oedogonium pringsheimii* withstand greater seasonal chemical variability. Their assemblages also remained at the air-water interface, where the stress conditions increased gradually with the formation of the dried salt crusts. Most of these species with resting benthic phases can harbor resting stages (akinetes, cysts, aplanospores, oospores, resting cells or colonies). The onset of the dry period favored the formation of resting cells occurring isolated (*A. flos-aquae*, *Oocystis eremosphaeria*, *D. viridis*, *P. cassubicum*), isolated or in pairs (*T. contracta*) or in chains (*N. spumigena*, *N. harveyana*).

The biofilm succession displayed flocculent or homogeneous layers in the early stages. These occurred as a gelatinous coating on the sediment surface, dominated by cyanobacteria. In the intermediary stages, parts of the biofilm were lifted from the bottom (by wind or water turbulence or seepage inflow), forming occasional floating algal mats on the water surface of the lagoon. Benthic or floating mucilaginous biofilms including detritus, mineral and salt particles became progressively stratified. Submerged cryptoendolithic biofilms formed mainly by *O. tenuis* and *Pleurocapsa entophysaloides* were also recognized as the salinity gradient increased up to saturation levels in brines pools. In the final stages, original benthic biofilms became dry, dispersed and exposed with the formation of a saline soil crust. The dry bed lagoon allowed benthic biofilm to be exposed to air. Exposed subaerial microphytobenthic communities with spatial and temporal species distribution in the mats displayed response in extracellular pigmentation: *Lyngbya aestuarii* as well as *P. entophysaloides* displayed thick cell walls and brown sheaths pigmented by scytonemin.

The spatial arrangement within the dried biofilms showed complex stratification patterns of sheaths. Cells of *Chroococcus dispersus* developed individual sheaths which became confluent, forming macroscopic mucilaginous

biofilms. *Chroococcus turgidus* sheaths tended to be copious or diffuse at low salinities (2-10 ppt), resulting in less-defining colonies that favored the formation of a cohesive biofilm. However, colonies with firm or hard sheaths embedding groups of cells developed during the gradual desiccation period. Soil particles and detritus were trapped within capsulated colonies of *C. turgidus* with their mucopolysaccharide structures (Figure 1c). Initial sediment moisture (rewetting), due to groundwater infiltration at the onset of the flooding period, removed evaporite salts and reactivated the dried biofilms, changing the stratified sheaths into diffuent mucilage in *C. turgidus*, *C. dispersus* and *C. giganteus* as well as in the filamentous cyanobacteria. The external surface of the organic mucilage dissolved into the external medium and eventually contributed to the pool of dissolved organic material excreted by the cells. Partial photooxidative damage of exposed *C. turgidus* colonies was recognized during desiccation, with the formation of pale dead cells; however, some cells could be reactivated to resume growth (Figure 1d).

The landscape of the exposed surface sediments in the Grande lagoon with the efflorescent salts alternated with mats of halophilic higher plants. Patches of halophytes such as *Salicornia fruticosa*, *Sesuvium portulacastrum* and *Baccopa monnieri* alternated with areas of deposition of crusts of evaporite mineral overlying brines and dried thalli of *Chara hornemannii*. Benthic cyanobacterial mats dominated on the exposed surface sediments in ephemeral pools and in the desiccated littoral lagoon. Sometimes sciophyte subaerial complex communities (*Chroococcus turgidus*, *C. dispersus*, *Oscillatoria tenuis*, *Lyngbya diguetii*) developed under the halophyte plants and below the dried fragile *Chara hornemannii* thalli in the bed of the dried saline lagoon.

Along the spatiotemporal changes in the species composition of the microphytobenthos biofilm, the dominance of cyanobacteria (55%) was recognized as primary producers followed by diatoms (26%) in the developing hypersaline benthic communities as well as in the desert environments. Besides, colonization of the filamentous chlorophytes *Rhizoclonium hieroglyphicum* with resting cells with thick sheaths and *Enteromorpha intestinalis* were frequently observed in the saline crusts. They were found to be tolerant to desiccation, can could later be reactivated to successfully colonize the fluctuating and dynamic ecosystem.

DISCUSSION

Cyanobacteria, a phylogenetically coherent group of oxygenic photoautotrophic prokaryotes, grow in a wide variety of habitats, including extreme saline environments. They are the dominant constituents of wetland microphytobenthic biofilm communities in many aquatic saline ecosystems, including the soft sediments of the Grande ephemeral lagoon. Hypersaline environments in this shallow and sheltered lagoon showed temporal and spatial differences in salinity up to desiccation due to large fluctuations of temperature related to high rates of evaporation and low precipitation in the coastal desert. Most euryhaline algae and cyanobacteria found in the lagoon have been reported for other saline environments as well. The cyanobacterial species found to dominate in the present study such as *Chroococcus turgidus*, *Gomphosphaeria aponina*, *Aphanathece stagnina*, *Spirulina subsalsa*, *Pleurocapsa entophysaloides*, *Nodularia spumigena*, *Microcoleus chthonoplastes*, *Oscillatoria limnetica*, *O. tenuis*, *Phormidium hypolimneticum*, *Lyngbya aestuarii*, and *Aphanathece* spp. also occur in other saline and hypersaline ecosystems (Campbell & Golubic 1985; Erlich & Dor 1985; Javor 1989; Montoya & Golubic 1991; Garcia-Pichel et al. 2001). Hypersaline waters are usually dominated by unicellular cyanobacteria or chlorophytes (Brock 1976; Borowitzka 1981; Mackay et al. 1984; Garcia-Pichel et al. 1998). The Grande coastal lagoon was mainly typified by coccoid colonies and filamentous cyanobacteria, unicellular diatoms and unicellular or filamentous chlorophytes.

In the Grande coastal lagoon, mats of soft, flocculent, well-defined irregular colonies of *Aphanathece stagnina* on the sediment surface formed cohesive mats containing bright green unicellular cyanobacteria within copious amounts of clear mucilage. These findings are in agreement with the report of this species from the hypersaline Gavish Sabkha, Sinai (Erlich & Dor 1985). *Aphanathece* formed mucilaginous coatings in the benthos of the Yallahs saline ponds, Jamaica, and Solar Lake, Sinai Peninsula, Egypt (Cohen et al. 1977; Krumbein et al. 1977; Golubic 1980; Jørgensen & Revsbech 1983). The unicellular cyanobacteria *Synechocystis* and *Synechococcus* could be ecophenes (ecomorphs) of the colonial *Aphanathece*. They were also recognized in the top slime layer of dome-shaped laminated mats (Reed 1986; Javor 1989).

The colonial coccoid cyanobacteria species *Gomphosphaeria aponina* was also reported from the saline Hot Lake, Washington and from the hypersaline habitats (90-180 ppt) of the Gavish Sabkha where it was associated with colonies of *Chroococcus turgidus* that formed a loose mat at a salinity range between 50 and 70 ppt (Bauld 1981;

Erlich & Dor 1985; Gerdes et al. 1985). Benthic mats with crusty colonies adhering firmly to hard substrate were formed by irregular nodular communities of *Pleurocapsa entophysaloides* at the Grande lagoon where it was also present as epiphytic thalli on *Lyngbya aestuarii*.

The benthic filamentous oscillatorian cyanobacterium *Microcoleus chthonoplastes* has a cosmopolitan distribution and its populations (field and cultures) are phenotypically and phylogenetically extremely coherent (Garcia-Pichel et al. 1996). It is a dominant constituent of microbial mats in hypersaline habitats such as the Solar Lake, Exportadora de Sal (Baja California Sur, Mexico), the Yallahs salt ponds, and shallow marine intertidal environments (Golubic 1980; Jørgensen & Revsbech 1983; Campbell & Golubic 1985; Garcia-Pichel et al. 1996; Montoya et al. 1999). *M. chthonoplastes* of the island of Mellum (southern North Sea), Germany, has accreted and fixed the sediment (Stal 2000). Cyanobacterial mats in Australia (Spencer Gulf and Shark Bay) are dominated by *M. chthonoplastes* (Bauld 1984; Javor 1989) and so is the Gavish Sabkha with a salinity range of 50-180 ppt (Erlich & Dor 1985). Coastal mature mats of *M. chthonoplastes* in the Solar Lake were intermingled with *Spirulina subsalsa* and *Aphanathece* sp. (Krumbein et al. 1977; Golubic 1980; Campbell & Golubic 1985), and it formed stratiform mats in the northern part of the saline Lake Coorong, South Australia, where it was associated with crenulate mats of *Calothrix crustacea* (Bauld 1981). *M. chthonoplastes* was associated with *Lyngbya aestuarii* forming extensive and compact layers in African saline ponds in Tunisia and Algeria as well as in the Mexican saline Laguna Mormona, where it occurred together with tufted and laminated mats of *L. aestuarii* which was also reported for the Gavish Sabkha (Hof & Freymy 1933; Bauld 1981; Erlich & Dor 1985). The hypersaline ponds of Yallahs with fluctuating salinities were colonized by *M. chthonoplastes*, *S. subsalsa*, *L. aestuarii*, and *Johannesbaptistia pellucida*. The last species has also been reported for the Gavish Sabkha (Golubic 1980; Erlich & Dor 1985). Also in the Grande coastal lagoon, *M. chthonoplastes* was mainly associated with *L. aestuarii*, *A. stagnina*, *S. subsalsa*, *C. crustacea*, and *J. pellucida*. The species have thus expanded their distribution range in extreme environments.

Although the benthic mats are composed primarily of cyanobacteria, eukaryotic algae such as diatoms are important constituents of the surface layer of complex laminated assemblages (Jørgensen & Revsbech 1983; Clavero et al. 2000). Benthic epipellic diatoms were not dominant in the microbial mats of the Grande coastal lagoon, but they were present in soft-sediment habitats at salinity values up to 90 ppt. However, diatoms have been reported from hypersaline ecosystems such as the Gavish

Sabkha and solar salterns with salinity values as high as 205–213 ppt (Davis 1978; Ehrlich & Dor 1985; Javor 1989). *Amphora coffeaeformis*, a common diatom, was reported from the South Arm of Great Salt Lake, from the Gavish Sabkha, and from Little Manitou Lake (Felix & Rushforth 1979; Erlich & Dor 1985; Hammer et al. 1983). *A. coffeaeformis* associated with *Navicula* sp. and *Nitzschia* sp. formed flat mats in the Solar Lake (Krumbein et al. 1977).

Unicellular chlorophytes such as the halophilic flagellate *Dunaliella viridis* and *D. salina* are widely distributed in hypersaline aquatic ecosystems. The benthic palmelloid stage formed mixed algal photosynthetic mats in the northern arm of Great Salt Lake (Post 1977) as well as the ones registered for the Huacho Salines, Lima (Barberena & Montoya 1990). The euryhaline prasinophyte *Tetraselmis contracta* colonized a few ponds of the Grande coastal lagoon during the initial flooding period. This species is common among the phytoplankton of British coastal and brackish waters ranging in salinity between 5 and 40 ppt (Butcher 1959).

Benthic biofilm colonization and species patch dynamics provided a laminated or flocculent colloidal texture and contributed to the sediment stabilization in the Grande coastal lagoon. The structural integrity of sediment assemblages is maintained by the cyanobacterial extracellular matrix that contains the extracellular polymeric substance (EPS). A highly hydrated EPS layer may provide protection against desiccation in some cyanobacterial species (Whitton 1987; De Philippis et al. 1998; Otero & Vincenzini 2004). The production of EPS in the aquatic environment is ecologically significant because it is related to the biogenic sediment stabilization (Cibic et al. 2007). The EPS also plays a functional role in motility within the biofilm. Motility is an important behavioral process for photoacclimation, allowing cyanobacterial cells to migrate into the photic zone of the sediment near the surface after periods of sediment mixing or deposition. *Oscillatoria tenuis*, *O. limnetica* and *Lyngbya diguetii* filaments are motile, and they were seen to migrate through the biofilm mucilage of *Chroococcus dispersus*.

Stressed microalgal communities of saline lagoons, solar salterns and harsh habitats within desert crusts display a morphological diversity of cyanobacteria with different community structures (Jørgensen & Revsbech 1983; Garcia-Pichel et al. 2001; Oren 2002). In the desert saline crust of the studied coastal lagoon, the capsulated morphotypes of *Chroococcus turgidus*, *C. dispersus*, *C. giganteus*, *Gomphosphaeria aponina*, filaments of *O. tenuis*, *Lyngbya diguetii*, *L. aestuarii* and *L. martensiana* were recognized in addition to *T. contracta*, *R. hieroglyphicum* and *E. intestinalis*. The thick

mucilaginous capsule that encases *C. turgidus* cells under hypersaline conditions might be a protective adaptation that permits the organism to flourish under conditions where most other species cannot survive saline desiccation, such as was established for the brown tidal alga *Aureoumbra lagunensis* from a hypersaline coastal lagoon where hypersalinity enhanced EPS production (Liu & Buskey 2000). *C. turgidus* has a remarkable morphological plasticity under natural growth, particularly influenced by salinity and desiccation. According to Whitton (1992), sheath production depends on environmental and culture conditions. Sheath formation by *C. turgidus* could be triggered by the presence of bacteria, as has also been recognized for field populations of *M. chthonoplastes* (Garcia-Pichel et al. 1998).

Phenotypic plasticity traits have also been observed in some cyanobacterial strains that grow slowly at high salinities. The tendency to form compact mucilage that slows down cell dispersal results in the formation of large colonies (Dor & Hornoff 1985; Garcia-Pichel et al. 1998). Significant size differences with smaller cells at higher salinities, as well as changes in shape were reported for *Cyanothece* or *Aphanothece* strain from a solar evaporation pond in Eilat, Israel (Yopp et al. 1978). These findings support the idea that morphological divergence is not always reflected in genetic diversity (Palinska et al. 1996). In other cyanobacteria, however, morphological and phylogenetic traits were congruent (Casamatta et al. 2005).

The dynamic benthic biofilm ecosystem has been considered a depositional environment that entraps particles and precipitates, in which the extracellular matrix controls preservation and viability of the species. The growth and reproductive phases of phytoplankton (cyanobacteria and microalgae) during the turbid state of the lagoon showed the heterogeneity and complexity of their adaptive strategies. *Tetraselmis contracta*, *Prorocentrum cassubicum*, *Aphanizomenon flos-aquae*, *Nodularia spumigena*, *Oedogonium pringsheimii* and other species have evolved an alternation of generations, between vegetative planktic and resting benthic phases. This combination offers them a strategy for successful colonization. The resting stages are tolerant to extreme conditions such as desiccation. They can survive buried in the sediment for many years, although the level of tolerance may vary with the species and the amount of sediment disturbance. The resting stages in the microphytobenthos can serve as refuge populations (seed bank) for recolonization following harsh times of unfavorable conditions, as was reported for other algal groups (Fryxell 1983; Gao & Ye 2007). Therefore, the shallow sediments of the Grande coastal lagoon are important for the recruitment of species from sediments, to serve as inoculum enabling development of future blooms.

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Managing Salt Lakes in the Neotropics: Challenges and Alternatives

The Case of Mar Chiquita, Argentina

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The Bañados del Río Dulce and Laguna Mar Chiquita Ramsar site (Córdoba province, central Argentina) includes a large, terminal salt lake (present area = 5000 km²) and its associated grasslands and wetlands. The area is very rich in biodiversity, particularly migratory shorebirds and three species of flamingos. Climate change has altered the region dramatically. Between 1970 and 2000 the rainfall regime in the Mar Chiquita basin increased by about 30%, which resulted in a 9 m increase in the lake's water level, together with a substantial decrease in water salinity, from 23‰ to 2.7‰ (Figure 1). Lower salinity levels allowed the Argentine silverside, or pejerrey (*Odontesthes bonariensis*) to expand into the lake in the early 1980s, becoming an important economic resource. Although the lake is still largely pristine, the region is becoming increasingly threatened by man-induced environmental problems. Main negative factors include: a) water appropriation in the upper tributary rivers, b) increasing water pollution, and c) unregulated sport hunting, including international bird-hunting tourism. Water appropriation is driven mostly by agriculture expansion and urban growth along the three main Mar Chiquita tributaries. A decrease in water availability and new projected dams in the upper tributaries may result in changes in the annual flooding

and fire regime, which in turn may have profound effects on the grasslands and wetlands that surround the lake. Water pollution originates mostly from urban and agricultural sources. Lead pollution is also a growing problem because of increased hunting pressure from international waterfowl hunting tourism. Unfortunately, lead ammunition is still allowed in wetlands under the present Argentine legislation. An integrated, long-term management plan for the area is an urgent need. Management priorities include a) water-use policies in the tributaries' river basin that ensure adequate water supply to the system, including safeguarding of the annual flooding pulse, b) control of nonpoint source agricultural pollution, c) banning lead ammunition use, and d) sustainable urban planning and development. The University of Córdoba, through the PROMAR initiative, is promoting development and implementation of a management plan for the site, following Ramsar guidelines and criteria. As a first step, a comprehensive baseline study has been published recently [Bucher, E.H., ed. 2006. Laguna Mar Chiquita y Bañados del río Dulce (Córdoba, Argentina). Academia Nacional de Ciencias, Córdoba].

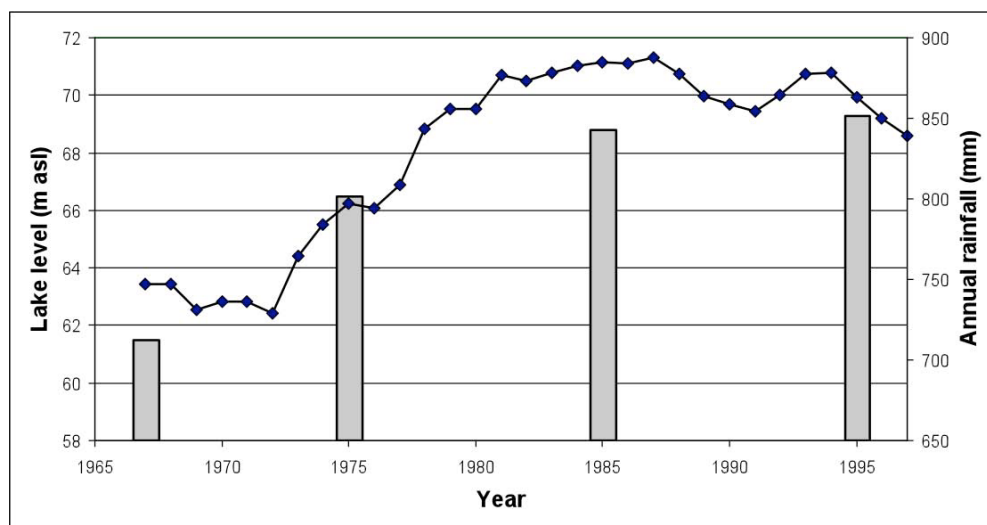


Figure 1—Mar Chiquita water level (line) and average annual rainfall for each decade in the lake's basin in 1967–1997.

Archaeal Diversity in the Dead Sea: Microbial Survival under Increasingly Harsh Conditions

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ABSTRACT

The Dead Sea is rapidly drying out. The lake is supersaturated with NaCl, and precipitation of halite from the water column has led to a decrease in sodium content, while concentrations of magnesium and calcium greatly increased, making the lake an ever more extreme environment for microbial life. In the past decades, blooms of algae (*Dunaliella*) and halophilic Archaea were twice observed in the lake (1980–1982 and 1992–1995), triggered by massive inflow of freshwater floods, but no conditions suitable for renewed microbial growth have occurred since. To examine whether the Dead Sea in its current state (density 1.24 g ml⁻¹, water activity about 0.67) still supports life of halophilic Archaea, we collected particulate matter from a depth of 5 m at an offshore station by means of tangential filtration. Presence of bacterioruberin carotenoids, albeit at low concentrations, in the particulate material showed that members of the *Halobacteriaceae* were still present in the lake's water column. Amplification of 16S rRNA genes from the biomass yielded genes with less than 95% identity with environmental sequences reported from other environments and only 89–95% identity with cultivated *Halobacteriaceae*. It is thus shown that the Dead Sea, in spite of the ever more adverse conditions to life, supports a unique and varied community of halophilic Archaea. We have also isolated a number of strains of *Halobacteriaceae* from the samples collected, and their characterization is currently in progress.

INTRODUCTION

During the nearly three decades that have passed since the start of a systematic monitoring program of the biological communities and processes in the Dead Sea, the lake has proven to be an ever changing environment, requiring constant adaptation of the microorganisms inhabiting its waters. Occasional rainy winters bring large amounts of floodwaters diluting the upper meters of the water column, triggering dense microbial blooms. When this happens, the density of red halophilic Archaea can be so high that the

entire lake obtains a red color. On the other hand, the lake is now drying out at an ever increasing rate, and this had important implications for the salt concentration and the ionic composition of its waters.

While many halophilic and halotolerant microorganisms are known to inhabit NaCl-dominated ('thalassohaline') brines up to halite saturation, only few organisms can live in the 'athalassohaline' environment of the Dead Sea, dominated by divalent cations. Studies in the past have shown that quantitatively the most important inhabitants of the water column are the unicellular green alga *Dunaliella*—the sole primary producer in the lake, and red extremely halophilic Archaea of the family *Halobacteriaceae* (Kaplan & Friedmann 1970; Oren 1988). Species first reported from the Dead Sea include *Haloferax volcanii* (Mullakhanbhai & Larsen 1975), *Haloarcula marismortui* (Oren et al. 1990), *Halorubrum sodomense* (Oren 1983a), and *Halobaculum gomorense* (Oren et al. 1995a). A recent addition to the list is *Haloplanus natans*, an unusual flat, gas-vesicle-containing organism isolated from an experimental mesocosm at Sedom (Elevi Bardavid et al. 2007; see also Oren et al. 2004, 2005). Other organisms have been obtained from the Dead Sea as well, including colorless members of the domain Bacteria, protozoa, and fungi (Oren 1988, 2003), as well as viruses (Oren et al. 1997). The quantitative importance of these components of the biota in governing the biological properties of the lake is as yet unclear.

In the years 1980–1982 and 1992–1995, dense blooms of microorganisms were observed in the entire Dead Sea. In both cases these blooms occurred following exceptionally rainy winters that caused the formation of a pycnocline at a depth varying between 5 and about 15 m, turning the holomictic regime of the lake into a meromictic one (Gavrieli et al. 1999; Gavrieli & Oren 2004). During these blooms the density of the biota was very high: up to 9×10^3 and 1.5×10^4 *Dunaliella* cells ml⁻¹ and up to 2×10^7 and 3.5×10^7 prokaryote cells ml⁻¹ were counted in 1980 and 1992, respectively (Oren & Shilo 1982; Oren 1983a, 1985, 1988, 1993; Oren & Gurevich 1993, 1995; Oren et al.

1995b, Oren 1997, 1999a, 1999b, 2000, 2002). These archaeal blooms imparted a red color to the entire lake. The blooms ended with the termination of the meromictic state and the renewed overturn of the water column (Oren 1985; Anati et al. 1995; Oren & Anati 1996). Field observations combined with laboratory simulations have shown that two conditions must be fulfilled for a microbial bloom to occur in the Dead Sea: the salinity of the upper water layers must become reduced to a sufficient extent, and phosphate, the limiting nutrient in the lake, must be available (Oren & Shilo 1985; Oren et al. 2004, 2005).

The overall negative water balance of the lake has caused a drop in water level, in the past decade over a meter per year (Yeichieli et al. 1998; Oren & Gavrieli 2002; Gavrieli & Oren 2004). The water column is now supersaturated with NaCl, and large amounts of halite have precipitated to the bottom. As a result, the ionic composition of the brines has changed dramatically: the Na^+ concentration decreased from 1.73 M in 1977 to 1.54 M in 2007, while Mg^{2+} increased from 1.81 M to 1.98 M. The lake's chemistry is thus becoming increasingly dominated by divalent cations (Table 1). The water activity (a_w) of the brines is now around 0.67, near the lowest level known to support life (Oren 2008). Due to the precipitation of halite, the total salt concentration has remained approximately constant at around 340 g l^{-1} . The pH of the brine is about 6.

Table 1—Changes in the ionic composition of the Dead Sea waters, 1977–2007. Data for 1977 were derived from Beyth (1980); data for 1996 and 2007 were provided by the Geological Survey of Israel and the Dead Sea Works Ltd. All concentrations are in mol l^{-1} .

	1977 (average)	1996 (average)	2007 (average)
Ion			
Na^+	1.730	1.590	1.540
K^+	0.180	0.200	0.210
Mg^{2+}	1.810	1.890	1.980
Ca^{2+}	0.430	0.440	0.470
Cl^-	6.340	6.340	6.480
Br^-	0.070	0.070	0.080
SO_4^{2-}	0.005	0.005	0.004

As conditions suitable for renewed microbial growth have not occurred since the 1992–1995 microbial bloom, and as conditions in the lake have since become ever more extreme due to the continuing drying out accompanied by a dramatic increase in the divalent/monovalent cation ratio, the question should be asked whether the Dead Sea may already now have become too extreme even for the most salt-tolerant and salt-requiring microorganisms, or whether

this unusual lake does still support different forms of life (Oren 1999b). We have therefore initiated a study, involving culture-independent molecular microbial ecology approaches as well as classic culture-dependent techniques, to examine the present state of the Dead Sea biota. It is our goal to compare the biological properties of the current Dead Sea both with the properties of the lake in earlier times when conditions were less extreme than presently, and with other, thalassohaline lakes dominated by halophilic Archaea. We here report the first data on our characterization of the *Halobacteriaceae* present in the water column in the beginning of 2007 and show that the Dead Sea still supports a small, but apparently unique community of halophilic Archaea adapted to life in this unusual lake.

MATERIALS AND METHODS

Sampling of Dead Sea and Concentration of the Biomass by Tangential Filtration

Samples were collected on February 5, 2007 and March 8, 2007 at a station 4 km east of Ein Gedi, at the location of a moored meteorological station (31° 25' N, 35° 26' E), where the depth of the lake is about 100 m. Water pumped through a hose from a depth of 5 m was immediately diluted with 10% (vol/vol) of filter-sterilized distilled water to prevent clogging of the tangential filtration filters due to the crystallization of halite from the NaCl-supersaturated brine. During the February 5, 2007 cruise, the water was prefiltered through glass fiber filters (Millipore AP2514250; nominal pore size 0.8–8 μm , diameter of the filtered area 11 cm) before it was fed into a tangential filtration system with a Millipore (Billerica, MA) Pellicon® 2 unit equipped with a C screen 0.22 μm Durapore® 0.5 m^2 cartridge. About 200 l of water could be passed through such a glass fiber filter before it became clogged with brownish material. After it had become apparent that the glass fiber prefilter effectively removed all prokaryotes from the water and that little material was subsequently collected by the tangential filtration setup, no prefiltration was used during the March 8, 2007 cruise, and the Dead Sea water, diluted as above with distilled water, was directly pumped into the tangential filtration unit.

Molecular Characterization of Archaeal 16S rRNA Genes from DNA Collected from Prefilters and Ultrafiltration Retentate

DNA was extracted according to the method of Massana et al. (1997). Archaeal 16S rRNA genes were amplified by PCR from DNA collected from the prefilters as well as from the concentrated Dead Sea water. For Archaea we used two specific primers: Ar20-F (5'-TTC CGG TTG ATC CYG CCR G-3') (DeLong et al. 1999) and Arch958R (5'-TCC GGC GTT GAM TCC ATT-3') (DeLong 1992).

Microscopic Estimation of Prokaryote and Algal Densities

Prokaryotic cells (Archaea and Bacteria combined) in Dead Sea water were enumerated microscopically using a Petroff-Hauser counting chamber after 380-fold concentration by centrifugation (40 min, 12000 x g). The relative accuracy of prokaryotic cell counts was estimated at $\pm 20\%$.

For the enumeration of *Dunaliella* cells, 5 ml portions of fixed samples were supplemented with 0.1 ml of 0.1 N iodine to stain intracellular starch. The samples were then filtered through Millipore filters (25 mm diameter, 5 μ m mean pore size, cat. no. SMWP-25). Filters were placed on microscope slides, and cells were counted under a 16x objective as outlined by Oren & Shilo (1982) and Oren et al. (1995b).

Pigment Determinations

The content of chlorophyll and carotenoids collected on the glass fiber prefilter was determined on sections (4.5-11 cm²) cut out of the filters following overnight extraction in 3 ml methanol/acetone (1:1, by volume). The extracts were cleared of particles by centrifugation, and their absorption spectra (400-700 nm) were measured in a Cary Varian model E1 scanning spectrophotometer, using the solvent as a blank. Archaeal bacterioruberin pigments were quantified based on an $E_{1\%}^{1\text{cm}}$ value of 2540 at 496 nm for α -bacterioruberin. As no significant amount of chlorophyll was detected in the samples, it was not necessary to apply a correction for the contribution of algal pigments to the total absorbance at this wavelength, as outlined in Oren & Gurevich (1995).

Isolation of Dead Sea Archaea on Hypersaline Growth Media

Material from collected on the glass fiber prefilter and ultrafiltration retentate was inoculated on a variety of growth media suitable for cultivation of a variety of halophilic Archaea. Best results in terms of colony recovery were obtained on a medium designed for halophilic Archaea from the Dead Sea (Oren 1983a; Oren et al. 1995a), which contained (g l⁻¹): NaCl, 125; MgCl₂·6H₂O, 160; K₂SO₄, 5; CaCl₂·2H₂O, 0.1; yeast extract, 1; peptone or casamino acids, 1; starch, 2; pH 7.0. The medium was solidified with 20 g l⁻¹ agar. Plates were incubated at 35°C.

RESULTS

To obtain an upper estimate of the numbers of prokaryotic cells in the February 15, 2007 sample, we concentrated the particles suspended in the water by centrifugation and enumerated these microscopically. This same technique had been used to monitor the rise and decline of archaeal blooms in the Dead Sea in the past (Oren 1983b, 1985, 1993; Anati et al. 1995; Oren & Gurevich 1995; Oren & Anati 1996). We counted 4.5×10^5 'bacteria-like particles' per ml in the water sample collected from 5 m depth. For comparison, numbers up to 2×10^7 and 3.5×10^7 were observed during the 1980 and the 1992 archaeal blooms (Oren 1983b; Oren & Gurevich 1995). The number of 4.5×10^5 'bacteria-like particles' ml⁻¹ may overestimate the true number of prokaryotic cells present in the sample as not all particles observed may have been prokaryotic cells. After filtration of 200 l of Dead Sea brine, the glass-fiber prefilterers were stained brown rather than pink-red or purple as were expected when all particles were halophilic Archaea.

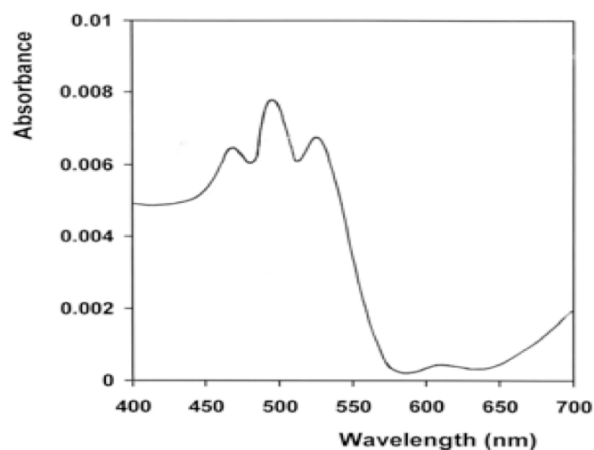


Figure 1—Presence of bacterioruberin carotenoids in the Dead Sea particulate material collected on February 15, 2007. A 4.5 cm² piece was cut out of a glass fiber prefilter (AP2514250; effective filtration diameter 11 cm) after filtration of 200 liter of diluted Dead Sea brine, extracted overnight with 3 ml of methanol/acetone 1:1 (vol/vol), and the absorption spectrum of the extract was recorded using the solvent as a blank.

The presence of members of the *Halobacteriaceae* on these prefilterers was unequivocally proven when pieces of these prefilterers were extracted in methanol/acetone 1:1 and the absorption spectra of the extracts recorded (Figure 1). The peaks at 496 and 530 nm and the shoulder at 470 nm are characteristic of the bacterioruberin carotenoids of the halophilic Archaea. No peaks of chlorophyll and algal carotenoids were found that would have indicated presence of *Dunaliella* or other phototrophs in the water column. Based on data including the volume of Dead Sea water that had passed through the filter, the filter area extracted and the specific absorbance of α -bacterioruberin, we estimated

the bacterioruberin content at $0.011 \mu\text{g l}^{-1}$. This value should be compared to the peak value of $14 \mu\text{g l}^{-1}$ observed during the 1992 bloom when total cell counts were $3.5 \times 10^7 \text{ ml}^{-1}$. When assuming a similar carotenoid content per cell in both communities, about 3×10^4 red, carotenoid-containing archaeal cells may be calculated to have been present per ml brine in early 2007. Taking all uncertainties and limitations of the approach into account, our calculation shows that in 2007 a small community of *Halobacteriaceae* was still found in the Dead Sea water column.

To obtain information on the nature of the Archaea present in the samples collected, and especially on the phylogenetic affiliation of the organisms, we amplified and sequenced archaeal 16S rRNA genes from DNA isolated from the glass fiber prefilters (February 15, 2007) and ultrafiltration retentate (March 15, 2007). Table 2 presents a representative selection of the sequences retrieved and their similarity with published 16S rRNA sequences of cultured haloarchaeal species and of ‘environmental sequences’. The data presented suggest that the community of halophilic Archaea was diverse. Similarity values with 16S rRNA sequences of the type strains of the published species within the family *Halobacteriaceae* showed low values only (87-96%); for the 14 sequences shown, the closest

phylogenetic affiliation was found with the species *Halorhabdus utahensis* (4x), *Halosimplex carlsbadense* (3x), *Halomicrobium mukohataei* (3x), *Halogeometricum borinquense* (1x), *Haloplanus natans* (1x), *Natronomonas pharaonis* (1x), and *Halalkalicoccus tibetensis* (1x). In some cases a somewhat higher similarity existed with ‘environmental sequences’ recovered from a variety of hypersaline environments worldwide, but still the Dead Sea sequences appear to be unique and do not closely resemble sequences recorded from other hypersaline habitats.

We also succeeded in isolating a number of colonies of red halophilic Archaea and other prokaryotes from the samples retrieved during the February-March 2007 cruises. For isolation of *Halobacteriaceae*, the high-magnesium (0.8 M) –low nutrient medium described in the Materials and Methods section proved to be superior to all other media tested. Use of similar media had in the past led to the isolation of *Halorubrum sodomense* and *Halobaculum gomorrense* (Oren 1983a; Oren et al. 1995a). On other, less saline media richer in organic nutrients a number of halophilic or halotolerant Bacteria were recovered, including members of the genus *Halobacillus* and the *Halomonas/Chromohalobacter* group. The isolates are currently being subjected to a taxonomic evaluation.

Table 2—Representative archaeal 16S rRNA sequences retrieved from the glass fiber prefilters (February 15, 2007) and ultrafiltration retentate (March 8, 2007) with prokaryote biomass collected from the Dead Sea, and their similarity with published 16S rRNA sequences of cultured haloarchaeal species and of environmental sequences.

	Sequenced Length (bp)	Closest Related Named Species and Other Cultured Strains	Closest Related Environmental Sequence
February 15, 2008			
1	755	<i>Halorhabdus utahensis</i> (94%); Ancient salt deposit isolate (McGenity et al. 2000) (95%)	Tuz Lake, Turkey (Burçin et al. unpublished) (97%)
2	908	<i>Halosimplex carlsbadense</i> (91%)	Tuz Lake, Turkey (Burçin et al. unpublished) (92%)
3	757	<i>Halogeometricum borinquense</i> (93%)	Australian saltern (Burns et al. 2004) (95%)
4	413	<i>Halorhabdus utahensis</i> (95%); Ancient salt deposit isolate (McGenity et al. 2000) (99%)	Saline soil (Walsh et al. 2005) (97%)
5	753	<i>Haloplanus natans</i> (96%)	
6	755	<i>Halomicrobium mukohataei</i> (89%); Ancient salt deposit isolate (McGenity et al. 2000) (99%)	Tuz Lake, Turkey (Burçin et al. unpublished) (92%)
7	541	<i>Natronomonas pharaonis</i> (89%); Ancient salt deposit isolate (McGenity et al. 2000) (99%)	Saltern, Korea (Park et al. unpublished) (92%)
March 8, 2007			
1	850	<i>Halosimplex carlsbadense</i> (90%)	Evaporite crust (Sahl et al. unpublished) (90%)
2	850	<i>Halosimplex carlsbadense</i> (90%)	Saline soil (Walsh et al. 2005) (90%)
3	790	<i>Halomicrobium mukohataei</i> (89%)	Alkaline, saline soil, Mexico (Valenzuela-Encinas et al. unpublished) (95%)
4	850	<i>Halalkalicoccus tibetensis</i> (87%)	Alkaline, saline soil, Mexico (Valenzuela-Encinas et al. unpublished) (95%)
5	1030	<i>Halomicrobium mukohataei</i> (92%)	Saline soil (Walsh et al. 2005) (93%)

DISCUSSION

The Dead Sea is a far more extreme environment for life than all ‘thalassohaline’, NaCl-dominated salt lakes. The a_w value for a saturated NaCl solution is about 0.75, while the a_w value of the Dead Sea waters is now as low as 0.67 (Oren 2008). Not only the low water activity makes the Dead Sea hostile to life, also the specific ionic composition is continuously becoming more difficult to tolerate by microorganisms. Divalent cations such as Mg^{2+} have a destabilizing, ‘chaotropic’ effect on proteins and other cellular components. Therefore the concentrations at which such ions are present, as well as the concentration ratio of such destabilizing ions and stabilising (‘kosmotropic’) ions such as Na^+ , determines whether or not microbial life may be possible. In the Na^+ -saturated Dead Sea, large amounts of NaCl have precipitated to the bottom in the past 25 years, leading to an ever increasing ratio of Mg^{2+}/Na^+ . As shown in the present paper, a small community of Archaea and other microorganisms still survives in the lake, but conditions have probably become too extreme for active growth, and the alga *Dunaliella* has not been seen in the water column during the past 12–13 years.

One of the indications for the continued presence of halophilic Archaea in the Dead Sea was the finding of bacterioruberin carotenoid pigments (Figure 1). It should be noted that the spectrophotometric pigment assay following extraction in organic solvents did not enable qualitative or quantitative assessment of the presence of retinal pigments that some members of the *Halobacteriaceae* possess and that enable the cells to use light energy to pump out protons and produce ATP (bacteriorhodopsin) or pump chloride ions into the cells (halorhodopsin). Such pigments may help the cells survive in situations in which the availability of organic substrates is limited, as is surely the case in the Dead Sea at times *Dunaliella*, the sole primary producer known from the lake, is absent from the water column. During the 1980–1981 bloom, bacteriorhodopsin was present in the archaeal community (Oren & Shilo 1981) and the pigment was shown to contribute to the energy metabolism of the cells (Oren 1983c). Whether such retinal pigments are also currently present in the Dead Sea and may contribute to the survival of the microbial communities cannot be assessed on the basis of the information obtained. Cultivation experiments using a variety of media have yielded a number of isolates, Archaea as well as Bacteria, and these are currently being characterized. It will be of special interest to see whether one or more of the archaeal isolates obtained may harbor a 16S rRNA gene that is

identical to one of the genes directly amplified from the community DNA (Table 1). In this way it can be assessed to what extent the isolates may or may not be representative for the total archaeal community. We also isolated a number of Bacteria (*Halobacillus* and organisms affiliated to the *Halomonas/Chromohalobacter* group). Based on the first results of our analyses of fosmid clones prepared from the community DNA of the March 8, 2007 sample (see below), it appears that most genes recovered are probably archaeal, and that the Bacteria are present at a lower level in the overall community. These data will be documented elsewhere.

Analysis of the 16S rRNA genes amplified from the community DNA yielded sequences affiliated with the genera *Halorhabdus*, *Halosimplex*, *Halomicrobium*, *Halogeometricum*, *Haloplanus*, *Natronomonas*, and *Halalkalicoccus*. It is interesting to note that none of the sequences showed presence of close relatives of those genera that have earlier been recovered from the Dead Sea: *Haloarcula*, *Haloferax*, *Halorubrum* and *Halobaculum*. There is no indication that organisms isolated and described from the Dead Sea such as *Har. marismortui*, *Hfx. volcanii*, *Hrr. sodomense* and *Hbc. gomorreense* were ever numerically dominant in the lake. It is more probable that these isolates happened to grow quite easily in enrichment media and on agar plates, so that they could be conveniently isolated and characterized. Biomass material from the 1992–1993 archaeal bloom in the Dead Sea has been preserved, and the application of a culture-independent, 16S rRNA-gene-based approach toward the characterization of the bloom community may in the future show to what extent the dominant type(s) of Archaea in that bloom have or have not already been cultured. Recovery of a 16S rRNA gene from the 2007 community with a relatively high (96%) similarity to *Haloplanus natans* is interesting. *Hpl. natans* (Elevi Bardavid et al. 2007) is an organism recently discovered (as a minor component of the community) in a red archaeal bloom that had developed in a 0.9 m³ mesocosm on the grounds of the Dead Sea Works Ltd. near the southern end of the lake. This mesocosm contained a mixture of Dead Sea water and Red Sea water, and had been supplemented with phosphate to stimulate growth of *Dunaliella* (Oren et al. 2004, 2005). The finding of 16S rRNA sequences affiliated with the genera *Natronomonas* and *Halalkalicoccus* was completely unexpected, as these genera consist thus far of alkaliphilic organisms only, while the Dead Sea has a slightly acidic pH.

We are now expanding the molecular study of the 2007 Dead Sea microbial community beyond the 16S rRNA gene characterization. A fosmid library has been prepared of the community DNA, and we are now in the process of sequencing as much DNA from this library as possible to obtain information not only on the phylogenetic affiliations of the organisms present in the community but also on their metabolic potential. We thus also hope to obtain an insight into the special adaptations that may be connected with life and survival in the present-day Dead Sea, one of the harshest environments known to support life.

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The Negative Water Balance of the Dead Sea; its Impact and the Future of the Lake

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The Dead Sea is one of the most saline lakes in the world (total dissolved salts ≈ 278 g/kg; density ≈ 1.240 g/ml) and forms the lowest exposed surface on Earth (-421 m, 2008). The lake is the last of a series of precursor lakes that evolved in the Dead Sea Rift valley since seawater first transgressed into the valley and formed the Sedom lagoon in the late Neogene. The unique Ca-chloride composition of these terminal lakes evolved through seawater evaporation, salt precipitation, water rock interaction and freshwater inflow. The Dead Sea came into existence in the early Holocene after the desiccation of its immediate precursor, Lake Lisan, in the late Pleistocene. Since then, its water level has been fluctuating in response to climatic changes. However, as of the middle of the 20th century, the Dead Sea experiences an "anthropogenic negative water balance", resulting in rapid water level decline of nearly 30 m, with increased rate of 1 m/yr over the last decade (Figure 1). This water level decline is mainly due to the diversion of water from the drainage basin for domestic and agricultural usages. The chemical industries at the shores of the lake contribute their share by pumping brines and evaporating them in evaporation ponds. The continuous

drop in water level has resulted in the drying of the Dead Sea southern basin, overturn of the water column which was meromictic for at least 400 years, onset of halite precipitation, retreat of shorelines and exposure of large mudflats, subsidence and development of sinkholes along its shore and collapse of the surrounding infrastructure. In order to stop the decline in water level plans are being considered to convey seawater from the Red Sea to the Dead Sea, while utilizing the > 400 m elevation difference between the seas to desalinize seawater. Yet, the mixing of seawater and/or desalination reject brine with Dead Sea brine will result in changes in the limnology of the Dead Sea that over the long run may change its characteristics and uniqueness. Initial one-dimensional model runs, in which water level is stabilized or raised by increasing inflow volumes, demonstrate the re-establishment of meromictic conditions, with continuous decrease in the density of the mixed layer, reaching values lower than those of the mid 20th century within 20-30 years of operation. It is thus important to quantify the mixing processes and verify that the benefits from the proposed project would not be outweighed by undesired environmental impacts.

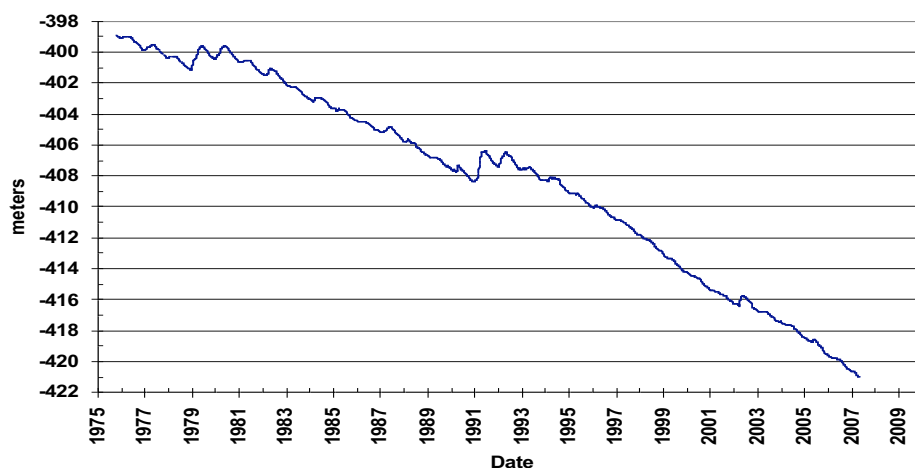


Figure 1—The Dead Sea water level, 1975–2008. Note the 2 meters rise in water level following massive runoff from the otherwise dammed Lake Kinneret (Sea of Galilee) and the Yarmouk River during the rainy winter of 1991–1992. The rise in water level resulted in dilution of the surface water and development of stratification which lasted only 3 years and was eroded by the continuous negative water balance of the lake. Data from the Israel Hydrological Service.

Long-term Mesocosm Simulation of Algal and Archaeal Blooms in the Dead Sea Following Dilution with Red Sea Water

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ABSTRACT

To understand the factors that determine the extent of blooms of the unicellular green alga *Dunaliella* and halophilic Archaea in the Dead Sea, and to predict the possible effects of the planned conveyance of Red Sea water to the Dead Sea, we performed simulation experiments in 0.9 m³ outdoor mesocosms on the grounds of the Dead Sea Works Ltd. at Sedom, as well as in the laboratory. The laboratory simulations showed that development of *Dunaliella* was possible only when Dead Sea water (340 g l⁻¹ total dissolved salts) was diluted with minimally 10% (by volume) of Red Sea water (40 g l⁻¹ total dissolved salts). Addition of phosphate was essential for the algae to grow, and growth rates and yields increased with increasing phosphate concentration and decreasing salinity. Field simulations in the mesocosms showed that development of algae was rapidly followed by development of dense blooms of red halophilic Archaea, which imparted an intensely red color to the ponds. While algal numbers declined after the peak of the bloom had been reached, number of halophilic Archaea and levels of archaeal pigments remained high for over two years. Although it should be realized that the closed system formed by the shallow ponds differs from the conditions in the lake, the results suggest that a microbial bloom, once formed, can remain present in the Dead Sea for months to years. These observations are important when attempting to predict how the biological properties of the lake may change in the future, and they have important implications for the planning of the Red Sea–Dead Sea conduit.

INTRODUCTION

The Dead Sea is located on the border between Israel and Jordan, has an area of about 630 km² and a maximum depth of 310 m. The lake presents fascinating challenges to the biologist who attempts to understand the biological processes and the limits of life in one of the most extreme environments on Earth. Its waters contain around 340 g l⁻¹ of salts, and have a highly unusual ionic composition: divalent cations (1.98 M Mg²⁺, 0.47 M Ca²⁺) dominate

over monovalent cations (1.54 M Na⁺, 0.21 M K⁺). The anions are 99% Cl⁻ (6.48 M) and 1% Br⁻ (0.08 M) (values for 2007). Sulfate concentrations are low (0.004 M), and the brine has a pH of about 6. The main water source to the Dead Sea was the Jordan River. However, diversion of water from its catchment area has decreased the Jordan River discharge to the Dead Sea to only about 10% of its natural flow (Lensky et al. 2005).

Only few microorganisms can live in such an environment. Quantitatively the most important inhabitants of the water column are the unicellular green alga *Dunaliella*—the sole primary producer in the lake, and extremely halophilic Archaea of the family *Halobacteriaceae*. Archaea first reported from the Dead Sea include *Haloferax volcanii*, *Haloarcula marismortui*, *Halorubrum sodomense*, and *Halobaculum gomorrense*. Other organisms have been isolated from the Dead Sea as well, including colorless members of the domain Bacteria, protozoa, and fungi (Oren 1988, 2003). Their quantitative importance in governing the biological properties of the lake has never been ascertained.

Systematic monitoring of the algal and prokaryotic communities in the water column of the Dead Sea since 1980 has yielded the following general picture: undiluted Dead Sea water is a too harsh environment even for the best salt-adapted microorganisms. However, exceptionally rainy winters can turn the holomictic regime into a meromictic one with the formation of a pycnocline at depths varying between 5 and about 15 m (Gavrieli & Oren 2004). When the surface waters become sufficiently diluted, dense blooms of algae and red Archaea develop in the upper meters of Dead Sea water column. Such blooms were recorded in 1980 (lasting until a renewed mixing of the water column in the end of 1982) and in 1992 (lasting until the end of 1995). During these blooms the density of the biota reached very high values: up to 9 x 10³ and 1.5 x 10⁴ *Dunaliella* cells ml⁻¹, and up to 2 x 10⁷ and 3.5 x 10⁷ archaeal cells ml⁻¹ were counted in 1980 and 1992, respectively (Oren 2000; Oren et al. 1995). These archaeal blooms imparted a red color to the entire lake. Field observations combined with laboratory simulations have

shown that two conditions must be fulfilled for a microbial bloom to occur in the Dead Sea: the upper water layers must become diluted to a sufficient extent, and phosphate, the limiting nutrient in the lake, must be available.

A thorough understanding of the biological phenomena in the Dead Sea and the factors that determine the nature and extent of biological blooms in the lake is of great importance when planning human interference in the properties of the lake. From the 1930s the water balance of the lake has generally been negative. Since the 1960s anthropogenic intervention through diversion of freshwater from the catchment area for irrigation and drinking water has greatly increased. As a result, during the 20th century the Dead Sea level has dropped by more than 20 m, and during the past decade it has dropped by approximately one meter per year, on the average (Gavrieli & Oren 2004). This negative water balance is attributed primarily to water pumping from Lake Kinneret to the Israel National Water Carrier and diversion of water from the Yarmouk River by Syria and Jordan. The latter constructed the King Abdullah Canal which runs along the eastern side of the Jordan Rift Valley and supplies Yarmouk water for irrigation in the region. About 40% of the water level decline of the Dead Sea is attributed to evaporation of Dead Sea brine in the evaporation ponds of the Israeli and Jordanian mineral industries located in the southern basin of the Dead Sea. These industries pump together about 500 million cubic meters from the Dead Sea into the evaporation ponds where halite (NaCl) and carnallite ($\text{KMgCl}_3 \cdot 6\text{H}_2\text{O}$) precipitate. At the end of the process less than 250 million cubic meters of concentrated end brines, composed mainly of Mg-Ca-Cl, are returned to the Dead Sea. The drop in water level is causing severe problems in the area for local infrastructure, tourism, and industrial activities.

Since the peace treaty between Israel and Jordan was established in 1994, the construction of the "Peace Conduit", connecting the Dead Sea with the Gulf of Aqaba (Red Sea) is being discussed. This planned water carrier is intended to counteract the drop in Dead Sea water level, mitigating damaging processes that currently occur in the Dead Sea and its surrounding area. The difference in elevation between the Red Sea and the Dead Sea (current surface level: -420 m) may be exploited for the generation of energy which can be used for seawater desalination (Oren et al. 2004; Gavrieli et al. 2005).

The present study, combining laboratory model experiments with simulations in outdoor ponds, was intended to provide answers to two basic questions: (1), what are the boundary conditions with respect to dilution and phosphate concentrations that enable the development of algae in the Dead Sea, and (2), how long may algal and archaeal blooms, once formed, remain in the lake when limnological conditions remain constant.

METHODS

Field-Scale Simulation Experiments in the Experimental Ponds at Sedom

Mixtures of Dead Sea water and Red Sea water were incubated in mesocosms on the grounds of the Dead Sea Works Ltd. at Sedom. The experimental setup consisted of white plastic tanks (1 x 1 x 1 m; Dolav, Kibbutz Dvir, Israel), buried 0.75 m in the ground. These tanks were filled with 900 l of mixtures of Dead Sea water (sampled from the channel that feeds the evaporation ponds of the Dead Sea Works Ltd. with water from the northern basin of the lake) and water from the Gulf of Aqaba, purified through a filter of 60-70 cm sand, eliminating particles larger than 20-30 μm . The first set of experiments performed at the site, initiated in July 2002, as well as further details of the experimental setup, have been documented by Oren et al. (2004). The experiments documented in the present paper started in 2002–2003, and are based on two ponds. One mesocosm ("no. 4") was filled with a mixture of 80% Dead Sea water and 20% Red Sea water, amended with 1 μM KH_2PO_4 and inoculated with 50 ml of brine from a mesocosm that had developed a bloom of *Dunaliella* and halophilic Archaea in the previous set of experiments. The second mesocosm ("no. 9") contained a 1:1 mixture of water from mesocosms no. 9 and 10 from the earlier experiment. Mesocosms 9 and 10 both contained a 70% Dead Sea water –30% Red Sea mixture and 1 and 10 μM KH_2PO_4 , respectively, thus the new experiment was based on brine that had received an equivalent concentration of 5.5 μM phosphate, and started at the high algal and archaeal densities that had developed in the first round of experiments as documented (Oren et al. 2004). The mesocosms were mixed daily, and their water level was kept constant by adding deionized water every 1-2 days, followed by thorough mixing. No conspicuous growth of algae or other organisms was ever observed attached to the walls or to the bottom of the containers. In November 2004, an additional portion of 1 μM KH_2PO_4 was added to mesocosm no. 4. The ponds were sampled once every two weeks for the determination of the density of the *Dunaliella* population, the halophilic archaeal community density, and the content of algal chlorophyll and archaeal carotenoids.

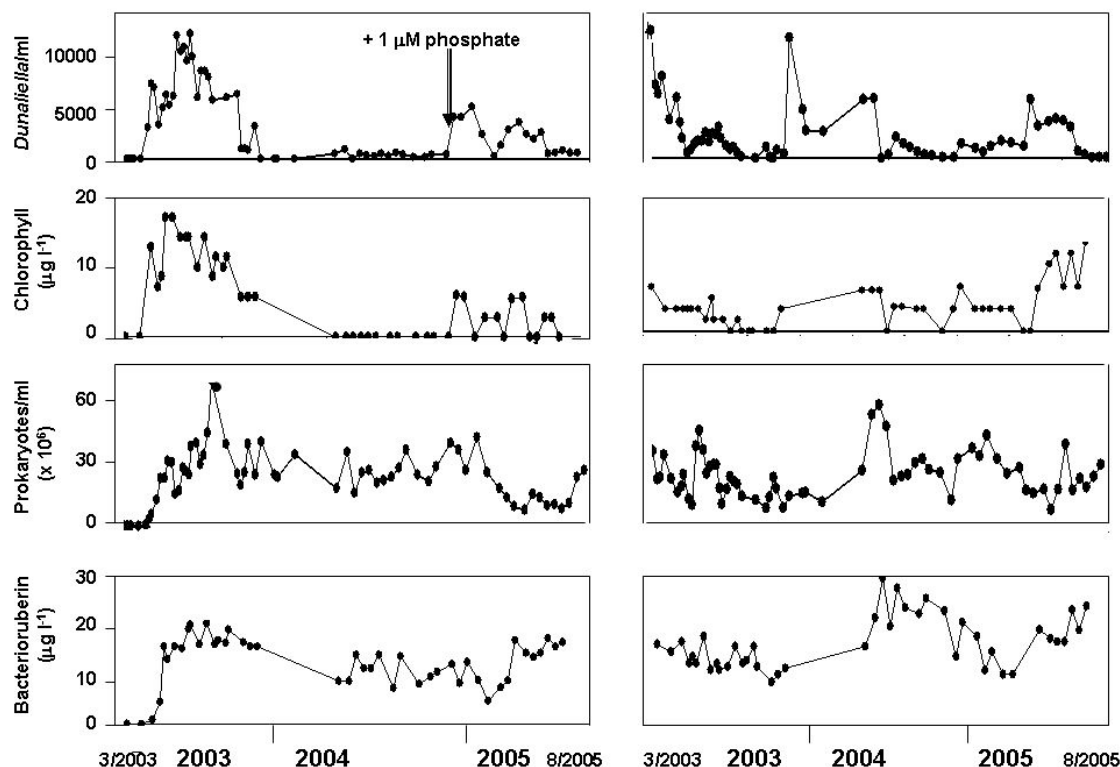


Figure 1—Left panels: numbers of *Dunaliella* cells, concentrations of chlorophyll, numbers of prokaryotic cells, and concentration of bacterioruberin carotenoids in an outdoor pond filled with a mixture of 80% Dead Sea water and 20% Red Sea water, and amended with 1 μM KH_2PO_4 from the start of the experiment in March 2003 until August 2005. An additional portion of 1 μM KH_2PO_4 was added in November 2004 (arrow). Right panels: numbers of *Dunaliella* cells, concentrations of chlorophyll, numbers of prokaryotic cells, and concentration of bacterioruberin carotenoids in an outdoor pond filled with a mixture of 70% Dead Sea water and 30% Red Sea water. In March 2003 the pond was filled with a 1:1 mixture of water from ponds no. 9 and 10 from an earlier experiment, which contained 70% Dead Sea water–30% Red Sea and 1 and 10 μM KH_2PO_4 (Oren et al. 2004), explaining the high initial values of biological parameters.

Laboratory-Scale Simulation Experiments of Micro-bial Development in Dead Sea–Red Sea Water Mixtures

To examine in further depth the effect of salinity and phosphate concentration on the development of *Dunaliella* in Dead Sea–Red Sea waters, we set up laboratory experiments in which 100 ml Erlenmeyer flasks were filled with 75 ml portions of Dead Sea water (sampled in July 2005 from a depth of 10 m at the deepest point of the lake 8 km east of Ein Gedi) and filtered Red Sea water as described above. Different concentrations of KH_2PO_4 were added, and all flasks were inoculated with a unialgal, non-axenic culture of *Dunaliella* from the Dead Sea in 80% Dead Sea water–20% Red Sea water to supply an inoculum of about 500 *Dunaliella* cells ml^{-1} . The flasks were incubated without agitation at 30°C under constant illumination ($100 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) by white fluorescent tubes. To prevent evaporation and to ensure constant salinity for the duration of the experiment, the flasks were closed with Parafilm. After 25 days samples were withdrawn for microscopic enumeration of *Dunaliella* cells and chlorophyll assay.

Enumeration of Algae and Halophilic Archaea

To count the density of the algal (*Dunaliella*) and halophilic archaeal communities, 50 ml of samples from the experimental ponds were fixed with 1 ml of 37% formaldehyde, previously cleared by filtration through a 0.2 μm pore size membrane filter. Samples were stored at room temperature until counted within 3–4 weeks. For the enumeration of *Dunaliella* cells, 2.5 ml portions of fixed samples were supplemented with 0.1 ml of 0.1 N iodine to stain intracellular starch. The samples were then filtered through Millipore filters (25 mm diameter, 5 μm mean pore size, cat. no. SMWP-25). Filters were placed on microscope slides, and cells were counted at 128x or 320x magnification.

A similar procedure was followed for the enumeration of algal cells in laboratory experiments. However, no prior fixation was used in this case, and variable volumes of water filtered were filtered according to the density of *Dunaliella* in the flasks. Cell numbers were calculated from the average number of cells per field and the field diameter,

calibrated with the aid of the grid of a Petroff-Hauser counting chamber (Oren & Shilo 1982; Oren et al. 1995). Prokaryotic cells (Archaea and Bacteria combined) were enumerated microscopically using a Petroff-Hauser counting chamber after 5-10-fold concentration by centrifugation (20 min, 12000 x g). The relative accuracy of the algal and prokaryotic cell counts was estimated at ± 10 and 20%, respectively.

Pigment Determinations

The content of chlorophyll and carotenoids was determined by filtering 30-50 ml sample portions through glass fiber filters (Whatman GF/C, 25 or 47 mm diameter) within 1 hour after sampling. Filters were kept at -20°C in the dark until further processing within 3-4 weeks. Filters were then extracted overnight in 2.5-5 ml methanol/acetone (1:1, by volume). The extracts were cleared of particles by centrifugation, and their absorption spectra (400-700 nm) were measured in a Cary Varian model E1 scanning spectrophotometer, using the solvent as a blank. Chlorophyll concentrations were calculated, based on a specific absorption of $73.5 \text{ l mg}^{-1} \text{ cm}^{-1}$ for chlorophyll *a* at 665 nm.

Archaeal bacterioruberin pigments were quantified based on a specific absorption of $25.4 \text{ l mg}^{-1} \text{ cm}^{-1}$ at 496 nm for α -bacterioruberin. A correction was made for the contribution of algal pigments to the total absorbance at this wavelength, as outlined in Oren et al. (2004).

RESULTS

Our outdoor simulation experiments in 0.9 m^3 mesocosms show that once a microbial bloom has formed in a Dead Sea–Red Sea water mixture, it can persist for over two years (Figure 1). The left panels of Figure 1 document a

bloom of *Dunaliella* and halophilic Archaea in a mixture of 80% Dead Sea water and 20% Red Sea water, supplemented with $1 \mu\text{M}$ orthophosphate. Algal numbers reached values of up to $1.2 \times 10^4 \text{ cells ml}^{-1}$ ($16 \mu\text{g chlorophyll l}^{-1}$) after 4 months, and then declined to low values. Mass development of algae was followed by the growth of halophilic Archaea, which are heterotrophic microorganisms that develop at the expense of organic compounds produced by the autotrophic algae. We counted up to 65×10^6 prokaryotic cells ml^{-1} , and their bacterioruberin carotenoids (up to $18 \mu\text{g l}^{-1}$) imparted an intensely red color to the brine. This community remained throughout the experiment, without any major decline for more than two years. To prove that further algal development was limited by the availability of phosphate after the initially added phosphate had been taken up by the microbial community and was incorporated in the biomass, we added an additional $1 \mu\text{M}$ orthophosphate in November 2004. This addition quickly resulted in a renewed development of *Dunaliella*. A similar long-living microbial bloom was obtained in a pond that had received a mixture of 70% Dead Sea water–30% Red Sea and $5.5 \mu\text{M}$ phosphate (Figure 1, right panels). This experiment was a continuation of an experiment set up in July 2002, documented earlier (Oren et al. 2004). It shows once more that such microbial blooms can be sustained for long periods—in this case for over three years.

The examples shown in Figure 1 are part of a more extensive set of experiments in which we examined the effect of different parameters on the timing and extent of microbial development in Dead Sea–Red Sea mixtures. Some of the early experiments have been described in an earlier paper (Oren et al. 2004). Without added phosphate no significant algal and archaeal blooms were observed. Insoluble finely powdered rock phosphate (apatite) did not trigger blooms (not shown).

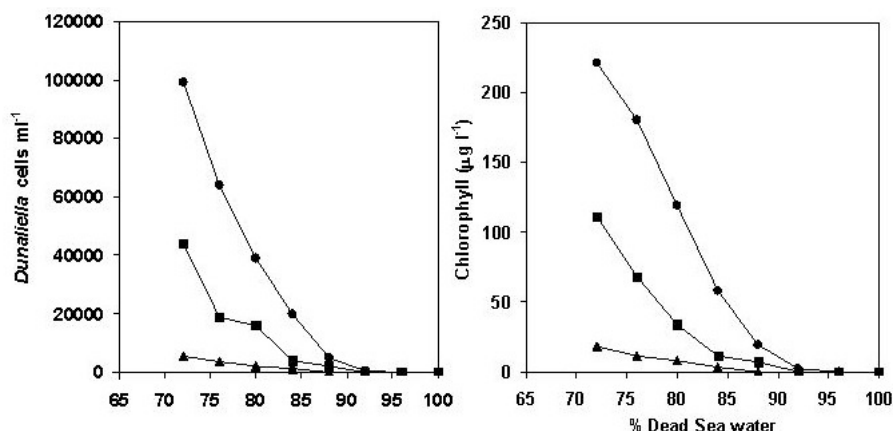


Figure 2—Development of *Dunaliella* cells and chlorophyll in a laboratory simulation experiment in which Dead Sea–Red Sea water mixtures were incubated for 25 days at 30°C in the light in the presence of 1 (▲), 2.5 (■) and 5 mM KH_2PO_4 (●) and an inoculum of 500 *Dunaliella* cells ml^{-1} , whereafter the density of *Dunaliella* cells (left panel) and the chlorophyll content of the water (right panel) were determined.

To further test the boundary conditions that enable the onset of an algal bloom in Dead Sea–Red Sea water mixtures, we set up a laboratory simulation experiment in which different mixtures were supplemented with different concentrations of orthophosphate and an inoculum of *Dunaliella*. After incubation in the light for four weeks, algae developed only when the concentration of Dead Sea water in the mixtures was below 90%, and the rate at which the cells multiplied increased with decreasing salinity of the water mixture. The extent of the algal growth obtained was a function of the concentration of phosphate added (Figure 2). No significant further changes were noted when incubation was continued for three additional weeks. These results confirm and extend laboratory simulation experiments performed in the early 1980s in which Dead Sea water was diluted with freshwater (Oren & Shilo 1985).

DISCUSSION

Compared to all other aquatic environments of lower salinity, the Dead Sea is a very simple ecosystem. Higher animals are absent, and protozoa, if they are present at all, do not appear to play a significant role in regulating community densities of unicellular algae and heterotrophic prokaryotes. The main players are one type of primary producer—the alga *Dunaliella*, and several species of halophilic Archaea (Kaplan & Friedmann 1970; Oren 1988, 1997). Figure 3 presents a general model of the biological processes that occur in the aerobic water column of the Dead Sea, the organisms involved, and some of the interrelationships between the biota. This model is based on observations of the dynamics of algal and archaeal communities in the lake, laboratory simulations, as well as field-scale simulations such as documented in the present study.

Undiluted Dead Sea water is too harsh an environment even for *Dunaliella*, the best salt-adapted alga known. Therefore algal blooms, and subsequent mass development of Archaea that live at the expense of organic material produced by the algae, can only occur after dilution with less saline water. Analysis of the biological events following the rainy winters of 1979–1980 and 1991–1992 have provided ample illustration of this (Oren & Shilo 1982; Oren 1983; Oren & Gurevich 1995; Oren et al. 1995), as have simulation experiments (Oren & Shilo 1985; Figure 2 in the present study). One of the organic compounds produced in massive amounts by *Dunaliella* is glycerol, used by the algae to provide osmotic stabilization (Ben-Amotz & Avron 1973). Evidence has accumulated that this glycerol is probably one of the major nutrients used by the halophilic Archaea.

Phosphate is clearly the limiting nutrient that governs the extent of microbial blooms in the lake. Inorganic nitrogen is plentifully available in the form of ammonium ions (Nissenbaum et al. 1990; Stiller & Nissenbaum 1999) but phosphate concentrations are low. Stiller & Nissenbaum (1999) and Nissenbaum et al. (1990) reported dissolved phosphate levels in the lake of about 1 μM . This value is approximate, due to the difficulty in performing high-precision chemical analyses in the presence of molar concentrations of other interfering salts. Furthermore, little information has been obtained on the spatial and temporal variation in the concentration of dissolved phosphate in the Dead Sea water column. In any case, the dramatic response of the *Dunaliella* community to phosphate addition in laboratory and field-scale simulation experiments shows its importance as a key nutrient controlling ecological processes in the Dead Sea. Following uptake by the algae, the phosphate becomes fixed in the algal and archaeal biomass. Addition of more phosphate provides the opportunity for renewed algal growth (Figure 1).

The simulation studies documented in Figure 1, as well as in an earlier publication (Oren et al. 2004), show that mass development of *Dunaliella* is generally followed by a rapid decline. The causes of this decline are still poorly understood. Following the 1992 spring bloom of the alga in the lake, the cells formed cyst-like structures, possibly zygotes, which sank to the bottom (Oren et al. 1995). Evidence has been obtained that such thick-walled cysts serve as the inoculum that enables rapid development of *Dunaliella* in the Dead Sea as soon as the upper water layers become diluted by freshwater floods (Oren 1999; Oren & Ben-Yosef 1997). However, we never observed formation of such cysts in the experimental outdoor ponds.

While algal blooms, both in the Dead Sea itself and in the pond simulation experiments, were always of limited duration, the Archaea remained present for over three years, both in the lake (Oren 1983; Oren & Gurevich 1995) and in the experimental ponds, and the mesocosms remained as brightly red colored as when the bloom first started (Figure 1). It has been suggested that the halophilic Archaea in the Dead Sea can to some extent use light energy absorbed by the retinal pigment bacteriorhodopsin as an energy source for maintenance (Oren & Shilo 1981; Oren 1983). Little is known about the factors that remove archaeal cells from the Dead Sea water column. Overturn of the water column with mixing of the Archaea-rich upper layer with the lower water masses has been a major factor in the decrease in prokaryote densities following the 1980–1982 and 1992–

1995 blooms (Oren 1985, 1988, 2000, 2003). Bacteriophages may also be involved in regulating archaeal community densities in the lake, as direct electron microscopic examination revealed large numbers of phage-like particles (Oren et al. 1997). However, their true impact on the community dynamics has never been ascertained (Oren 1999).

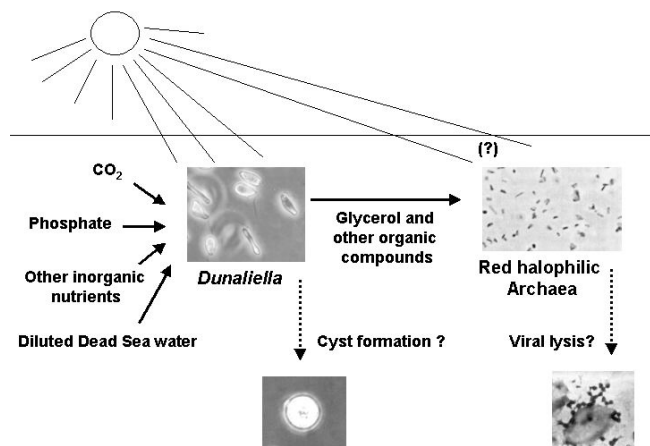


Figure 3—Schematic representation of the processes that govern the development of algal and archaeal blooms in the Dead Sea water column.

Although the microcosms simulate the events observed during natural microbial blooms in the Dead Sea to a large extent, it should be realized that the closed system formed by the shallow ponds differs from the conditions in the lake in certain important aspects. In the lake, particles can sediment to the bottom, and the nutrients bound to them will then no longer be available in the upper water layer. In the mixed microcosm systems, nutrients once added will remain present for indefinite times.

The Dead Sea, while unique in its ionic composition, is not the only hypersaline lake whose biology is dominated by *Dunaliella* and halophilic Archaea. The properties of the Dead Sea as a biotope can be compared to some extent with those of Great Salt Lake, Utah. The water level and the salt concentrations of Great Salt Lake have also been subject to major fluctuations in the past century (Stephens 1990). There has never been a systematic monitoring program of the communities of algae and of prokaryotes, and measurements of community densities and dynamics have been infrequent. Nitrogen rather than phosphorus is the inorganic nutrient that limits algal production (Post 1977; Stephens & Gillespie 1976). After more than two decades in which hardly any microbiological studies were performed in the lake, interest in the archaea and other

microorganisms in Great Salt Lake has recently been renewed (Baxter et al. 2005).

Understanding the factors that trigger the development of microbial blooms and determine their longevity is important in the planning of the Red Sea–Dead Sea water carrier (Gavrieli et al. 2005; Oren et al. 2004). A permanent stratification is likely to become established as the upper layers of the lake will become diluted with much less dense Red Sea water. When the upper layers become diluted by more than 10% by the waters from the Red Sea, combined with any flood waters that naturally enter the Dead Sea each winter, conditions are established for the development of blooms. The extent of these blooms will be a direct function of the availability of phosphate. The waters of the Gulf of Aqaba are very low in phosphate (concentrations in surface waters are generally between 0–5 nM, with maximal values up to 50 nM following winter mixing) (Stihl et al. 2001; A.F. Post, personal communication), but other sources (phosphate entering with flood waters from the catchment area, anthropogenic sources) can be quantitatively far more important. The results of simulation experiments documented in this study show that the conditions that lead to the formation of a microbial bloom in the Dead Sea are now quite well understood. They also show that such blooms, once formed, can remain present for long periods and determine to a large extent the properties of the lake for many years.

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Diel Variability and Community Metabolism in African Soda Lakes

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ABSTRACT

Information theory based indices of predictability were used to quantify seasonal differences in diel variability of dissolved oxygen and temperature in Lakes Elmenteita and Sonachi. Predictability of patterns in diel variability was high in both lakes for water temperatures and dissolved oxygen. Community metabolism was measured in Lake Elmenteita, a shallow, Kenyan soda lake based on a series of vertical profiles of dissolved oxygen and temperature measured about monthly over several days for 13 periods from February 1973 to May 1974. Variations in areal oxygen content at successive intervals throughout each day and night were corrected for air-water oxygen exchange to calculate net free water oxygen change. Maximal rates of increase usually occurred in late morning or early afternoon and ranged from 0.3 to 2.1 g O₂ m⁻² h⁻¹. Rates of change were summed to determine night-time respiration and gross photosynthesis (day-time sum plus night-time respiration); gross photosynthesis ranged from 1.5 to 18.2 g O₂ m⁻² d⁻¹.

INTRODUCTION

In tropical lakes where diel changes often exceed seasonal changes in physicochemical conditions, planktonic organisms must tolerate the full range of conditions since their generation time is longer than one day. However, the diel variability itself varies, and the predictability of the periodicity in the diel cycles is likely to have ecological relevance. Indeed, patterns of temporal fluctuation are of long-standing and broad interest in ecology and limnology (Colwell 1974; Talling & Lemoalle 1998). While time series analysis, such as spectral or wavelet analysis, are powerful techniques (Platt & Denman 1975; Keitt & Fischer 2006), insufficient data often preclude their application to ecological systems. Alternatively, the information theory based statistics derived by Colwell (1974) provide a suitable approach to measure the predictability of diel cycles in lakes.

Evidence that inland waters are an important component in the processing of carbon on a global scale has increased interest in the metabolism of lakes (Cole et al. 2007). Since photosynthetic and respiratory activities are indicated by variations in dissolved oxygen and carbon dioxide, measurements of these gases in lakes provide metrics of metabolism. Although recent technological advances have improved capabilities to determine concentrations of these

gases, the theoretical basis for calculations of metabolism and studies in a variety of ecosystems date back over 50 years. In particular, pronounced diel variation in thermal stratification and dissolved oxygen has been documented in productive, tropical African lakes (e.g., Talling 1957; Ganf & Horne 1975), and these diel variations permit robust calculations of community metabolism based on free-water changes in dissolved oxygen (e.g., Melack & Kilham 1974; Melack 1982).

Alkaline, saline (soda) lakes in tropical eastern Africa undergo strong diel heating and cooling with related variations in dissolved oxygen. Measurements of diel cycles of temperature and dissolved oxygen over one to several days about monthly for a period spanning 15 months in Lakes Elmenteita and Sonachi permit evaluation of the periodicity in the diel cycles and calculation of community metabolism. These results support Duarte et al. (2008) who suggest saline lakes with pH above 9 tend to be net autotrophic and demonstrate the regularity of diel variations in equatorial saline lakes.

MATERIALS AND METHODS

Lake Elmenteita (0°27'S, 36°15'E) lies at 1776 m above sea level in central Kenya within a 590 km² endorheic basin. The lake was ca. 20 km² in area and ranged in mean depth from 0.65 to 1.1 m during the period of study from February 1973 to May 1974 (Melack 1976). The alkalinity and pH (ca. 9.9) are high. Electrical conductance varied from 19.1 to 40.2 mS cm⁻¹ as the lake experienced evaporative concentration during low rainfall. No aquatic macrophytes grew in the lake, and concentrations of chlorophyll in phytoplankton ranged from 16 to 310 mg m⁻³. Further limnological information about Lake Elmenteita is provided in Melack (1979a, 1981, 1988), Melack et al. (1983), Tuite (1981) and Kalff (1983). January and February usually have the highest temperatures, least rainfall and highest winds; climate statistics are summarized in Melack (1981, 1988).

Lake Sonachi (0°47'S, 36°16'E) lies in a volcanic crater 1980 m above sea level in central Kenya. During the period study (February 1973 to May 1974) the lake decreased in area from 16.1 ha to 14.7 ha and in maximum depth from 6.5 to 5.5 m. No streams enter or leave the lake. Lake Sonachi was chemically stratified with a mixolimnion varying from 6.6 to 8.4 mS cm⁻¹, a monimolimnion varying

from 10.3 to 17 mS cm⁻¹ and a chemocline at about 4 m during the period of study. The waters are high in alkalinity and pH (9.6). Chlorophyll concentrations ranged from 29 to 74 mg m⁻³. Further limnological information is provided in Melack (1981, 1982), Melack et al. (1981) and MacIntyre & Melack (1982) and references cited in those papers.

Measurements were made at a station located off a point in the southeastern end of the Lake Elmenteita and accessed by inflatable boat. In Lake Sonachi measurements were made mid-lake from a floating platform that permitted day and night presence. The study spanned February 1973 to May 1974 with measurements made about monthly over one to three for 13 periods in Lake Elmenteita and 10 periods in Lake Sonachi.

The vertical distribution of dissolved oxygen was measured with a submersible Clark polarographic electrode and Yellow Springs Instruments (YSI) model 51A meter (precision ca. 0.2 mg O₂ l⁻¹). The Miller method (Ellis & Kanamori 1973) was used to calibrate the polarographic electrode. The vertical distribution of temperature was measured with a thermistor and Wheatstone bridge circuit (YSI model 51A meter readable to 0.05°C or a YSI model 46 meter readable to 0.05°C). The thermistors were calibrated against a certified thermometer accurate to 0.01°C. Measurements were made every 0.2 m or 0.5 m in Lake Elmenteita and every 0.5 m in Lake Sonachi. Wind speeds were measured periodically through the day and night with a hand-held anemometer at about 1 m above the water surface, and concurrent observations of wave heights were recorded.

To characterize temporal variability, Colwell's (1974) information theory based statistics were applied to temperature and dissolved oxygen data from Lakes Elmenteita and Sonachi. Before computing the statistics, the data were interpolated linearly to create a matrix with regular times. The values of the statistics depend on the grouping in increments of the temperatures or dissolved oxygen concentrations. In Lake Elmenteita, calculations were done using 1 and 2°C intervals for temperature and 1.5 and 3 mg l⁻¹ intervals for dissolved oxygen. In Lake Sonachi, calculations were done using 1, 2 and 3°C intervals and 1.5 and 3 mg l⁻¹ intervals. In the time domain, the calculations were done for each contiguous set of data, for aggregates of 2 or 3 periods and for all the periods.

Community metabolism was calculated based on the series of vertical profiles of dissolved oxygen and temperature. Variations in areal oxygen content at successive intervals throughout each day and night were corrected for air-water oxygen exchange to calculate free water oxygen change. Methodological details for the approach applied to Lake

Sonachi are provided in Melack (1982), and those applied to Lake Elmenteita are described below.

The air-water exchange of oxygen (F , mg O₂ m⁻² h⁻¹) was calculated with the expression:

$$F = D/T (C_w - C_{sat})$$

Where T is the thickness of the hypothetical stagnant boundary layer (m), D is the diffusion coefficient for oxygen (m² h⁻¹) at the approximate salinity and temperature of the lake, C_w is the concentration of oxygen measured in the lake (mg m⁻³), and C_{sat} is the oxygen concentration in water at saturation (mg m⁻³). Measurements of T , as a function of wind speed, provided in Peng & Broecker (1980) were used. Though recent formulations of gas exchange as a function of wind speed are available, they do not significantly alter the values used here.

To determine the solubility of oxygen in soda lakes, samples of lake water from four Kenyan soda lakes (Elmenteita, Nakuru, Simbi and Sonachi) with conductances ranging from 7.7 to 34.1 mS cm⁻¹ plus standard seawater and distilled water were air-equilibrated at 20°C as recommended by Carpenter (1966). Concentrations of dissolved oxygen were measured using an in-line gas stripper and injected into a gas chromatograph with a helium carrier and silica gel molecular sieve column and detected with thermal conductivity. The distilled water and seawater values were within 0.3% of those in Carpenter (1966). Since the conductance of Lake Elmenteita changed during the course of the study, the relation between oxygen solubility and conductance was used to determine the concentration of dissolved oxygen at equilibrium after adjusting for altitude and temperature.

Procedures for calculation of lake metabolism based on free-water measurements of dissolved oxygen, proposed in the 1950s (Odum 1956; Talling 1957), have been modified based on practical and theoretical perspectives (e.g., Odum & Hoskin 1958; Welch 1968; Bella 1970; Hornberger & Kelly 1974) and, recently, based on the availability of reliable probes that record continuously (Lauster et al. 2006; Staehr & Sand-Jensen 2007; Coloso et al. 2008). The approach applied here is similar that used by Melack & Kilham (1974) and produces results comparable to those published for other tropical African lakes.

The amount of dissolved oxygen per square meter was determined by planimetry of plots of vertical profiles uncorrected for changes in lake volume with depth. The rate of change of areal concentrations per hour was then calculated and corrected for air-water exchange. Net

daytime oxygen (NOC_d) change was determined by planimetry of the rate of change plots. Average nighttime respiration (R_n) was determined for each set of diel plots and added to the NOC_d to calculate gross photosynthesis (GP_d) during daylight. The assumption that nighttime respiration can be applied during the day may not be correct, but with no practical alternative, this assumption continues to be routinely made (e.g., Staehr & Sand-Jensen 2007).

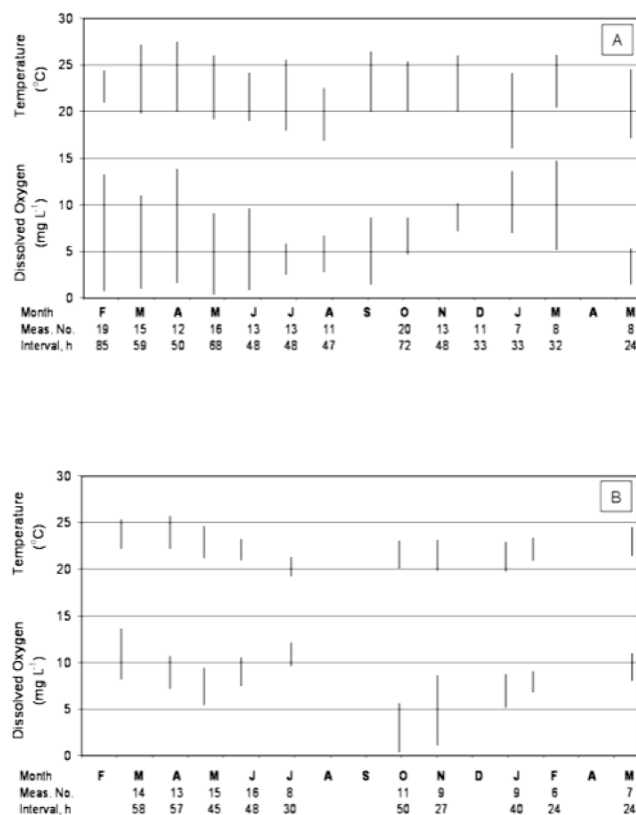


Figure 1—(a) Diel range from maximum to minimum for water temperature and dissolved oxygen at 0.2 m depth in Lake Elmenteita from February 1973 to May 1974. Interval is the length of the period in hours over which measurements (meas. no.) were made. (b) Diel range from maximum to minimum for water temperature and dissolved oxygen at 0.5 m depth in Lake Sonachi from March 1973 to May 1974. Interval is the length of the period in hours over which measurements (meas. no.) were made.

RESULTS AND DISCUSSION

Temporal Variability of Temperature and Dissolved Oxygen

In Lake Elmenteita, diel variations in water temperature and dissolved oxygen were determined from February 1973 to May 1974 during 13 periods that spanned between 24 and 85 hours with between 7 to 20 profiles during each period (Figure 1A). At 0.2 m over all the periods, maximum and

minimum temperatures ranged from 27.4°C to 16.1°C, and dissolved oxygen ranged from 14.7 mg l⁻¹ to 0.4 mg l⁻¹. The maximum diel differences during a contiguous sequence of days were 8.0°C and 12.5 mg l⁻¹, and minimum differences were 3.4°C and 2.9 mg l⁻¹. The magnitudes and ranges of temperature and dissolved oxygen near the bottom were slightly less than those at 0.2 m; dissolved oxygen near the bottom reached zero or nearly zero during the measurements from February to May 1973.

Vertical stratification of dissolved oxygen in Lake Elmenteita varied on diel and monthly time scales. In mid-February 1973 the lake had a dense suspension of phytoplankton with approximately 300 mg chlorophyll m⁻³ and Secchi disk transparency of 0.12 m (Melack 1979a 1988) and pronounced diel variation in the magnitude and vertical distribution of dissolved oxygen (Figure 2A). In mid-June 1973 diel variation and stratification of dissolved oxygen had lessened in concert with the decline in chlorophyll to 160 mg m⁻³ and increased Secchi disk transparency to 0.2 m (Figure 2B). By late August 1973 dissolved oxygen nearly lacked vertical stratification and had muted diel variation during a period with persistent winds generally between 4 to 8 m s⁻¹, chlorophyll of about 40 mg l⁻¹ and Secchi disk transparency of 0.35 m (Figure 2C).

In Lake Sonachi, diel variations in water temperature and dissolved oxygen were determined from March 1973 to May 1974 during 10 periods that spanned between 58 and 24 hours with between 16 to 6 profiles during each period (Figure 1B). At 0.5 m over all the periods, maximum and minimum temperatures ranged from 25.3°C to 19.3°C, and dissolved oxygen ranged from 13.6 mg l⁻¹ to 0.4 mg l⁻¹. The maximum diel differences during a contiguous sequence of days were 3.5°C and 7.5 mg l⁻¹, and minimum differences were 1.3°C and 2.2 mg l⁻¹. Diel and monthly variations in vertical profiles of dissolved oxygen in Lake Sonachi are described in Melack (1982). As a consequence of persistent chemical stratification (MacIntyre & Melack 1982), anoxia was perennial below the chemocline at about 4 m and on occasion reached 2 m.

Predictability (P) of diel patterns in temperature and dissolved oxygen has two components: constancy (C) and contingency (M) (Colwell 1974). Predictability ranges from 0 to 1, and C and M contribute varying proportions. P is maximal if the same temperature or dissolved oxygen concentration occurs at the same time every day and minimal if the values are equally probable at any time. Constancy is complete if the state is the same at all times for all days. Contingency is complete if the state is different

at each time, but the pattern is the same on every day. Hence, a periodic diel cycle would have high predictability because of high contingency. As an example of the range of values of P, Colwell (1974) analyzed 10 years of monthly precipitation data for four locations. Uaupes, located in the humid rainforests of Brazil, had a P value of 0.75, while Miami, Florida, had a P value of 0.46, and Acapulco, Mexico, and Bella Coola, British Columbia, had intermediate values.

Table 1—Predictability of diel variations in water temperature (at 0.2 m and grouped in 1°C intervals) and dissolved oxygen (at 0.2 m and grouped in 1.5 mg O₂ l⁻¹ intervals) in Lake Elmenteita for 5 periods in 1973 and 1974. First number is predictability; within parentheses first number is the percent contribution of constancy to predictability and second number is the percent contribution of contingency to predictability; see text for further explanation.

	Temperature	Oxygen
February	0.84 (67,33)	0.66 (25,75)
March-April	0.68 (37,63)	0.58 (20,80)
May-June	0.68 (45,55)	0.67 (31,69)
July-August	0.71 (38,62)	0.83 (67,33)
September-November	0.75 (31,69)	0.48 (63,37)

Table 2—Predictability of diel variations in water temperature (at 0.5 m and grouped in increments of 1°C intervals) and dissolved oxygen (at 0.5 m and grouped in increments of 1.5 mg O₂ l⁻¹ intervals) in Lake Sonachi for 4 periods in 1973 and 1974. First number is predictability; within parentheses first number is the percent contribution of constancy to predictability and second number is the percent contribution of contingency to predictability; (see text for further explanation).

	Temperature	Oxygen
March-April	0.74 (50,50)	0.71 (68,32)
May-June	0.66 (50,50)	0.72 (74,21)
October-November	0.66 (54,46)	0.65 (45,55)
January-February	0.64 (72,28)	0.82 (64,36)

To evaluate the P, C and M of the diel periodicities of temperature and dissolved oxygen, data from 0.2 m grouped in increments of 1°C and 1.5 mg l⁻¹ intervals in Lake Elmenteita and from 0.5 m grouped in increments of 1°C and 1.5 mg l⁻¹ intervals in Lake Sonachi are presented for

sets of periods with similar numbers of measurements spanning several diel cycles (Figure 1). These data are representative of the diel patterns in the water column in Lake Elmenteita and in the mixolimnion of Lake Sonachi.

The predictability and the percentage contributions of C and M differ through time for the two parameters and between the two lakes (Tables 1 and 2). In Lake Elmenteita, predictability of the diel patterns of temperature was high and similar (0.68 to 0.84) among the periods; with the exception of February 1973, contingency accounted for a greater proportion of P than constancy. Diel patterns in dissolved oxygen had slightly lower and more variable P than those for temperature; in three of the five periods contingency was more important than constancy. In Lake Sonachi, predictability of the diel patterns of temperature was high and similar (0.64 to 0.74) among the periods. Contingency and constancy accounted for about equal proportions of P except for the January-February period when constancy dominated. Diel patterns in dissolved oxygen also had high and similar P (0.65 to 0.82); in three of the four periods constancy was more important than contingency. As the separate sets of contiguous data are aggregated to include all the periods, the predictabilities decrease to about half the values in Tables 1 and 2. This is an expected result because of the shifting ranges of values evident in Figure 1. Although diel periodicity is present, the temperature or dissolved oxygen values at a particular time of day varies among the periods resulting in lower P.

To evaluate the implications for planktonic organisms of the differences in P, C and M requires experimental studies and comparative investigations from a variety of lakes. Although time series of diel measurements are available for few tropical lakes (e.g., Lake George, Uganda, Ganf & Viner 1973; Lake Calado, Brazil, Melack & Fisher 1983), the current availability of temperature and dissolved oxygen probes and data logging systems should encourage further investigation of temporal patterns in tropical lakes.

Community Metabolism

Diel changes in dissolved oxygen, expressed as g O₂ m⁻², for the three periods in Figure 3, illustrate a strong day to night alternation from increase to decrease and the reduction in amplitude associated with the decline in phytoplankton abundance from February to August (see text below). In February, the values ranged from a minimum of 0.5 g O₂ m⁻² to a maximum of 9.8 g O₂ m⁻² while in August the values ranged from a minimum of 2.2 g O₂ m⁻² to a maximum of 5.4 g O₂ m⁻².

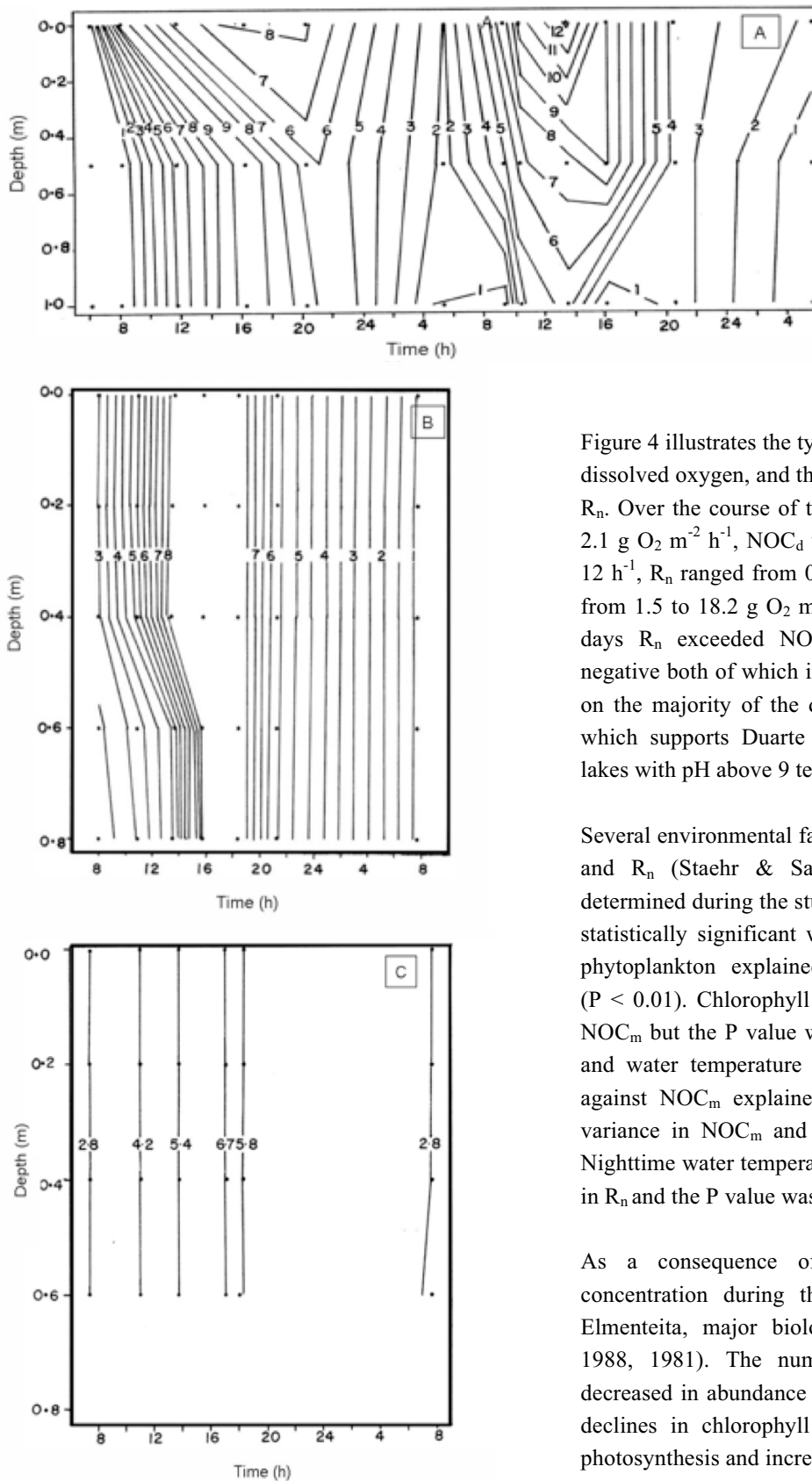


Figure 2—Time-depth diagrams of dissolved oxygen ($\text{mg O}_2 \text{l}^{-1}$) in Lake Elmenteita (A) 17 to 19 February 1973, (B) 20 to 21 June 1973 and (C) 24 to 25 August 1973. Dots represent measurement depths and times.

Figure 4 illustrates the typical diel cycle in rate of change of dissolved oxygen, and the area represented by NOC_d and by R_n . Over the course of the study NOC_m varied from 0.3 to $2.1 \text{ g O}_2 \text{ m}^{-2} \text{ h}^{-1}$, NOC_d varied from -0.9 to $10.4 \text{ g O}_2 \text{ m}^{-2} \text{ 12 h}^{-1}$, R_n ranged from $0.9 \text{ g O}_2 \text{ m}^{-2} \text{ 12 h}^{-1}$, and GP_d varied from 1.5 to $18.2 \text{ g O}_2 \text{ m}^{-2} \text{ 12 h}^{-1}$ (Table 3). On 7 of the 16 days R_n exceeded NOC_d and on one day NOC_d was negative both of which indicate net heterotrophy. However, on the majority of the days the lake was net autotrophic, which supports Duarte et al. (2008) who suggest saline lakes with pH above 9 tend to be net autotrophic.

Several environmental factors may influence NOC_m , NOC_d and R_n (Staehr & Sand-Jensen 2007), but few were determined during the study and weak correlations were not statistically significant with one exception: chlorophyll in phytoplankton explained 54% of the variance in NOC_d ($P < 0.01$). Chlorophyll explained 23% of the variance of NOC_m but the P value was 0.08. Regressions of insolation and water temperature and chlorophyll in phytoplankton against NOC_m explained in each case only 15% of the variance in NOC_m and P values were greater than 0.20. Nighttime water temperature explained 19% of the variance in R_n and the P value was 0.18.

As a consequence of low rainfall and evaporative concentration during the course of the study in Lake Elmenteita, major biological changes occurred (Melack 1988, 1981). The numerically dominant phytoplankton decreased in abundance precipitously in parallel with large declines in chlorophyll concentration and phytoplankton photosynthesis and increased transparency and benthic algal photosynthesis. Therefore, the variations in community metabolism reflect these changes and a shift in the relative importance of planktonic versus benthic photosynthesis and respiration.

Diel dissolved oxygen variations are influenced by photosynthesis and respiration, other oxidation and reduction reactions, mixing and advection within the lake and air-water gas exchanges. In the shallow, productive, well oxygenated waters of Lake Elmenteita, biological processes are likely to be dominant, although air-water gas exchange and possibly advection will influence the diel oxygen balance. Daytime variations in dissolved oxygen are fairly well characterized based on profiles measured every few hours (Figures 1, 2 and 3).

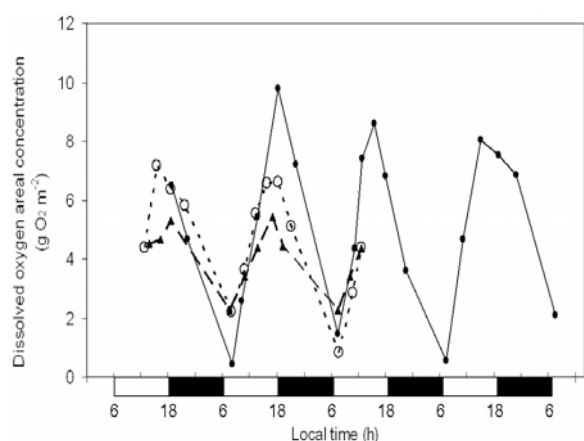


Figure 3—Diel variations in areal dissolved oxygen ($\text{g O}_2 \text{m}^{-2}$) in Lake Elmenteita over several days in February (●), June (○) and August (▲) 1973.

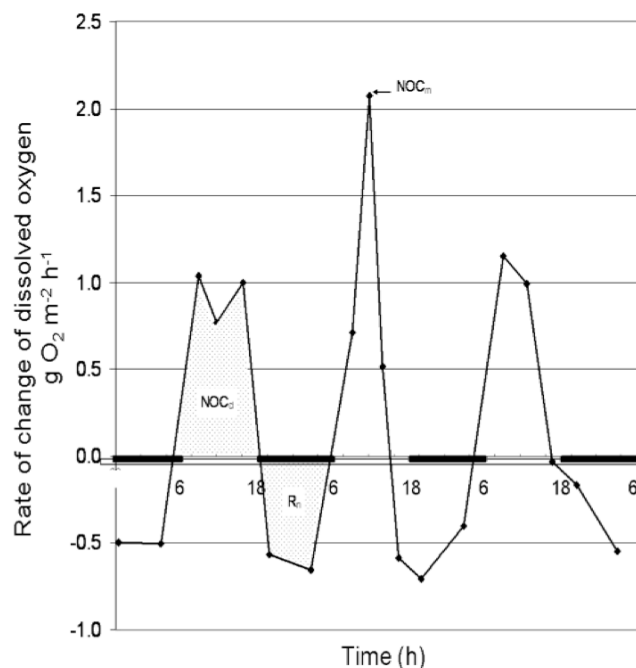


Figure 4—Rate of change of air-water exchange corrected dissolved oxygen in Lake Elmenteita 16 to 20 February 1973. The horizontal solid bar indicates night and the open bar indicates day. The area under the positive rate of change curve and above the open bar represents NOC_d and the highest value is NOC_m . R_n is the area above the negative rate of change line and the solid horizontal bar.

Table 3—Metabolic measurements based on free water, dissolved oxygen measurements in Lake Elmenteita from February 1973 to May 1974. NOC_m is maximal, daytime net change in dissolved oxygen, NOC_d is day-time net dissolved oxygen change, R_n is nighttime respiration and GP_d is the gross photosynthesis during day-time. See Figure 4 and text for further explanation.

Date	NOC_m $\text{g O}_2 \text{m}^{-2} \text{h}^{-1}$	NOC_d $\text{g O}_2 \text{m}^{-2} (12 \text{ h})^{-1}$	R_n $\text{g O}_2 \text{m}^{-2} (12 \text{ h})^{-1}$	GP_d $\text{g O}_2 \text{m}^{-2} (12 \text{ h})^{-1}$
17-February-73	1.0	9.3	5.4	15.4
18-February-73	2.1	6.5	4.9	13.4
19-February-73	1.2	8.1	4.6	14.2
21-March-73	0.8	2.9	5.6	9.6
20-April-73	1.3	10.4	5.8	18.2
20-May-73	1.6	4.7	6.6	8.3
21-May-73	0.7	3.7	4.4	8.4
20-June-73	0.8	6.9	2.9	10.4
21-July-73	0.3	-0.9	1.9	1.5
24-August-73	0.4	1.1	3.3	4.9
1-October-73	0.5	1.4	4.2	4.7
2-October-73	0.7	3.0	1.2	6.3
1-November-73	1.0	3.2	1.5	4.9
14-December-73	0.5	1.8	0.9	4.9
28-January-74	1.0	-	-	-
7-March-74	1.1	5.5	3.2	10.6
9-May-74	0.3	1.1	1.7	3.3

Measurements of photosynthesis by phytoplankton and benthic algae (Melack 1981, 1988) demonstrate high rates. Air-water gas exchange is related to wind speeds and oxygen saturation in surficial waters. Winds were generally low to moderate (calm to 4 m s⁻¹), but gusts associated with afternoon storms ranged from 5 to 10 m s⁻¹. Surficial waters were up to 9 mg O₂ l⁻¹ above or 5.8 mg O₂ l⁻¹ below saturation, but mostly were within 1 to 3 mg O₂ l⁻¹ of saturation. Synoptic sampling throughout the lake during 9 months revealed uniform Secchi disk transparency and limited variation in abundances of the most important phytoplankton (Melack 1976, 1988); these results suggest advection of water with different characteristics is unlikely.

Table 4—Free water estimates of metabolism in eastern African soda lakes. NOC_m is maximal, day-time net change in dissolved oxygen, and GP_d is the gross photosynthesis. Asterisks denote air-water exchange corrected values. Sources of data are as follows: (1) Melack 1982; (2) Melack & Kilham 1974; (3) Melack 1979b; (4) Talling et al. 1973.

Lake	Country	NOC _m g O ₂ m ⁻² h ⁻¹	GP _d g O ₂ m ⁻² (12 h) ⁻¹
Sonachi ¹	Kenya	0.8 to 4.85*	-0.7 to 18.7*
Nakuru ²	Kenya	2.8, 2	36, 31*
Simbi ³	Kenya	2.8 to 12	--
Elmenteita	Kenya	0.3 to 2.1*	1.5 to 18.2*
Aranguadi ⁴	Ethiopia	3 to 6	43, 57
Kilotes ⁴	Ethiopia	0.9 to 4.5	11, 12

When estimates of gross photosynthesis obtained from free water approaches are compared with those from bottled samples, the free water estimates are often higher (cf. Melack 1982). In Lake Elmenteita, sequential deployments of bottled samples were made to obtain measurements of gross photosynthesis by phytoplankton in concert with the free-water profiles of dissolved oxygen (Melack 1976). A subset of these measurements made around mid-day, called GPb, were compared to gross photosynthesis calculated as NOC plus average hourly R_n, called GPh, for approximately the same intervals as the bottle measurements. The ratio GPh:GPb varied from 1 to 2 with an average of 1.7 (n = 9) and indicates that the bottle measurements are likely to underestimate gross photosynthesis.

Free water estimates of community metabolism are available from only five other saline lakes in eastern Africa, and only Lake Sonachi has more than a couple of

measurements of daily gross photosynthesis (Table 4). Maximal daytime net dissolved oxygen changes and daily gross photosynthesis in Lake Elmenteita are on the low end of the range reported for these lakes, but high in comparison to many inland waters. The highest values reported for Lakes Aranguadi, Simbi and Nakuru occurred during periods with very abundant *Spirulina platensis* and are near the upper limits found in natural lakes.

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Effect of Osmotic Downshock Treatment on the Yield of Ectoine Synthesized by *Halomonas* sp. EG6

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The halophilic bacterium strain EG6 was isolated from the Burg el Arab solar saltern lake in Egypt. Strain EG6 can grow in a wide range of NaCl concentrations up to 4 M. Its phylogenetic position was established by 16S rRNA gene sequencing as a member of the genus *Halomonas*, showing with 94, 95 and 97% similarity to *Halomonas ventosae* DSM 15911, *H. elongata* ATCC 33173 and *H. eurihalina* ATCC 49336, respectively. Morphological, physiological and biochemical tests were performed to characterize strain EG6. It produced ectoine as the main compatible solute for osmotic stress adaptation. Our aim was to establish the best conditions of osmotic downshock treatment required for high yield of ectoine production, comparing strain EG6 with the type strain *H. elongata* IFO (NBRC) 15536. We subjected strain EG6 to osmotic downshock treatment with different NaCl concentrations from 2 M to 0-0.7 M. HPLC and NMR analyses were carried out to quantify ectoine concentration and confirm its purity. Cells of strain EG6 had a great flexibility to withstand sudden changes in NaCl concentration, even when cells were subjected to osmotic downshock treatment from 2 M to 0.3 M NaCl. A total yield of ectoine of about 3.7 g/l was released after 7 days of osmotic downshock. This yield was the highest obtained when using a range of NaCl concentrations (0-0.7 M) for osmotic downshock

(Figure 1). On the other hand, *H. elongata* IFO15536 only released 2.89 g/l ectoine under the same conditions. For comparison, *Brevibacterium* sp. JCM 6894 released 2.46 mg/l as the highest yield of ectoine during 7 days of osmotic downshock treatment from 2 M to deionized water. These results indicate that strain EG6 can compensate the loss of ectoine with high efficiency. The percentage of release was calculated during osmotic downshock under the above-described conditions. The highest released rates were observed for a downshock from 2 to 0-0.2 M NaCl (95%–83%), but the concentrations of ectoine synthesized were low, while a moderate release rate (72%) was obtained for cells subjected to osmotic downshock from 2 to 0.3 M NaCl. This confirmed that the maximum yield of ectoine released not only depends on the amount of ectoine synthesized by the cells but also on the release rate. Growth of strain EG6 was highly increased during downshock treatment as compared to *H. elongata* IFO 15536. Thus, we concluded that 0.3 M NaCl was the optimum condition to obtain the highest yield of ectoine from strain EG6. The ectoine was found to be of high purity, suitable to be used for large scale ectoine production. *Halomonas* sp. EG6 may represent a new species that might be of interest for future studies.

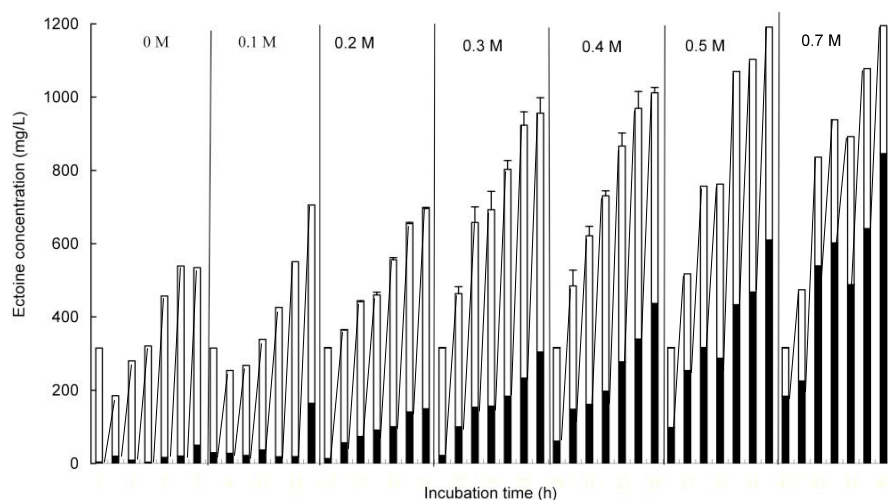


Figure 1—Synthesis and release of ectoine from strain EG6. Cells were grown in LB medium containing 2 M NaCl at 30°C for 24 h. After centrifugation, cells were subjected to osmotic downshock in the presence of different NaCl concentrations (0-0.7 M) for 10 minutes at 30°C. Subsequently the downshocked cells were incubated for further 24 hours in fresh medium with the same NaCl concentrations prior to shock. Open and shaded parts in each column indicate the amounts of ectoine released and remaining in the cells, respectively. Each culture was sampled 24, 48, 72, 96, 120, 144 and 168 hours after downshock. The values are the means \pm SD from two independent experiments.

Biodiversity of Inland Saltscapes of the Iberian Peninsula

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ABSTRACT

The Iberian Peninsula hosts a wide variety of inland saltscapes, from man-made solar evaporation salterns to hypersaline lakes and salt rivers, most of them unknown in other European countries. The present contribution deals with this diversity of saltscapes, briefly introduces their main features and locations, and presents a general overview of their conservation status. Some of their natural values are not even well understood by the scientists themselves, and therefore they are very rare and fragile and in need of protection. However, many of these natural saltscapes have been and are currently under threat, having been desiccated, used as waste dumps or polluted with irrigation runoff. With respect to artificial salinas, these have been halting their activity in the last decades. Generally, historically salt-making operations occurred where hypersaline environments already existed. These locations were converted into commercial operations. As these, too, are being phased out, the loss of production has negatively affected the halophilic plant and animal communities. These results are based on empirical field observations, and a comprehensive inventory and status report of Iberian saltscapes is needed to support the ideas presented here.

INTRODUCTION: IBERIAN SALTSCAPES

The presence of salts (especially common salt or sodium chloride, which will be referred to as “salt” hereonwards) in nature affects the behavior and physiology of individual species as well as the composition and diversity of plant and animal communities in many different ways. This effect does not only depend on extrinsic factors such as latitude, altitude, topography or climatic conditions of the site in which salt is present. Intrinsic factors such as the origin of the salt (brine sources, underground brine flows, rock salt, saline rivers, etc.), the dynamics of the salt delivery (seasonal, permanent), its concentration in water, soil or even air, etc. also affect all living beings at the site and often in different ways. Given the enormous amount of distinct landscape types that are associated to the presence of salt in nature and in fact define specific ecosystems (Montes & Martino 1987; Williams 1998), the Association of Friends of Inland Salinas (AFIS) has coined the term “saltscapes” to define them as “any landscape type whose elements are strongly influenced by the presence of salt and forms a defined ecosystem”. This definition is vague enough to include almost any type of saline habitat,

regardless of salinity, seasonality or origin of the salt and also includes some cultural landscape types that exist due to the action of man in some of these saltscapes, in search of salt as a mineral resource. Some saltscapes have been artificially created almost from scratch, by pumping underground brine for salt production, which otherwise would have never reached the surface. This is the case of many inland solar evaporation salinas, which have also been defined by the Association of Friends of Inland Salinas as those “saltscapes dominated by solar evaporation salt making sites fed by evaporitic brine sources” (Hueso & Carrasco 2008c). This type of saltscapes, inland salinas, is a typical Iberian phenomenon—even though it exists in many other regions of the world, but certainly in lesser amounts or concentrations—with remains of over 500 solar evaporation salt making sites away from the sea (Carrasco & Hueso 2008). However, many other types of inland saltscapes occur in Spain and Portugal, some examples of which are natural brine springs, hypersaline streams, salt meadows, salt/saline lakes, salt steppes, etc. Most of these saltscapes originate from the old Tethys Sea, which roughly covered the eastern half of the Iberian Peninsula 200 million years ago during the Triassic period (Figure 1) (Montes & Martino 1987; Comín & Alonso 1988). This sea evaporated and reinundated certain areas periodically, creating large evaporitic deposits in this region. Depending on later geological events (such as tectonic and karstic movements), these deposits have become more or less accessible for nature or man, in the form of layers, domes, diapirs, etc. Other saltscapes originate from the Miocene period, much later (5-10 million years ago), but with a similar geological evolution, and yet others are of endorheic origin, such as most saline lakes (Montes & Martino 1987; Comín & Alonso 1988). Some of these saltscapes include habitats protected by law, as will be seen below.

Although a proper inventory of saltscapes is pending, the regional authorities in Spain have started to conduct their own wetland inventories (M. Bernués, pers. comm.), including saline wetlands. The Association of Friends of Inland Salinas is now compiling this information in order to list all inland saline wetlands contained in the regional inventories and in other relevant references (Hueso & Carrasco, in preparation). Earlier work by Pardo (1948) resulted in a catalogue of the 2500 Spanish lakes existing at the time, in which he included 80 inland salt lakes. A later survey that focused only on continental Spain obtained a total of almost 5000 wetlands, of which 99 were inland saline wetlands and salterns (DGOH 1991). As can be seen

in coastal environments, as salinity quickly disappears on a short distance from its source (spring, stream, lake, etc.). Hypersaline sites are dominated by plant species typical of seashores and coastal salt marshes, but are rare and fragile when found far inland and at relatively high altitudes. Examples of these species are *Salicornia* sp., *Suaeda* sp., *Salsola* sp., *Arthrocnemum* sp., *Limonium* sp., *Glaux maritima*, etc. Waterplants such as *Ruppia maritima*, *Chara* sp., *Riella helicophylla*, etc. are also typical of hypersaline wetlands, and many are protected by European and regional laws (Montes & Martino 1987; Comín & Alonso 1988; Casado & Montes 1995; Sadoul et al. 1998; Cirujano & Medina 2002; Martín et al. 2003; Hueso & Carrasco 2008a).

Another particular type of saline wetland is represented by the hypersaline temporary streams or “ramblas” or “wadi complexes”, which are found in the southeastern tip of the Iberian Peninsula. These streams are “wide, usually dry channels flowing only in flash flood events, although sometimes small permanent or temporary streams, springs

and pools can be formed by groundwater seepage” (Vidal-Abarca et al. 1992; Moreno et al. 1996). Endemic to this type of habitat are a number of aquatic *Coleoptera* and *Heteroptera* (López-González 1997; Barahona et al. 2005; Velasco et al. 2006; Abellán et al. 2007; Sánchez-Fernández 2008).

In Portugal, hardly any scientific or policy literature can be found on local saltscapes. Most of these appear to be associated with estuarine or inland salterns (R. Neves, personal communication). However, it is worth noting that Portugal hosts an interesting example of a saline fen, located at the old Junqueira salt making site, a unique saltscapes within the Iberian Peninsula (Dias 2005).

With respect to other types of saltscapes, little literature exists and even less an inventory of sites, except those that have been protected (see Table 1). Also pending is an inventory of cultural values associated with wetlands, which is especially needed in the case of saltscapes (Viñals et al. 2002, 2005).

Table 1—Habitat types recognized by the 1992 European Commission Habitats Directive that can be considered as saltscapes (European Commission 1999).

Annex 1 ¹ code nr.	Habitat type
13	ATLANTIC AND CONTINENTAL SALT MARSHES AND SALT MEADOWS
1310	<i>Salicornia</i> and other annuals colonizing mud and sand Formations composed mostly or predominantly of annuals, in particular <i>Chenopodiaceae</i> of the genus <i>Salicornia</i> or grasses, colonizing periodically inundated muds and sands of marine or interior salt marshes.
1340*	Inland salt meadows Non-coastal natural salt basins made up of different habitat types consisting of zones of seepage of saline water, running or stagnant saline water, with typical halophilous vegetation and of reed beds at the edge of brackish waters.
14	MEDITERRANEAN AND THERMO-ATLANTIC SALT MARSHES AND SALT MEADOWS
1410	Mediterranean salt meadows (<i>Juncetalia maritimi</i>) Various mediterranean communities and tall rush saltmarshes dominated by <i>Juncus maritimus</i> and/or <i>J. acutus</i> ; short rush, sedge and clover saltmarshes and humid meadows behind the littoral, rich in annual plant species and in <i>Fabacea</i> , Mediterranean halo-psammophile meadows; Iberian salt meadows; halophilous marshes along the coast and the coastal lagoons; humid halophilous moors.
1420	Mediterranean and thermo-Atlantic halophilous scrubs (<i>Sarcocornetea fruticosi</i>) Perennial vegetation of marine saline muds (schorre) mainly composed of scrubs, essentially with a Mediterranean-Atlantic distribution
1430	Halo-nitrophilous scrubs (<i>Pegano-Salsoletea</i>) Halo-nitrophilous scrubs (matorrals) belonging to the <i>Pegano-Salsoletea</i> class, typical of dry soils under arid climates, sometimes including taller, denser brushes.
15	SALT AND GYPSUM INLAND STEPPES
1510*	Mediterranean salt steppes (<i>Limonietalia</i>) Associations rich in perennial, rosette-forming <i>Limonium</i> spp. or esparto grass (<i>Lygeum spartum</i>), occupying, along Mediterranean coasts and on the fringes of Iberian salt basins, soils temporarily permeated (though not inundated) by saline water and subject to extreme summer drying, with formation of salt efflorescence.

The Protection of the Natural Heritage of Iberian Saltscapes

The protection of saltscapes has been a quite recent phenomenon and is still far from representative, as will be argued below. The first declarations of protected areas in the world, at the end of the 19th century, focused on romantic, scenic and wild mountainscapes. At that time, wetlands were considered a nuisance and even a threat to public health, as they hosted parasites and insects that could easily transmit infectious diseases. Decades later, attention was finally placed on wetlands, partially due to the 1972 Ramsar Convention on Wetlands (Mioduzewski 2006; O'Connell 2003). Again, many lesser—especially inland—wetlands were missed by Ramsar, as one of the main requisites to belong to the Convention was to host bird species or communities of international importance. Many small saline wetlands lack this feature, due to seasonality of the water table, fluctuating salinity, size of the wetland, distance to larger wetlands, off the main flyways of migrating birds, etc. (Casado & Montes 1991). Typical features of saltscapes such as habitat-specific plant species or communities, microbial diversity or rarity and/or fragility of halophilic organisms are just starting to be widely recognized by the scientific community. These features have also started to be acknowledged by policy makers and this has helped broaden the declaration criteria of Ramsar sites, allowing the declaration of smaller wetlands. In Spain and Portugal, 80 Ramsar sites have been declared to date (Ramsar 2008). Of them, over one third (30 sites; 38%) protect some sort of saltcape (see Table 3). It is worth noting that, in the case of Spain, more than half of the Ramsar sites devoted to the protection of saltscapes do protect inland saltscapes (15 of 25 sites or 60%), most of which are lakes and lagoons in central and eastern Spain. In Portugal, however, all five Ramsar sites protecting saltscapes are located at the mouth of important rivers, where coastal solar evaporation salinas exist.

On the other hand, the European Commission launched an ambitious project in order to efficiently protect the most representative species and habitats of the EU by creating the Natura 2000 (European Commission 2008) network. To this end, among other actions, a comprehensive catalogue of European habitats was prepared. Some of the habitats were considered in danger of disappearing and enjoy a special priority status when it comes to their protection. The final list of habitats contains six that could be considered as saltscapes (see Table 1), as these have been defined above.

Once this catalogue of habitats was made, each member state had the responsibility to designate areas to protect a representative part of them, by creating the so called “Sites

of Community Interest” or SCIs. These sites may contain one or several habitats and form part of the Natura 2000 network, together with the so called, “Special Protection Areas” or SPAs, devoted to the protection of birds. Only recently have the member states designated both their SCIs as their SPAs. The next step is to educate both policy makers as the general public about the (legal) implications and opportunities of a certain area belonging to the Natura 2000 network, in order to be truly efficient. This is an ongoing process. But this would require a much deeper discussion that falls well beyond the scope of this paper.

Table 2—Number of designated SCIs per country and habitat type (European Commission classification system, see Table 1).

Country	Habitat type					
	1310	1340*	1410	1420	1430	1510*
Belgium	3					
Cyprus	1		1	1		
Denmark	34	6				
Estonia	2					
France	55	10	15	21		
Germany	13	49				
Greece	7		9	8		1
Ireland	23		35	2		
Italy	49		53	45	7	41
Latvia	3					
Malta			1	3		
Netherlands	21					
Poland		1				
Portugal	9		8	8	7	2
Slovenia		2				
Spain	79		73	124	55	68
Sweden	33					
United Kingdom	18	1		5		
Total SCIs	350	69	195	217	69	112

A brief look at the number of designated SCIs per country and per habitat type quickly shows that the Mediterranean countries in general and Spain in particular are leading in the number of designated saltscapes (Table 2). Portugal, however more modestly, also hosts most of the saltcape habitats. An exception to this is habitat type 1340* (inland salt meadows), which belongs to the continental biogeographic region, not represented in most Mediterranean countries and not at all in Spain or Portugal. Of all other saltscapes represented by these habitat types, Spain and Portugal host 46% of all of the protected designated SCIs in the EU.

These figures give an idea of the importance and representativeness of these saltscapes in the Iberian context. However, Table 2 only gives an estimate of *protected* saltscapes in the EU. It is rather difficult to estimate the total number of valuable saltscapes in a certain region, as many of them are seasonal, intermittent or artificially created by soil erosion, salt water intrusion or excessive irrigation (Williams 1998). It has to be assumed that the number of saltscapes protected within the Natura 2000 network in the different EU countries is only representative of their total number. Also, bearing in mind that hardly any inland solar evaporation salt making facilities exist outside Iberia, and that Spain and Portugal host (the remains of) 500 such sites (Carrasco & Hueso 2008), the Iberian peninsula may be considered as the European reservoir of saltscapes both from a qualitative as well as a quantitative point of view and as such they should be protected (González Bernáldez 1987; Montes & Martino 1987; Casado & Montes 1991; Guerrero & de Wit 1992).

A completely different way to look at the protection of saltscapes is their conservation as cultural heritage. The Spanish legislation may designate historic buildings, sites, or other tangible heritage as “Good of Cultural Interest” (GCI), which confers them with a protection status and enforces their owners and the local and regional authorities, to protect and maintain them. Most GCIs are churches, monasteries, castles and fortifications. However, more recently, other more modest sites have started to be declared as GCI. Among the relatively new designated sites are a number of salt making sites. Of the almost 1.6×10^4 declared GCIs, 17 are salt making sites, of which 35% (6 sites) are inland (Table 3). The declaration of a site as a GCI normally only affects the man-made structures (buildings, engines, infrastructures) found in it and not its surrounding landscape or natural values. In fact, only in two cases (Salinas de Añana, in the Basque Country, and Salinas de Saelices de la Sal, in Castile–La Mancha) do two protection statuses overlap (SCI and GCI).

Some regional governments in Spain are starting to understand cultural heritage as part of a geographic context and refer to it as “cultural landscapes” or “territories” (i.e. Castile and León, Aragón). Several non-governmental organizations devoted to the protection of industrial, archaeological and mining heritage are using the concept for the protection of whole mining districts (International Committee for the Conservation of the Industrial Heritage/TICCIH, Spanish Society for the Defense of Geological and Mining Heritage/SEDPGYM, Industrial Archaeology Association/INCUNA, Spanish Society for History of Archaeology/SEHA, etc.), including also their

natural (mainly geological, but not only) heritage. This trend may be useful for saltscapes too, especially in those sites where salt has been obtained by man, as both the cultural and material values are tightly interwoven.

Table 3—Number of protected saltscapes in Spain according to different protection categories and type of saltcape.

Type	SCI ¹	Ramsar ²	GCI ³
Coastal salinas	4	7	11
Inland salinas	8	1	6
Other saltscapes	173	17	0
Total saltscapes	185	25	17

¹SCI: Site of Community Importance: Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora (EUNIS 2008).

²Ramsar: Ramsar Convention on Wetlands, 1972 (Ramsar 2008).

³GCI: Good of Cultural Interest: Law 16/1985 of Spanish Historic Heritage (historic sites, monuments, etc.) (Ministerio de Cultura 2008).

Threats and Challenges to Iberian Saltscapes

Despite their importance and value, wetlands are being threatened globally, for different reasons: drainage, overpopulation, eutrophication, intensification of agriculture, overexploitation, pollution, alteration of ecological conditions, etc. Many of these actions are related to each other and create synergic effects: since 1900, more than half of the world’s wetlands have disappeared (Casado & Montes 1991, 1995; Barbier 1993; Pearce & Crivelli 1994; Schuyt & Brander 2004). Saltscapes in general and saline wetlands are no different in this respect; they have been under threat almost everywhere around the world and predictably will be in the next decades (Williams 1986, 1993, 1998, 2002; Jellison 2003). Many of them continue to be destroyed or irreparably damaged (Williams 1986, 1993). A team of researchers from the ecology departments of the three universities of Madrid, Spain has estimated a loss of between 40 and 70% of the total wetland area in Spain since the 18th century. In the Upper Guadiana watershed, 90% of the wetlands disappeared between 1953 and 1973, as a result of agricultural land reclamation and hygienic measures against malaria (Álvarez 2007). In the case of saline wetlands, this loss has been reduced to 30%, mainly due to the conservation of large salt lakes such as Gallocanta and Fuentedepiedra. A larger percentage of smaller saline wetlands has disappeared (16%) or is highly altered (35%) (Casado & Montes 1991). One of them is La Janda Lagoon, in southern Spain, the southernmost saline lake in Europe (Montes & Martino 1987). As will be seen in the discussion below, saltscapes in general and saline wetlands in particular suffer threats that are common to other wetlands in general, but others are specific to them:

Ignorance

One of the most important threats to saltscapes is, in fact, ignorance of their existence and therefore their natural, cultural and even economic values. Scientists such as Williams (1981, 1986, 1998) or Margalef (1983, 1994) have repeatedly defended the study of saline inland water while limnology has typically restricted itself to fresh water. Policy makers, however, seem not to value fresh and saline water to the same extent. To illustrate this, the Association of Friends of Inland Salinas has repeatedly and fruitlessly tried to include an event related to saline wetlands within the NGO forum of the Universal Expo on Water that was celebrated during the spring and summer of 2008 in Zaragoza (Spain). The little attention that has been paid to Iberian wetlands has directly been related to their bird diversity and abundance (i.e. Doñana), but Spain contains large numbers of small wetlands, too small to host interesting bird communities, but with more hidden natural values. This is especially so in the case of Iberian inland saline wetlands, as they are unique within Europe (see above) and host specific ecological functions and values not found in other types of wetland (Casado & Montes 1991).

Abandonment

The abandonment of inland saltscapes in Spain is tightly related to the so called rural exodus that had its peak during the second half of the 20th century, but in fact had started decades earlier. Traditional activities such as extensive agriculture, animal husbandry, and handicrafts were quickly abandoned at the prospect of a better life in the then fast growing cities. This is also the case for inland traditional salinas. In artificial saltscapes such as inland salterns, the abandonment of traditional salt making has resulted in the disappearance of valuable halophilic communities, which are being replaced by generalist or opportunist plant or animal species as soon as salinity decreases. As a result, these saltscapes are abandoned and follow an uncontrolled ecological succession towards degradation (Carrasco & Hueso 2006; Hueso & Carrasco 2008b, 2008c).

Agriculture and Irrigation Runoff

In the second half of the 20th century, agriculture in Spain underwent an important transformation process, from extensive, low impact, to intensive practices, with comprehensive land reclamations and irrigation projects in large, arid areas (Casado & Montes 1991). One of the affected areas, the Monegros desert, located in one of the salt lake districts in northeastern Spain, has since lost 50% of its wetlands and 30% of the remaining ones have been invaded by non-native vegetation (Castañeda & Herrero 2007). Another associated effect of agricultural

intensification is, depending on the quantity of water used, eutrophication of saline wetlands or further salinization of water bodies and/or soil (Guerrero & de Wit 1992).

Infrastructures and Water Reservoirs

Previously, authorities did not hesitate to destroy wetlands to build infrastructures, but today they must search for alternative locations, as present environmental impact assessment legislation requires. Water reservoirs have been built on saline wetlands such as Mar de Ontígola, El Atance, Estanca de Alcañiz (central Spain) or Sariñena (northeastern Spain) (Montes & Martino 1987; personal observation) and roads have covered the remains of traditional salt making sites as Tordelrábano (central Spain) (personal observation).

Wild Recreation and Vandalism

Even though overpopulation is not an issue in inland rural Spain, certain human activities are highly damaging to wetlands. Especially fragile are saline wetlands, as they are usually dry during the summer months, a period in which outdoor activities are more popular and people have more leisure time. A lack of public awareness and enforcement of nature protection laws causes many people to practice uncontrolled outdoor activities such as motor racing, mountain biking, or even 4x4 wheel driving on dry wetland beds that offer large, flat, isolated areas for these purposes. Such activities severely damage these fragile habitats (personal observation). Another issue is sheer vandalism, which is practiced mainly upon the cultural heritage of saltscapes because most of its buildings are abandoned and have become an easy prey for collectors of tools, machinery, etc. or for people wishing to renovate their home with “authentic” pieces of wood, stone, tile, etc. (Carrasco & Hueso 2006; personal observation).

Drainage, Desiccation and Waste (Water) Dumping

Although not currently a common practice, in the past many wetlands have been drained or desiccated for health purposes. In fact, an important part of early limnological studies in Spain was focused on wetland drainage and desiccation (Álvarez 2007). A few decades ago, they were considered the source of infectious diseases, especially malaria. Later, wetlands were often used as wastewater dumps which therefore reinforced the image of unhealthy sites (Comín & Alonso 1988; Casado & Montes 1991; Álvarez 2007; personal observation). Montes & Martino (1987) cite a number of saline wetlands used as waste dumps: Las Eras and Camino de Villafranca (central Spain) and Laguna del Gobierno (southern Spain).

Climate Change

A recent evaluation of the threat of climate change on Spanish wetlands concludes that seasonal saline wetlands of endorheic origin may disappear altogether. This is especially the case for the smaller ones. Other possible consequences of climate change may be the alteration of ionic composition, eutrophication, and hypersalinization (Álvarez et al. 2005).

The future management of saltscapes faces numerous challenges in Iberia (Montes & Martino 1987; Comín et al. 1999; Álvarez et al. 2005; Hueso & Carrasco 2008c). Wetlands in general and saline lakes in particular host a number of values (natural, cultural, intangible) and functions (regulation of natural processes; production of commodities and raw materials such as salt, cosmetics, edible plants, microorganisms for biotechnological and industrial applications, etc.; economic activities such as agriculture, husbandry, tourism, recreation, health care, etc.; and education through research, interpretation, etc.) that make them complex habitats that require complex and site-specific approaches (Hammer 1986; Skinner & Zalewski 1995; Viñals et al. 2002; Schuyt & Brander 2004; Hueso & Carrasco 2008c). However, an attempt is made here to classify the most important challenges ahead of saltcape managers.

Raise Awareness of Stakeholders

Ignorance of the values of saltscapes is widespread. Therefore an important task for saltcape managers is to raise awareness of them at different levels (local community, visitors, owners, policy makers, general public, etc.). Saltscapes, thanks to their unique combination of natural and cultural values along with their diversity, offer excellent grounds for educational and interpretation activities from a multidisciplinary perspective (Hueso & Carrasco 2008c).

Saltscapes as Systems

Conservation measures should not only be designed at the wetland level but also at the much larger watershed level. This is especially important for Spanish wetlands in general, which have a very high ratio watershed to wetland surface. This is not always taken into account when designing management plans (Álvarez 2007). The same can be said about the socioeconomic aspects of wetland management, in which many levels of stakeholders are involved (local community, policy makers, owners, non-

government organizations, and even non-users, who simply value their existence) (Schuyt & Brander 2004). Therefore, management measures should be planned in a broader context and in a longer term than is usually the case, and involve the coordination of multidisciplinary teams (Hueso & Carrasco 2008c).

Increase (Applied) Research on Inland Saline Wetland Values

Basic research on saltscapes is still needed. Several authors have suggested a number of issues of interest, such as diversity and dynamics and halophilic microorganisms, long term limnological processes, ecological modeling of saline ecosystems, biogeochemistry of saltscapes, cultural tangible and intangible values of saltscapes, etc. (Guerrero & de Wit 1992; Viñals et al. 2002, 2005; Álvarez 2007). Applied research towards the optimal use of saltcape resources (i.e. for biotechnology, electronics, pharmaceutical uses, etc.) may also help them to recover in a sound and even profitable way (Hueso & Carrasco 2008c).

Invest in Sound (Socio-Economic) Recovery Projects

In the case of inland saltscapes, and especially, in traditional salt making sites, preference should be given to projects that recover wetland functions through the restoration of the cultural and natural heritage, increase conservation awareness, recognize the spiritual values of wetlands for the local communities and value cultural traditions for the benefit of everyone (Viñals et al. 2002, 2005). An interesting example of a coordinated recovery project of cultural, socioeconomic and natural values of a saltcape is the Master Plan for the Salinas de Añana salt inland salt making site Basque country (Lasagabaster et al. 2003).

CONCLUSION

Spain hosts a great diversity and abundance of saltscapes, both at the European and at the global level. Although protection efforts have been implemented in the last decades, knowledge of the cultural and natural values of these saltscapes is still patchy as compared to other landscapes and wetlands. Saltscapes still need to be properly catalogued, studied, and the information disseminated in order to reach efficient levels of protection and sound use, optimizing their values and functions in a sustainable way.

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The Role of Non-Governmental Organizations in the Conservation and Management of Saltscapes in Spain - The Case of the Association of Friends of Inland Salinas

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Saltscapes need a keen eye to be appreciated, as they do not offer instant, pleasant experiences. It is an important challenge for most non-governmental organizations devoted to their protection and dissemination. There are two main types of organizations that deal with saltscapes in Spain: nature conservation and cultural heritage associations. In the first case, their interest is nature conservation in general, and they may act at different geographical levels (local, regional, national) and may organize volunteer activities related to their goals. In the second case, their interest is mainly the local cultural heritage and this may include (historic) salt making sites. Only a few cultural associations primarily deal with the local salt making heritage and their specific interests and activities usually depend on the scientific, technical or professional backgrounds of the leaders of the organization. Often they cooperate with regional authorities to organize summer work camps to restore part of the salt heritage. These types of non-governmental organizations act at a local level and often ignore each other's existence. However, in 2002 the Association of Friends of Inland Salinas (ACASI) was established with the aim to preserve, study and disseminate the cultural and natural values of saltscapes. In Spain, it is the only organization that is specialized in salt heritage in general that acts both at the national and the international level. The association works with two main groups of

stakeholders: schools and the general public and organizations; or, specialized public agencies (public authorities, managers, owners, scientists, etc.). Its main goal is to disseminate knowledge on saltscapes by means of field trips, exhibitions, and publications, and by organizing courses, conferences, and salt heritage related events. It also offers technical assistance and consults on issues such as planning, management, tourism, and sound local socioeconomic development in (former) salt making areas. The ACASI performs research on the cultural and natural values of saltscapes and functions as a network for people and organizations interested in salt heritage. In general, non-governmental organizations dealing more or less specifically with salt heritage in Spain have certain weaknesses that are hard to overcome: they generally have a very local scope of action; they work in rural and sociodemographically poor environments; they suffer from a permanent shortage of human, technical and financial resources; they usually have less than 100 paying members; and they obtain little cooperation (if not indifference or even opposition) from the local and regional authorities. Finally, the participation of the local population is highly variable, usually depending on personal relations. All of these are difficult to solve, but with patience, enthusiasm and hard work, it is possible to cope with these difficulties and successfully work towards preserving the saltscapes.



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Threatened *Artemia* Biodiversity in the Iberian and Western Mediterranean Region

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Two anthropogenic activities that lead to a decrease in biodiversity are the destruction of habitat and the introduction of exotic species. The autochthonous species of the genus *Artemia* are being eliminated from hypersaline ecosystems in the Old World after the introduction of *A. franciscana* from America. This report summarizes data about the present distribution of the invasive *A. franciscana* populations identified in the Iberian and Western Mediterranean area. In Spain the most important invaded solar saltern complex is located at the Cadiz salt marshes. The recent finding of *A. franciscana* in the salterns of Isla Cristina (Huelva Province) shows its range extension from the Portuguese Algarve District to the East, invading the neighbouring Spanish salterns, and from where it has been likely progressing northward, invading up to the Portuguese Aveiro District salterns. Two more important hypersaline environments in Spain have been invaded by *A. franciscana*, the Fuente de Piedra hypersaline inland lagoon in the province of Málaga, and the river Ebro Delta salterns in the province of Tarragona (Mediterranean shore). These are important hotspots for potential brine shrimp dispersion, being among the most important nesting areas for flamingos in the Iberian Peninsula. The presence of *A. franciscana* in the salt marshes and salterns of Salin de Giraud evidences the spread of the invasive species into the Mediterranean French Departments (Languedoc-

Roussillon and Provence), probably as a consequence of their location in the West Mediterranean flyway, East of Gibraltar. We have also recently discovered *A. franciscana* populations in La Guérande salterns in the French Loire Atlantique Department. In the winter and spring of 2007 we used open-air mesocosms to study competitive elimination of the autochthonous species by the invasive *A. franciscana*. In winter when cold temperatures extended maturation to 40 days, both the invasive and native populations coexisted (Figure 1). However, even under a daily thermal range as low as 10°-20°C, the invasive species reached maturity earlier than the autochthonous species. Other fitness traits (interbrood interval; fecundity) also provide *A. franciscana* a competitive advantage. In mid-April temperatures increased markedly, together with a clear decline on phytoplankton availability. Under these conditions all species, except *A. franciscana*, shifted egg production from ovoviviparous recruitment of nauplii to production of cysts (oviparism). By late May *A. franciscana* females showed balanced levels of ovoviviparism and oviparism as a result of declining phytoplankton availability. All the other competing species had disappeared from the water column (Figure 1). In a four month period the invasive *A. franciscana* species had outcompeted the autochthonous bisexual species *A. salina* and the two parthenogenetic strains.

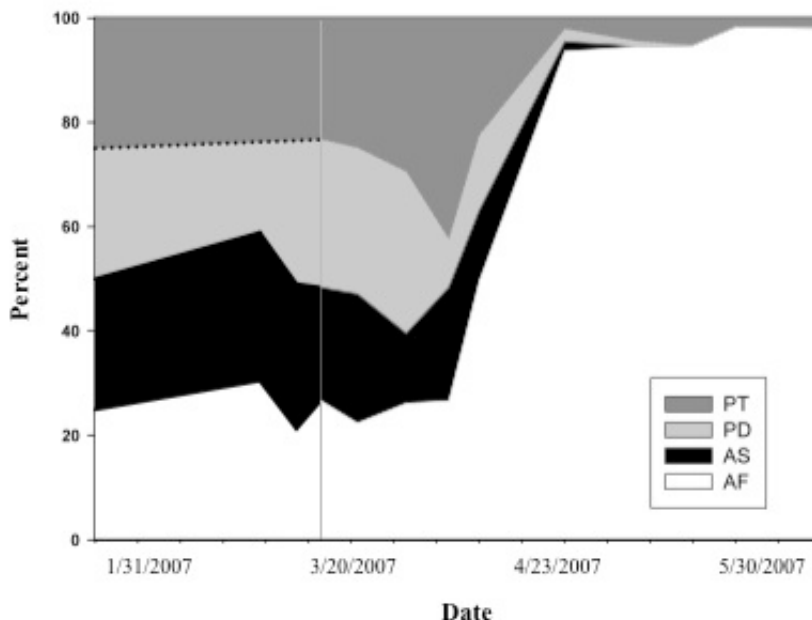


Figure 1—Changes in population composition in terms of percentages for the different species present in the competition open air mesocosm experiment conducted during the period January–May 2007: AF (invasive *A. franciscana*), AS (autochthonous bisexual *A. salina*), PD and PT (autochthonous parthenogenetic *Artemia*, diploid and tetraploid, respectively).

The Role of Parasites in the Successful Invasion of Mediterranean Salterns by the Exotic Invasive *Artemia franciscana*

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Artemia species (Branchiopoda, Anostraca) play an important role as intermediate hosts of parasite cestodes (Cyclophyllidae), facilitating transmission of the parasite to the avian hosts by predation. When exotic invasive species escape from their coevolved parasites and encounter new parasites in the invaded environment, they can experience a demographic release (enemy release hypothesis), becoming highly competitive and an important threat to biodiversity. Here we report the presence of American *A. franciscana* in a Mediterranean saltern where this exotic species eliminated autochthonous *Artemia* species. To assess whether invasive *A. franciscana* is parasitized by cestodes to the same extent as native *Artemia* species, we studied the natural infection of *A. salina* and *A. parthenogenetica* from non-invaded Mediterranean salterns: San Pedro del Pinatar (Murcia Province), Bras del Port and La Mata lagoon (Alicante Province) and parasitized *A. franciscana* in the invaded saltern La Trinitat (Tarragona Province). To compare the infection levels, we used the prevalence (number of infected hosts divided by the total number of hosts examined), mean intensity (total number of parasites found in a sample divided by the number of hosts infected) and mean abundance (total number of parasites found in a sample divided by the total number of hosts examined). The present study included three samples of the two bisexual

species of *Artemia* and five samples of *A. parthenogenetica*. We collected them during 2007 and 2008 and between 150 and 600 individual *Artemia* were examined from each sample in search of parasites. The mean prevalence was only 25% in the *A. franciscana* species compared to 35% in *A. salina* and 52% in *A. parthenogenetica*. The mean abundance of the cestode infection was 0.38/individual in the *A. franciscana* species compared to 0.47 in *A. salina* and to 1.30 in *A. parthenogenetica*. The highest mean intensity of cestode infection was found in *A. parthenogenetica* (2.20/*Artemia*). Bisexual species, *A. franciscana* and *A. salina*, showed similar mean intensities: 1.25 and 1.20, respectively. Ten cestode species, most hymenolepidid cestodes (*Flamingolepis liguloides* and *Confluaria podicipina*) were found in the autochthonous brine shrimp species (Figure 1). In the *A. franciscana* population we found only eight parasite species, mainly dilepidids (*Eurycestus avoceti*). *Confluaria podicipina* and *Wardium gvozdevi* never appeared in the invasive species. In conclusion, cestode parasites may play a role in the competitive interaction between native and invasive brine shrimps. Before we can conclude if these reduced levels of infection in *A. franciscana* are consistent with the enemy release hypothesis further work will be required.

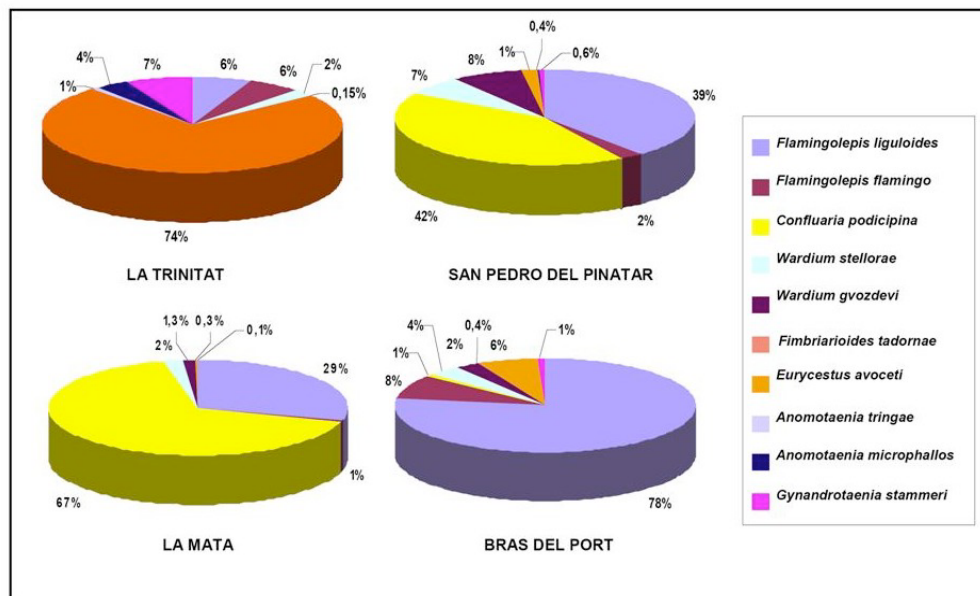


Figure 1—Cestode species recorded in brine shrimp populations in the Mediterranean salterns studied: La Trinitat, invaded with *A. franciscana*, and San Pedro del Pinatar, La Mata and Bras del Port as non-invaded salterns.

Natural Curative Factors of the Sotvino Salt Lakes

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There are about 29 deposits of subthermal (20-35°C), thermal (35-42°C) and highly thermal (> 42°C) mineral waters in Transcarpathia, Ukraine. They differ in chemical composition (nitrate, methane-containing, carbonic-acidic, sodium chloride, siliceous, iodine- and bromide-rich), total salinity, and temperature. The most interesting of these are the Sotvino Salt Lakes. Due to the specific geographic location on the south-east of the Carpathian Mountains, this region has distinctive atmospheric and climatic features. The natural resources of the Sotvino Salt Lakes include subthermal saline water up to salt-saturated brine, curative silt mud, and presence of halophilic bacteria. The karstic salt lakes were formed where the Sotvino rock salt massive emerged to the surface. The largest is Kunigunda Lake, with a total area of about 800-1000 m² and a depth of up to 8 m. The total salt content of the brine changes with the depth of the lake and also depends on the season; maximal levels reach 247-270 g/l. The water contains bromides and sodium chloride, and belongs to the type of Ust'-Kachka waters. The main chemical components of the water are given in Table 1 and its properties are described by Kurlov's formula as follows:

$$\text{Br}_{0.540} \text{M}_{247} \text{Cl}_{99} \text{pH}_{6.4} \text{T}_{22}^{\circ\text{C}}, \\ (\text{Na} + \text{K})_{99} \text{Ca}_1$$

where M (“Mineralization”) represents the total salt concentration.

In addition to these chemical properties, the lakes have unique photobiological characteristics. The solar spectrum of ultraviolet light reaching the lake is rather restricted because of a continuous haze that develops over the surface of water. The silt mud of the lake is slightly

alkaline, finely dispersed, with a clay skeleton and a pronounced colloidal complex, highly-saline sodium-chloride composition of the liquid phase. The organic material content is low. The mud may be diluted with brine for medical use. Halophilic bacteria live in the Sotvino Salt Lakes and these may contribute to the curative properties of the salt-saturated brine. They contribute a specific photosensitization effect to the brine solution. After removing the mud or the brine, these bacteria remain on the skin. Subsequent exposure to UV radiation causes a specific photochemical effect, which improves the skin barrier function and reduces inflammation of the skin. These three major factors, the lake, the sun and the halophilic microorganisms, serve as the basis for the treatment of different illnesses at the lakes, particularly skin diseases. Thus, the Sotvino salt lakes have a great potential as health resorts with possibilities for balneotherapy, phototherapy, climatotherapy (temperature, humidity, sunlight), heliotherapy, and thalassotherapy, providing treatment of specific health problems and new medication-free approaches in the treatment of different diseases.

Table 1—Chemical composition of mineral water of Kunigunda Lake.

Cations	g/l
Na ⁺ + K ⁺	95.60
Mg ²⁺	0.12
Ca ²⁺	1.05
Anions	g/l
Cl ⁻	147.37
SO ₄ ²⁻	2.01
Br ⁻	0.54
HCO ₃ ⁻	0.31

The Role of Phytoplankton in Sediment Formation in Lakes with Pelogenous Potential

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The peloids formed in lakes and reservoirs with saline water differ in composition, depending on the organic and the inorganic compounds that participate in their formation. Based on the contribution of plant material in the formation of sediments in pelogenous lakes, the micro- and macrophyte populations in Braila Salt Lake, Fundata Lake, Techirghiol Lake and Amara Lake, differing in physico-chemical characteristics, were studied in the years 1983–2005, to monitor their presence and distribution in lentic facies and to elucidate the structure of the phytoplankton communities (Figure 1). Samples have been collected from pelogenous lakes during the summer season, when the plankton is well developed, from a number of representative sampling stations. Total plankton was analyzed qualitatively, and quantitative determinations were performed for phytoplankton. Samples were also collected for the determination of the physical and chemical properties of the water and its microbiological quality, determining the level of the bacterial pollution. After identification of the phytoplankton species and the determination of their abundance, the following types were found to play an important role in the productivity of these lakes with pelogenous potential:

–Filamentous algae: *Cladophora crystallina*, *Enteromorpha clathrata*, *Spirogyra tenuissima*, *Ulothrix zonata*, *Rhizoclonium hieroglyphicum*. These species contributed important amounts of organic material in Techirghiol, Fundata and Braila Salt Lakes.

–Submerged macrophytes: *Potamogeton pectinatus*, *Myriophyllum spicatum*; these represent the major source of organic material in the Fundata and Amara lakes.

–Emergent macrophytic vegetation: *Phragmites communis*, *Typha angustifolia*; these are important in the ecosystem of the therapeutic lakes Amara and Fundata.

Microalgae (cyanobacteria, diatoms, euglenophyta) contribute a smaller, but constant amount of organic material to the peloidogenesis process in all four studied lakes. When the salt composition of the lakes increases to values exceeding 100 g/l, as happens for example in Braila Salt Lake, the biological diversity and biomass are reduced, and the contribution of organic material is accordingly lower, relative to the amounts of inorganic material deposited.

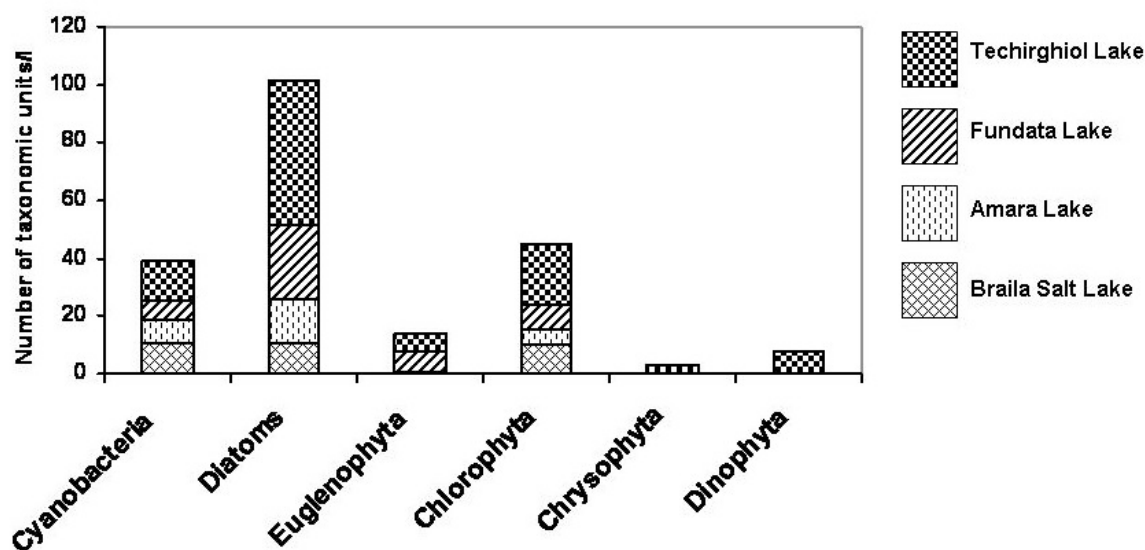


Figure 1–Variation of phytoplankton composition in lakes of different salinities (1983–2005).

The Aral Sea: Water Level, Salinity and Long-Term Changes in Biological Communities of an Endangered Ecosystem - Past, Present and Future

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This paper is dedicated to the memory of Prof. William Williams

ABSTRACT

The Aral Sea, which before 1960 was the fourth largest lake in the world, has now split into four separate water bodies. This break-up and desiccation mainly resulted from upstream irrigation withdrawals from the two main influent rivers, the Syr Darya and the Amu Darya. The negative effects on the lake ecosystem due to declining water level and increasing salinity, as well as the profound socioeconomic and human impacts to the riparian populations, have been well documented. This paper focuses on the conservation and rehabilitation efforts for four remaining key water bodies of the Aral Sea: the Northern (Small) Aral and its ecosystem; the Southern (Large) Aral and its ecosystem; the delta and deltaic water bodies of the Syr Darya; and the delta and deltaic water bodies of the Amu Darya. It is encouraging to note the reversal of the degradation in the Northern Aral after the creation of a dike at Berg's Strait in 1992. The dike washed out in 1999 but has been replaced with a new structurally sound dike. The water level in the Northern Aral has increased several meters and salinity is returning to levels that can sustain the pre-1960 ecosystem. However, much less success has been obtained regarding the Southern Aral which continues its retreat and salinization. There have been recent efforts also in the deltas and deltaic regions of the Syr Darya and Amu Darya, the rehabilitation of Sudochie Lake probably being the most famous. These restoration projects are critiqued in this paper and recommendations for future actions are given.

The Aral Sea and its Biodiversity

The Aral Sea was the fourth largest lake in the world by water surface area in 1960 (Figure 1). At that time its area was $6.75 \times 10^4 \text{ km}^2$ (Large Aral $6.14 \times 10^4 \text{ km}^2$, Small Aral 6118 km^2), and the volume was 1089 km^3 (Large Aral 1007 km^3 , Small Aral 82 km^3). The Aral Sea was 53.4 m above ocean level with a maximum depth of 69 m. It was a slightly saline lake with an average salinity of about 10 g/l. The Aral Sea was inhabited by about 12 species of fish and

about 160 species of free-living invertebrates excluding Protozoa and small-size Metazoa (Atlas of the Aral Sea Invertebrates 1974).

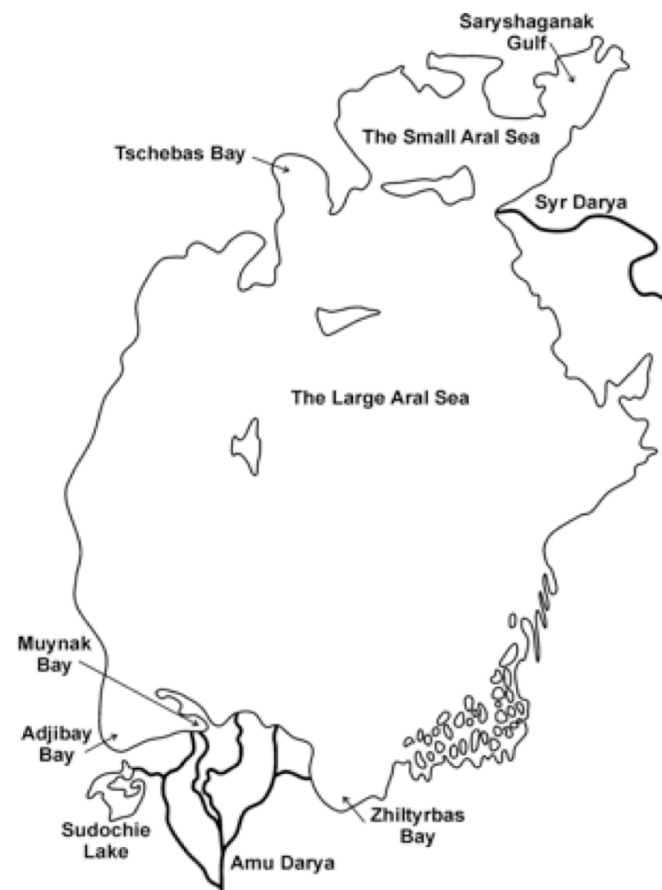


Figure 1—The Aral Sea in 1960.

Since 1960 the Aral Sea has steadily become shallower, mainly as a result of upstream water withdrawals for irrigation. By 2007 the area of the lake was reduced to about $1.4 \times 10^4 \text{ km}^2$ (21% compared to the area in 1960) with a volume of 102 km^3 (9% of 1960). The Large Aral was $1.1 \times 10^4 \text{ km}^2$ (17% of 1960), had a volume of 75 km^3 (8% of 1960) and a salinity that ranged from 100 g/l to well above that figure. The corresponding values for the Small Aral were 3258 km^2 (53% of 1960) and 27 km^3 (33% of 1960), with an average salinity of about 10 g/l (Table 1).

Table 1—Changes in the main hydrological and hydrobiological characteristics of the Aral Sea.

Aral Sea and its constituent parts	Level, meters above sea level	Area (km ²)	Volume (km ³)	Average salinity (‰)	Number of free-living invertebrate species		Number of fish species	
					aboriginal	alien	aboriginal	alien
Aral Sea, 1901	53.60			10	148	-	18	-
Aral Sea, 1961	53.40	66511	1089.0	10	148	5	18	11
Small Aral Sea, 1989	40.60		20.0	30	7*	4*	-	6
Large Aral Sea, 1989	39.07	37410	350.0	30	7*	5*	-	6
Small Aral Sea, 2007	42.00	3487	27.0	11-14	10*	6*	3	7
Tschebas Bay, 2007	30.00	105	0.2	90	8	2	-	-
Eastern Large Aral Sea, 2007	29.50	6117	9.5	120-160	-	1	-	-
Western Large Aral Sea, 2007	29.50		58.0	100	8	2	-	-

* – without Protozoa, small Metazoa and rare species.

Values for the level, area, and volume of the Small Aral for 1989 were derived from the map *Dinamika Aralskogo Morya*, 1990. Values for 2007 were calculated by interpolation from 1989 map data and data for the area and volume at 43 meters (Bortnik 1990). The area for Tschebas Bay and the eastern and western Large Aral were measured based on the August 11, 2007 MODIS 250 meter natural color image, using a polar planimeter and reference areas derived from pixel counts.

Citizens of Kazakhstan like to call the Small (northern) Aral Sea “Kazaral”, which means “Kazakh Aral”. People in Uzbekistan also sometimes use the name “Uzarl”, meaning “Uzbek Aral”, instead of Large (southern) Aral Sea. We believe that on future maps there will be four main water bodies at the place of the former Aral: Kazaral, Western Uzarl, Eastern Uzarl, and the remnant of Tschebas Bay (Figure 2).

Prior to introduction of fish species and free-living invertebrates to the Aral Sea that started in the 1920s, the following aboriginal organisms were identified: fishes–12, Coelenterata–1, Turbellaria–12, Rotatoria–58, Oligochaeta–10, Cladocera–14, Copepoda–7, Harpacticoida–15, Ostracoda–11, Malacostraca–1, Hydracarina–7, Bivalvia–9, Gastropoda–3; Total–160, not including Protozoa and some other small-size Metazoa (Atlas of the Aral Sea Invertebrates 1974). From the middle of the 19th century until 1961 the shape and salinity of the Aral Sea practically didn’t change. However, due to intentional and accidental introductions that started in the 1920s the number of free-living animals grew substantially. In the Aral Sea appeared: fishes–21, Mysidacea–5, Decapoda–3, Copepoda–3, Polychaeta–1, Bivalvia–4: a total of 37 new species (Aladin et al. 2004). The clam *Abra ovata* and the worm *Nereis diversicolor* introduced by man are of great importance for fish nutrition. *Rhithropanopeus harrisi tridentata* was introduced accidentally, and disturbs the lake sediments.

Originally the Aral Sea contained freshwater, transitional freshwater-brackish water, brackish water and transitional brackish water-marine ecosystems. Brackish water ecosystems occupied the largest area (Figure 3). By the end of the 1980s, due to the increase in salinity, marine ecosystems appeared in the Aral Sea and occupied the largest area instead of the brackish water ecosystems (Figure 4). Presently, all parts of the Large Aral are occupied by hyperhaline ecosystems. In the Small Aral the transitional brackish water-marine ecosystems prevail due to the salinity decrease (Figure 5).

Since 1989, when the level had dropped by about 13 m and reached about +40 m, the Aral Sea has been divided into the Large and Small Aral with a total area of 4.0×10^4 km² (60% of 1960); volume 333 km³ (33% from 1960) and an average salinity of 30 g/l (3 times higher than in 1960) (Table 1). After 1989, all discharge of the Syr Darya went to the Small Aral, leading to a decrease in salinity (Aladin et al. 1995).

Following the division of the Aral Sea, its volume decreased from 1000 km³ to 400 km³ by 2001 and to 108 km³ by 2005, with the Large Aral Sea accounting for 85 km³ and the Small Aral Sea 23 km³ of the total. Salinity in the Large Aral continued to rise and reached 90 g/l (western part, depth 21 m) and 160 g/l (eastern part, depth 28.3 m) in 2005, while in the Small Aral it decreased and reached 17 g/l in 2005 (Asarin & Bortnik 1987; Bortnik & Chistyayeva 1990; Uzglavgidromet 1994–2003; Water Balance models 1990–2006; Ptichnikov 2000, 2002; Final Report 2004; Expedition 2005, 2007).

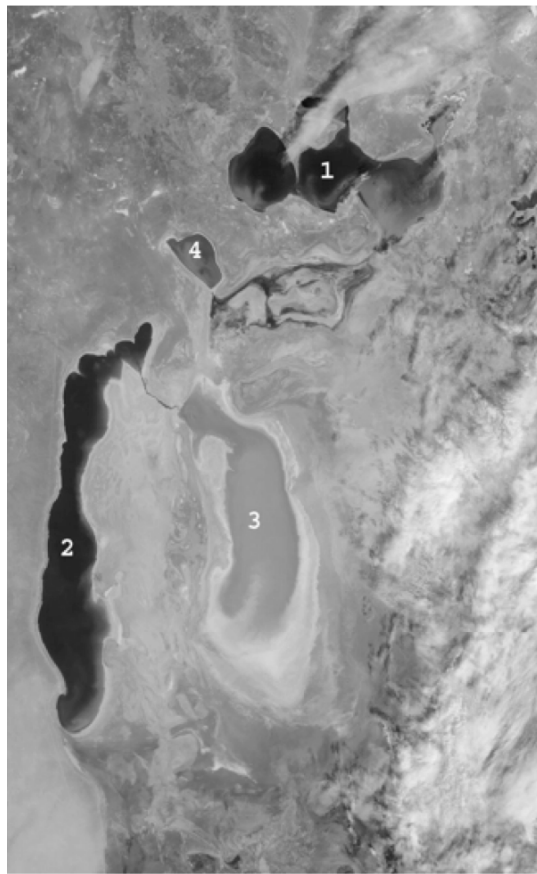


Figure 2—Modern view of the Aral Sea from space. 1—The Small Aral Sea (“Kazal”); 2—The Western Large Aral Sea (Western “Uzral”); 3—The Eastern Large Aral Sea (Eastern “Uzral”); 4—Tschebas Bay.

After the separation of the Aral Sea in 1989, when the average salinity was about 30 g/l, the following free-living animals survived: fishes—10; Rotatoria—3; Cladocera—2; Copepoda—2; Ostracoda—1; Decapoda—2; Bivalvia—2; Gastropoda—> 2; Polychaeta—1; a total of > 25. The zooplankton community was composed of the following invertebrates: Rotatoria—*Synchaeta vorax*, *S. cecilia*, *S. gyrina*; Cladocera—*Podonevadne camptonyx*, *Evadne anonyx*; Copepoda—*Calanipeda aquaedulcis*, *Halicyclops rotundipes aralensis*; larvae of Bivalvia. The zoobenthos community included Bivalvia—*Abra ovata*, *Cerastoderma isthmicum*; Gastropoda—*Caspihydrobia* spp.; Polychaeta—*Nereis diversicolor*; Ostracoda—*Cyprideis torosa*; Decapoda—*Palaemon elegans*, *Rhithropanopeus harrisi tridentata*. Fishes were represented by *Pungitius platygaster*, *Clupea harengus membras*, *Platichthys flesus*, *Atherina boyeri caspia*, *Knipowitschia caucasicus*, *Neogobius fluviatilis*, *N. melanostomus*, *N. syrman*, *N. kessleri*, and *Proterororchinus marmoratus*.

Conservation and Rehabilitation of the Aral Sea and its Ecosystems

The desiccation and salinization of the Aral Sea had serious negative impact not only on aquatic ecosystems but also on the environment of surrounding areas and the life of local people. To improve this situation, there are four main ways to conserve and rehabilitate the Aral Sea and its ecosystems, as first discussed at the United Nations Environment Programme (UNEP) meeting held in September 1992 in Geneva:

1. Conservation and rehabilitation of the Small Aral;
2. Conservation and rehabilitation of the Large Aral;
3. Conservation and rehabilitation of the delta and deltaic water bodies of Syr Darya;
4. Conservation and rehabilitation of the delta and deltaic water bodies of Amu Darya.

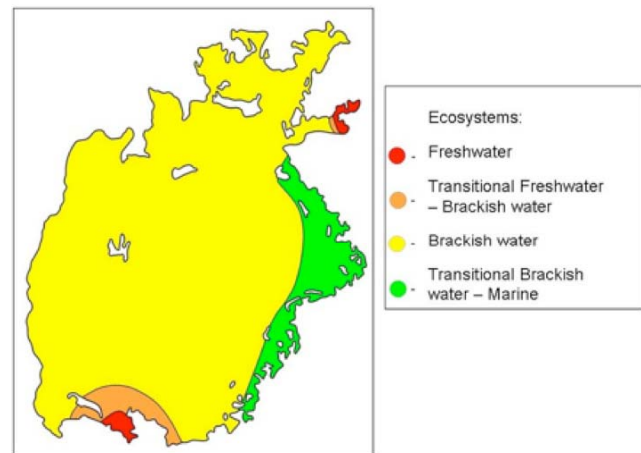


Figure 3—Ecosystems in the Aral Sea as a function of salinity before the onset of salinization.

Conservation and Rehabilitation of the Small Aral and its Ecosystems

Discharge of water from the Small Aral occurs primarily in spring-early summer which is the peak period of water flow for the Syr Darya. Since August 2005 outflow is controlled by a discharge structure (gates) in the dike. The dike in Berg strait is preserving the Small (northern) Aral, contributing to the rehabilitation of its biodiversity. The old dike (Figure 6) was built according to our proposal in August 1992 (Aladin et al. 1995). It existed, with periodic partial breaches, until April 1999, when after the water level rose to +43.5 m a catastrophic breach occurred that destroyed the dike. While the dike existed, the number of organisms increased, showing that even such a short period allowed partial rehabilitation of the biodiversity in the Small Aral.

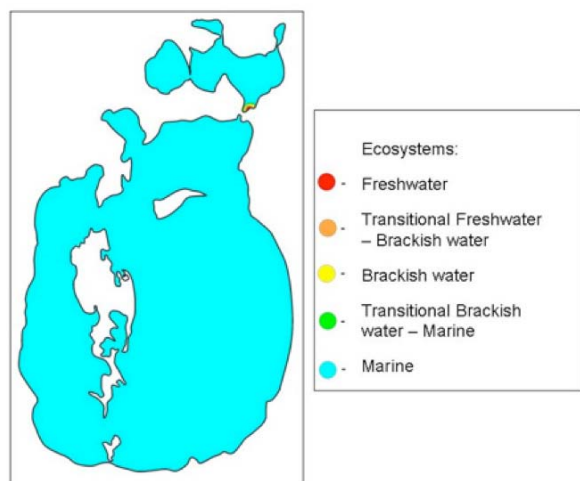


Figure 4–Ecosystems in relation to salinity at the division of the Aral Sea.

Since the separation of the Small Aral Sea from the Large Aral in 1989, the number of organisms in the lake increased as the salinity decreased to about half the earlier value, reaching about 17 g/l in 2005. At that time, the Small Aral's area was 2804 km² (47% from 1960), volume 23 km³ (28% from 1960), and level +40.4 m a.s.l. Our survey in September 2007 showed the following number of species: fishes–12; Rotatoria–3; Cladocera–2; Copepoda–2; Ostracoda–2; Decapoda–2; Bivalvia–2; Gastropoda– > 1; Polychaeta–1; total: > 27. The zoobenthos of the Small Aral Sea today consists of the following: Bivalvia–*Abra ovata*, *Cerastoderma isthmicum*; Gastropoda–*Caspihydrobia* spp.; Polychaeta–*Nereis diversicolor*; Ostracoda–*Cyprideis torosa*, *Eucypris inflata*; Decapoda–*Palaemon elegans*; Insecta: Chironomidae larvae. Fishes of the Small Aral are: *Clupea harengus membras*, *Platichthys flesus*, *Atherina boyeri caspia*, *Knipowitschia caucasica*, *Neogobius fluviatilis*, *N. melanostomus*, *N. syrmian*, *N. kessleri*, *Pungitius platygaster*, *Proterorichinus marmoratus*, *Ctenopharyngodon idella*, and *Sander lucioperca*. After construction of the first dike in Berg Strait in 1992, fishing on the Small Aral resumed. According to reports of fishermen in 2004, silver carp (*Ctenopharyngodon idella*) reappeared in the Small Aral.

The Russian company “Zarubezhvodstroy” built the most recent dike in Berg strait. It was completed in autumn 2005. In the spring of 2006 the level of the Small Aral had reached the designated mark of 42 m, well ahead of schedule. The Small Aral area in 2007 was about 3258 km², with a volume of 27 km³. The dike in Berg strait improved the brackish water environment of the Small Aral (Figure 7).

Conservation and Rehabilitation of the Large Aral and its Ecosystems

Since the Aral Sea divided into two lakes in the late 1980s, the level of the Large Aral Sea has constantly been declining. Since the beginning of 2003, when the level in the Large Aral Sea had dropped by 23 m and reached an elevation of about +30 m, the Large Aral Sea has practically been divided into the eastern Large Aral, the western Large Aral, and Tschebas Bay. The salinity in the western part in September 2007 was 100 g/l. It is considerably higher in the eastern Large Aral, where it may reach 150-160 g/l. In both the eastern and western Large Aral, salinity has increased so much that most species that previously inhabited the lake have disappeared. At the end of the 20th century, the brine shrimp *Artemia salina* (*A. parthenogenetica*) appeared in the Large Aral Sea. Industrial harvesting by the international company INVE Aquaculture was being considered, but in 2005 the company postponed activities until salinity would increase to levels more favorable for brine shrimp.

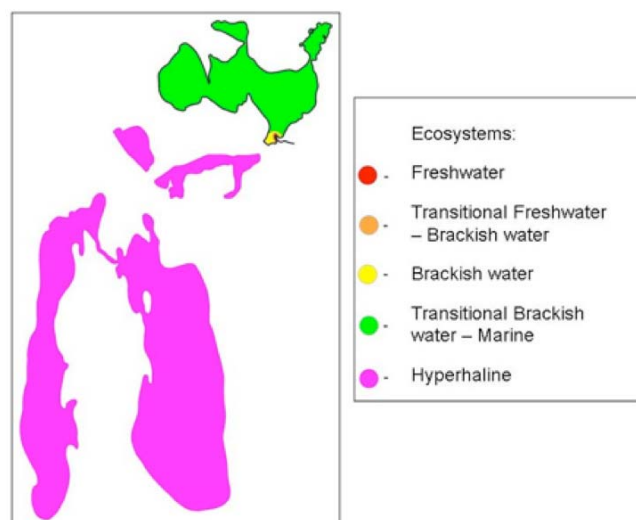


Figure 5–Ecosystems in relation to the present-day salinity.

In 2007 the zooplankton of the western Large Aral Sea consisted of: Infusoria–*Fabrea salina*; Rotatoria–*Brachionus plicatilis*, *Hexarthra fennica*; Branchiopoda–*Artemia salina* (*A. parthenogenetica*). In addition it is possible to find Cladocera–*Moina mongolica* and Copepoda–*Halicyclops rotundipes aralensis*. The zoobenthos consists of: Infusoria–*Frontonia* sp.; Turbellaria–*Mecynostomum agile*; Polychaeta–*Nereis diversicolor*; Ostracoda–*Cyprideis torosa*, *Eucypris inflata*; also it is possible that Gastropoda–*Caspihydrobia* spp. and Bivalvia–*Abra ovata* still survive. In Tschebas Bay zooplankton and zoobenthos resemble those of the western

Large Aral Sea. During this time the high salinity in the Eastern Aral Sea (150-160 g/l) restricted the zooplankton community to *Artemia salina*. No living macro- and meso-Metazoa were found in the zoobenthos of the eastern Aral Sea.

By 2005 all fishes of the Large Aral Sea had disappeared. Nevertheless, there is a chance that in places in the western Large Aral, where salinity is lower due to inflow of underground waters, some highly salinity-tolerant fish species could still survive: *Pungitius platygaster*, *Platichthys flesus*, *Atherina boyeri caspia*, and *Neogobius melanostomus*. There is unconfirmed oral information that flounder (*Platichthys flesus*) has been observed in Tschebas Bay in water with a salinity of 80-90 g/l. Also there is unofficial information that in the remnants of the strait between the western Large Aral and the eastern Large Aral, *Atherina boyeri caspia* was found in water with 60-80 g/l salinity. Only a few free-living invertebrates could survive such high salinity conditions: Infusoria-2; Rotatoria-3; Cladocera-2; Copepoda-2; Ostracoda-2; Branchiopoda-1; Decapoda-2; Bivalvia-2; Gastropoda - > 2; Polychaeta-1 for a total of > 18.

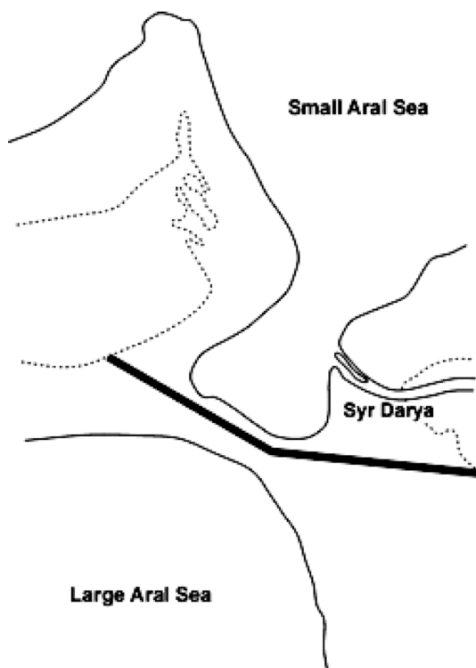


Figure 6–The dike in the Berg Strait (Aladin et al. 1995).

It may be desirable to provide more water to the eastern Large Aral from the Small Aral via the Berg Strait dike and water discharge from the Mezhdurechensky reservoir on Amu Darya via the Ak Darya river bed. The western Large Aral Sea could, perhaps, maintain its level using groundwater flow from the Amu Darya delta and the Ustjurt plateau. Realization of this project will help protect biodiversity of salt tolerant hydrobionts.

In 2005 a special water discharge facility (dike, water way and water discharge gates) was constructed to supply the Eastern depression of Large Aral with Amu Darya water from the Mezhdurechensky reservoir via the Ak Darya river bed. Unfortunately the completed spillway and water gates were destroyed by water in the autumn of 2005. Now this complex is under restoration.

Conservation and Rehabilitation of the Delta and Deltaic Water Bodies of the Syr Darya

After the construction of the first dike in Berg Strait in the summer of 1992 additional rehabilitation projects were initiated. The Syr Darya delta shifted slightly northwards and a number of freshwater reservoirs were built. Along the lower Syr Darya near the Small Aral several freshwater lakes have been rehabilitated: Tuschibas, Kamyslybas, Zhalanashkol, Karasholan, and others. These small projects allow restoring freshwater fisheries, hunting, and trapping. Fish farms were also renewed and more young fish are released into the local water bodies. Fish farms are also planned to reintroduce sturgeon into the Small Aral.

Conservation and Rehabilitation of the Delta and Deltaic Water Bodies of the Amu Darya

The Uzbekistan branch of the International Fund for Saving the Aral Sea (IFAS) in cooperation with other national institutions prepared a plan for rehabilitation of the Amu Darya delta. In the lower reaches of the Amu Darya several freshwater and brackish water reservoirs and lakes were established. One of the most successful projects is Sudochie Lake. Sudochie Lake is now completely filled and is contributing some water to the western Large Aral Sea via underground flow. Reeds, aquatic birds and hydrobionts have almost recovered in Sudochie Lake. Other former Aral Sea bays have or could be rehabilitated, including Sarbas, Muynak, Adjibay and Zhilyrbas. Fisheries and hunting activities have been resumed in the rehabilitated areas.

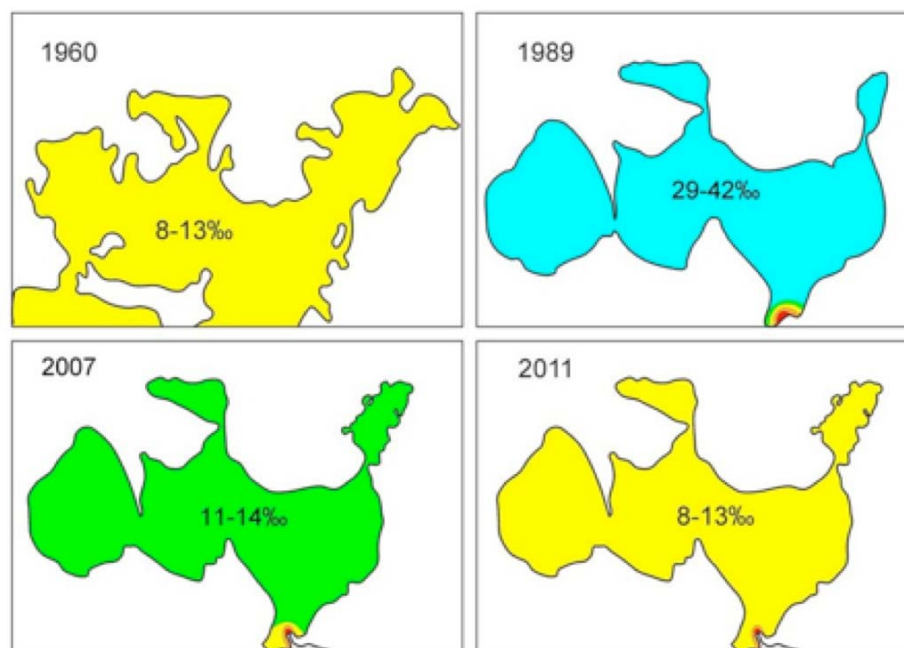


Figure 7—Decrease of salinity of the brackish water in the Small (Northern) Aral Sea as a result of the building of the dike in the Berg Strait funded by Global Environment Facility (GEF) and the Kazakhstan government.

Evidence of Desiccation of the Aral Sea in the Middle Ages

As the Aral dried, remnants of saxauls plants (*Haloxylon aphyllum*) were found on its former bottom. Some stumps were also found underwater close to the modern shoreline (Aladin & Plotnikov 1995). Radiocarbon analysis dated these to medieval times. For more paleoenvironmental reconstruction of medieval desiccation, special corings in the Aral Sea were made under the CLIMAN project (INTAS). At the end of the 20th century, Kazakh hunters found ruins of a medieval mausoleum (Kerdery) on the dried bottom. In 1960, the area was under about 20 m of water. Bones of *Homo sapiens* and domestic animals, millstones, elements of ceramics, and other artifacts were found near the mausoleum. All these findings were studied by an international team of archeologists, also under the CLIMAN project (Boroffka et al. 2005). Recently remnants of medieval river beds on the former Aral Sea bottom were also detected on satellite images. Preliminary investigations on this matter were made by D. Piriulin (personal communication).

An Alternative Second Phase of the Small Aral Rehabilitation Project

In our opinion, the future of the Aral Sea is connected with oil and gas extraction. Oil and gas drilling rigs are now widespread on the former Aral Sea bottom. A gas condensate plant was built not far from Muynak. Local decision makers even permanently closed the channel that

formally fed water into the Muynak reservoir. The gate has been closed in order to lower the groundwater level in the area. A high water table level promotes softening of the ground, endangering drill towers so that they could fall over or start to lean.

The dike in Berg's Strait has caused an increase in the level of the Small (northern) Aral Sea to +42 m a.s.l. Presently the average salinity in the Small (northern) Aral Sea is about 10 g/l. For further amelioration of the situation, irrigation efficiency has to be increased to improve the water balance. It is possible to increase the height of the present dike somewhat and raise the level up to +45 m a.s.l. This will allow an enlargement of the volume and area of the Small (northern) Aral Sea.

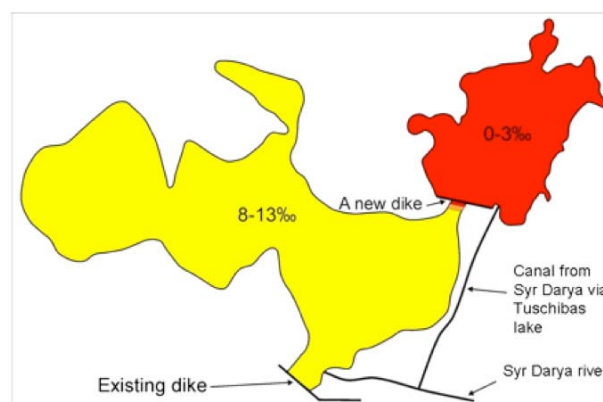


Figure 8—A variant of the second phase of the Small Aral Sea rehabilitation project.

Another possible variant of the second phase of the “Kazaral” rehabilitation project is shown in Figure 8. It would involve construction of a new dike at the mouth of the Saryshaganak Gulf to raise its level to near its 1960s mark of +53 m. Part of the flow of the Syr Darya would be diverted northward to fill the reservoir.

The second phase of the project will allow further improvement of the health of the local people, a decrease in unemployment and an increase of living standards as well as income to the local families. The local economy also will be improved (fishery, shipping, etc.). Finally, the local microclimate around the Small (northern) Aral Sea will become much more agreeable.

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The Phytoplankton of the Present-Day Central Caspian Sea

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During 2004–2008 the seasonal species succession of phytoplankton species and the quantitative structure of the phytoplankton community were examined in the area of the central part of Caspian Sea. A change in the type of diatom species dominant in the phytoplankton was noted: instead of the large-cell diatoms *Pseudosolenia calcar-avis* and *Dactyliosolen fragillissimus*, which traditionally dominated in the 1960s–1980s, *Pseudo-nitzschia seriata*, *Cerataulina pelagica*, and *Chaetoceros peruvianus*, all new species for Caspian Sea, now prevail during the spring-summer bloom.

A winter bloom of *Cerataulina pelagica* was for the first time observed in this area in the second half of February 2008 (up to 10^6 cells/l) at a temperature of 11.4°C when the water layer was uniform in temperature down to a depth of 120 m. The bloom occurred down to 100 m depth, and cells were practically equally distributed in the upper 50 m. The phytoplankton biomass in the upper layer reached 5.6 g/m^3 , with half that value at a depth of 100 m. The *C. pelagica* bloom was accompanied by development of *Pseudo-nitzschia seriata* (up to 10^6 cells/l).

Pseudo-nitzschia seriata and *Cerataulina pelagica* were the main dominant species in the Black Sea phytoplankton during the 1970s–1980s, but according to our observations the coccolithophorid *Emiliania huxleyi* and the pennate diatom *Pseudo-nitzschia pseudodelicatissima* presently dominate there. Our research in the central Caspian Sea showed presence of *E. huxleyi* and *P. pseudodelicatissima* in the plankton phytocenosis. In addition, some dinoflagellate species new for the Caspian Sea and the coccolithophorid *Braarudosphaera bugelowii* were found.

The change in the diatom component of the bloom phytoplankton community has led to a decrease in ecosystem productivity. Despite the fact that during May and October–November the diatoms bloom biomass reached $0.15\text{--}0.2 \text{ g C/m}^3$, it was twice as low as in the 1970s–1980s when *Pseudosolenia calcar-avis* dominated. The change in dominant diatom species and the increasing role of picoplankton in the primary production of the ecosystem demonstrate that the stability of the pelagic ecosystem in the central Caspian Sea is currently limited.



This is a map of the Caspian Sea including a small locator map. The drainage basin of the Caspian Sea is in yellow. The map is based on USGS and Digital Chart of the World data. Note the Aral Sea boundaries are circa 1960, not current boundaries. Wikipedia Commons at en.wikipedia.org. January 8, 2007. Accessed February 2009.

Characterization of a Eukaryotic Picoplankton Alga, Strain DGN-Z1, Isolated from a Soda Lake in Inner Mongolia, China

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ABSTRACT

Dagenoer Soda Lake is located on the Xilinhaote plateau in Inner Mongolia at an elevation of 1289 m. Blooms of one predominant kind of picoplankton algae were found in the lake throughout the year. A strain of this picoplankton alga, designated DGN-Z1, was isolated in axenic culture. Its cells are spherical or oval, 2-3 μm in diameter; it grows optimally at 0.5-1 M NaCl, and tolerates pH values from 7 to 12. Phylogenetic analysis based on sequence similarity of the 18S rRNA gene suggested that it belongs to the green algal species *Picocystis salinarum*.

INTRODUCTION

Planktonic organisms can be classified in different categories: bacterioplankton (mainly consisting of heterotrophic prokaryotes), phytoplankton (including cyanobacteria and eukaryotes), and zooplankton (formed by eukaryotic unicellular and multicellular organisms). In addition to this subdivision, organisms can be classified according to their size. Perhaps the simplest scheme is that of Dussart (1965) who divided plankton using a logarithmic size scale: macroplankton (200-2000 μm), microplankton (20-200 μm), and nanoplankton (2-20 μm). Sieburth et al. (1978) extended this classification scheme and added the terms picoplankton (0.2-2 μm) and femtoplankton (0.02-0.2 μm). The great importance of algal activity and the size of the picoplankton communities in the global primary production of aquatic ecosystems have been extensively documented in the literature (Craig 1985; Stockner 1988, 1989). In oligotrophic lakes, between 50 and 70% of the annual carbon fixation is attributed to organisms that pass through 1-2 μm pore size filters (Munawar & Fahnenstiel 1982, Callieri & Stockner 2002). Ten percent of the primary biomass in the sea was produced by the smallest free-living eukaryote (Fouillard et al. 2004).

Planktonic organisms can further be subdivided based on their physiological properties and their taxonomic affiliation (Stockner et al. 1978; Malone 1980). In the past decade, molecular biology method have been applied to the analysis of the diversity of small eukaryotes in the ocean,

and the diversity of small plankton was shown to be very high (López-García et al. 2001; Moon-van der Staay et al. 2001; Moreira & López-García 2002).

In this paper we report the isolation and characterization of a small phytoplankton species from Dagenoer Soda Lake, an inland soda lake located in the Xilinhaote area of Inner Mongolia, Autonomous Region of China. The primary production of plankton in this lake is very high (Huo et al. 2005). One type of small green plankton is dominant throughout the year, even when the lake is covered with ice (Figure 1). We here document the physiological properties of the organism as well as its phylogenetic affiliation, based on molecular methods.

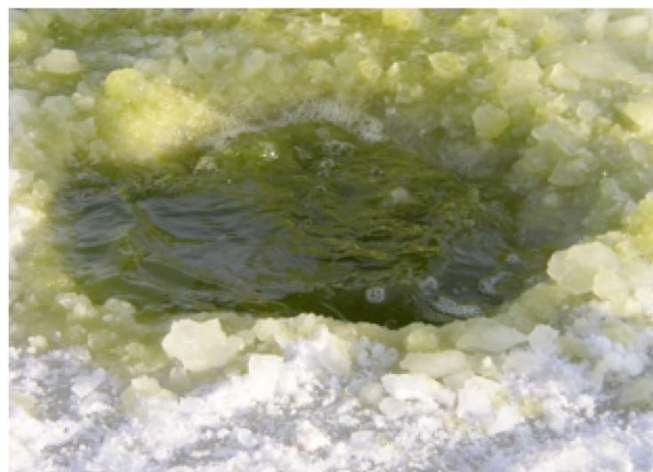


Figure 1—A bloom of picoplankton algae in Dagenoer soda lake covered with ice in winter.

MATERIALS AND METHODS

The Sample Collection Site

Dagenoer Soda Lake is located at 42°40'54"N, 115°50'37"E, in the Xilinhaote area of Inner Mongolia, Autonomous Region of China, at an altitude of 1297 m. The lake covers an area of approximately 2.1 km² and has a maximum depth of 1.1 m. The water has a salinity of 18.8‰ and a pH of 10. Four samples were collected in August, September, October and December 2003.

Isolation of an Axenic Culture of a Picoplankton Species

Clonal cultures were established by streaking water samples on 1% agar medium (Ds medium) which contains (in g l⁻¹): NaNO₃, 0.42; NaH₂PO₄·2H₂O, 0.156; NaHCO₃, 0.84; KCl, 0.074; MgSO₄·7H₂O, 1.23; CaCl₂·2H₂O, 0.044; 0.5 ml l⁻¹ of solution of 1% ferric citrate; and trace elements (in mg l⁻¹): H₃BO₃, 286; MnCl₂·4H₂O, 18.1; ZnSO₄·7H₂O, 2.2; CuSO₄·5H₂O, 0.79; g (NH₄)₆Mo₇O₂₄·4H₂O, 0.39. Cultures were incubated at room temperature under a 12 hours light–12 hours dark regime. Single colonies were picked and transferred to 5 ml of the same medium without agar in 20 mm capped glass test tubes. Cultures were established by serial restreaking on agar and isolating single colonies. The absence of bacteria was established by streaking on nutrient media (Lewin et al. 2000).

Scanning Electron Microscopy

Cells were harvested from a culture in the exponential growth phase, and collected by centrifugation at 4000 rpm for 10 minutes at 4°C. Then the cells were fixed with 2.5% glutaraldehyde, 1% osmium tetroxide, and 30 mM HEPES buffer (pH 7.2) at 4°C for 15 minutes. After rinsing twice with distilled water, they were dehydrated in an ethanol series (30%, 50%, 75%, 90%, 2×100%), and examined in a Hitachi S-570 electron microscope at 12 kV.

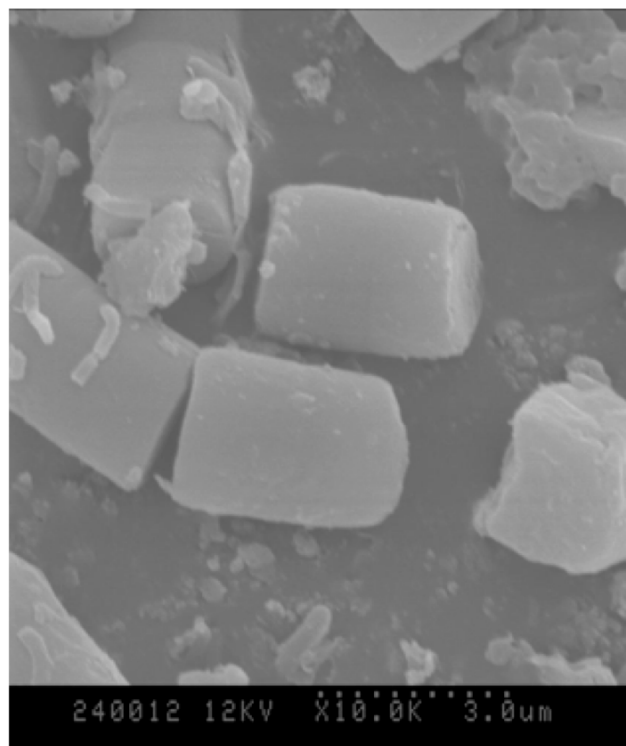


Figure 2—Strain DGN-Z1 observed in the scanning electron microscope.

Growth Experiments at Different Salinities and pH Values

Portions of 20 ml of exponentially growing cultures were transferred to flasks containing growth medium as described above, in which the salinity was varied between 0.5 and 5 M and pH between 7 and 12. Cultures were incubated at room temperature under a 12 hours light–12 hours dark regime, and the flasks were shaken three times a day. Algal growth was monitored by measuring OD_{540nm} in a spectrophotometer.

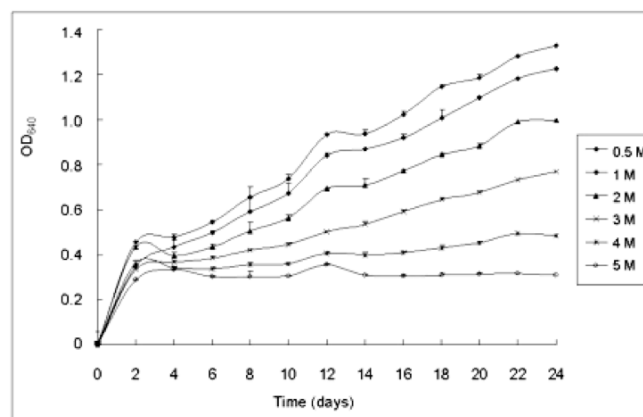


Figure 3—Growth of strain DGN-Z1 at different salt concentrations.

DNA Extraction, and Amplification, Cloning, and Sequencing of the 18S rRNA Gene

Portions of cells (1 ml) were harvested from an exponentially growing culture by centrifugation (4°C, 4000 rpm, 10 min). The supernatant was discarded and the cells were incubated for 60 minutes in lysis buffer (500 μl TE buffer, 60 μl 10% SDS, 30 μl 20 mg ml⁻¹ proteinase K) in a water bath at 55°C. DNA was extracted after addition of 1 volume phenol-chloroform-isoamyl alcohol (25:24:1, pH 7.9) by brief vortexing, followed by centrifugation (12000 rpm, 10 min). The aqueous phase from each sample was mixed with 0.1 volume of 5 M NaCl and two volumes of 96% (vol/vol) ethanol and left overnight at -80°C. Each sample was centrifuged (12000 rpm, 30 min, 4°C), and the DNA pellet was washed with 70% ethanol. Finally, the extracted DNA was stored at -80°C in autoclaved deionized water. PCR was performed with the primers DMA1: 5' to CGG GAT CCG TAG TCA TAT GCT TGT CTC 3', DMA2: 5' to CGG AAT TCC TTC TGC AGG TTC ACC 3' (Olmos et al. 2000). The reaction was carried out in an Eppendorf tube with 50 μl solution containing 1 U of Taq polymerase, 1 x buffer B with 1.5 mM Mg²⁺, 10 nmol of deoxynucleoside triphosphate (Promega Cor.), 50 pmol of each primer, and approximately 50 ng of extracted template DNA. PCR reactions were performed in a thermocycler

(Biorad iCycler) under the following conditions: hot start for 30 s at 94°C, 30 PCR cycles and 10 minute elongation at 72°C. Each single cycle consisted of a 30 s 94°C denaturing step, a 30 s annealing step at 55°C, and a 30 s elongation step at 72°C. PCR products were gel purified on 1% (wt/vol) low-melting-point agarose gels, cloned using the pGEM-T vector (Promega), and transformed according to manufacturer's manual. Individual colonies were picked randomly for sequencing of the plasmid inserts. Plasmid DNA was sequenced using a ABI377 PE (Perkin Elmer) sequencing machine.

Phylogenetic Analysis

Sequence data were BLAST analyzed against the GenBank database (Altschul et al. 1997). Clone sequences were deposited in the GenBank. Sequences were aligned with their closest relatives and representative cultured and uncultured picoplankton species by using DNAMAN software. Phylogenetic trees were constructed from the alignment sequences by using Jukes-Cantor distance matrices for inferring the tree topology and neighbor joining and maximum-parsimony for bootstrap analysis (Saitou & Nei 1987).

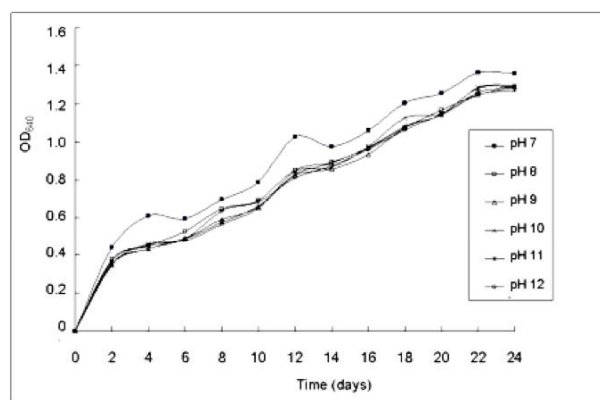


Figure 4—Growth of strain DGN-Z1 at different pH values.

RESULTS

One type of green picoplankton algae was dominant in Dagenoer Soda Lake throughout the year, even when the water was covered with ice in winter (Figure 1). A strain of this species, designated DGN-Z1, was isolated in axenic culture. Its cells are spherical or oval, and measure 2-3 μ m in diameter (Figure 2). The optimal NaCl concentration for growth of strain DGN-Z1 was 0.5-1 M; no growth was obtained in 4 and 5 M (Figure 3). The isolate grew well at pH 7-12 (Figure 4).

The 18S rRNA gene sequence (GenBank accession number EU935604) was used for phylogenetic tree construction together with other picoplankton algae sequences such as AF153314 (*Picocystis salinarum*), EF440183 (*Nanochloris* sp. ant-2), Y15814 (*Ostreococcus*), AB183605 (*Imantonia*), EU106816 (*Pelagomonas calceolata*), EU106738 (*Bolidamonas* sp. RCC852), AF123596 (*Bolidamonas mediterranea*), and AY254857 (*Florenciella parvula*). The resulting phylogenetic tree is shown in Figure 5. The sequence similarity analysis and the phylogenetic analysis of the 18S rRNA gene suggested that the strain belongs to the species *Picocystis salinarum*.

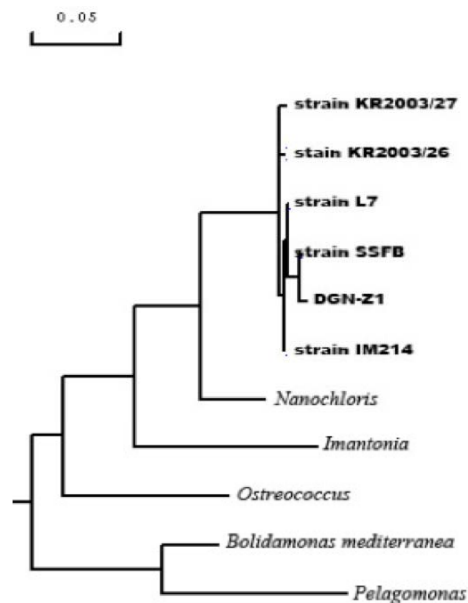


Figure 5—Phylogenetic tree based on 18S rRNA gene sequences, showing the phylogenetic position of strain DGN-Z1. Sequences used: five strains of *Picocystis salinarum*: DQ267705 (strain KR2003/27), DQ267704 (strain KR2003/26), AF153313 (strain L7), AF153314 (strain IM214), AF125167 (strain SSFB); in addition EF440183 (*Nanochloris* sp. ant-2), Y15814 (*Ostreococcus*), AB183605 (*Imantonia*), EU106816 (*Pelagomonas calceolata*), EU106738 (*Bolidamonas* sp. RCC852), AF123596 (*Bolidamonas mediterranea*), AY254857 (*Florenciella parvula*), and EU935604 (DGN-Z1).

DISCUSSION

Although autotrophic picoplankton can contribute high primary production and if of great ecological importance, little is known about the taxonomy of the organisms involved. Among the smallest eukaryotes are various autotrophic picoplanktonic chlorophytes, variously assigned to *Bathycoccus* Eikrem & Throndsen, *Ostreococcus* Courties & Chrétiennot-Dinet in Chrétiennot-Dinet et al., *Pycnococcus* Guillard in Guillard et al. (Eikrem & Throndsen 1990; Guillard et al. 1991; Courties et al. 1994, 1998), *Chlorella* Beijerinck, *Choricystis* (Skuja) Fott, *Nanochlorum* C. Wilhelm, Eisenbeis, A. Wild & R. Zahn

(Andreoli et al. 1978; Huss & Sogin 1990; Krienitz et al. 1996; Huss et al. 1999), and *Mychonastes*. Simpson & van Valkenburg (Kalina & Puncochgova 1987; Hanagata 1998; Krienitz et al. 1999). In Dagenoer Soda Lake, the predominant tiny alga represented by isolate DGN-Z1 may be *Picocystis salinarum*, based on 18S rRNA phylogenetic analyses. The species *Picocystis salinarum* was first isolated from a San Francisco Bay saltern and was described and named by Lewin et al. It has several unique features, notably in cell shape, cell wall and pigment composition (Lewin et al. 2000). It grows in saltern ponds or salt lakes at a salinity of about 18%. The cell wall of *Picocystis salinarum* has a sugar composition quite different from that of any species of the Trebouxiophyceae (to which *Nanochlorum* and *Choricystis* belong) or the Chlorophyceae, including *Mychonastes* and some 40 strains of *Chlorella* assigned to nine species (Takeda & Hirokawa 1984; Takeda 1988a, 1988b, 1991, 1993; Krienitz et al. 1999), in which the fibrous components are mainly composed of glucose accompanied by mannose or glucosamine. Isolate DGN-Z1 appears to be cold-tolerant (Figure 1), and on the basis of its combined tolerance to high salt concentrations, high pH and low temperature it may represent a new type.

ACKNOWLEDGEMENTS

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Zabuye Salt Lake Solar Pond in Tibet, China: Construction and Operational Experience

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ABSTRACT

We describe the construction of the Zabuye Salt Lake solar pond and our experience during its operation. The salinity gradient was experimentally determined in the pond, which has a surface area of about 3588 m², at different operation conditions and modes of operation. The method for establishing a salinity and temperature gradient can save large amounts of fresh water during the establishment of a temperature and salinity gradient in a solar pond. A technology to control solar pond operation was developed on the basis of our experimental results and is now being used to operate the pond.

INTRODUCTION

The structure and basic properties of solar ponds have been studied in China since about 1977 (Xu & Li 1983). Experiments have been performed in a wide geographical area (Figure 1) including Zhengzhou, GanSu, BeiJing, GuangZhou, GuangXi, and other sites (Song et al. 1984; Meng & Zheng 1991; Zheng & Meng 1991; Wang et al. 1992; Li 1994, 1995). The mechanism of operation, the heating regime, and the possible fields of application were reported by Li (1989, 1995). These studies have shown that solar ponds can be applied to supply heat to a marsh gas pool used to produce methane gas from organic waste and to aquaculture ponds. In practice, solar ponds have been successfully applied in the production of Glauber's salt in XinJiang (Yang et al. 1990; Ma et al. 1998; Ding et al. 1997; Ding 2002, 2003) and in aquaculture during winter in ShanDong (Chao & Song 2003). Solar ponds have also been successfully used for the production of lithium carbonate in Tibet since 2002 (Luo 2003a, 2003b).

Zabuye Salt Lake is located in central Tibet (Figure 1), about 1050 km from Zabuye in the direction of Lhasa, at an altitude of 4421 m. The air in this area is very dry. The lake brine is hydrochemically unique, forming a comparatively complex and special mineral assemblage characterized by alkaline minerals rich in lithium, boron, potassium, and sodium carbonate. The lake brine stores a huge amount of lithium carbonate in the form of the recently named mineral

zabuyelite (Li₂CO₃) (Zheng et al. 1989). The average annual temperature is about 2.2°C with about 229 days per year below freezing. As a result of the high insolation in the Zabuye Salt Lake area, there are about 248 days of sunshine per year, the highest radiation is about 10⁷ kJ m⁻² yr⁻¹.



Figure 1—The location of experimental solar ponds in China operated since about 1977.

Solar energy is the first economic alternative energy resource for exploiting the salt lake due to its remote location and the local climate. Mian-ping Zheng first suggested in 1996 that solar ponds could be used to exploit the lithium resource from Zabuye Salt Lake. Later, a solar pond was constructed and tests were initiated, and a pond with surface area of 40 m² and a depth of 1.4 m was constructed in 1999. This pond was used to study the possibility of supplying the heat necessary for producing lithium carbonate (Zhao 2003; Zhao et al. 2004). In 2000, the construction of a solar pond with a surface area of 1250 m² and a depth of 2.5 m was started near Zabuye Salt Lake as a collaborative effort between the Chinese Academy of Geological Sciences (CAGS) and the Tibet Zabuye High-Tech Lithium Industry Company Ltd. (ZBY). In this phase of the study it was shown that the solar pond could be directly used for the production of lithium carbonate (Luo 2003a, 2003b; Luo & Zheng 2004a, 2004b). A large solar

pond for producing lithium carbonate was built in October 2002 with a surface area of 3588 m² and a depth of 4 m resulting from collaboration between Tianjin University of Science & Technology, ZBY and CAGS.

To operate the solar pond, the bottom 2-2.5 m was filled with saturated lithium carbonate brine to form the lower convective zone (LCZ). The upper part of the pond was filled with fresh water for establishing the non-convective zone (NCZ). After the NCZ layer is formed, the fresh water was filled to a depth of about 25 cm. This newly formed fresh water layer is called the upper convective zone (UCZ). The distribution of temperature and salinity in the pond, the mode of establishing the salinity gradient and the way the stability of the pond is maintained are very important for the recovery rate of the lithium carbonate and the production capacity of the pond. The method of the LCZ, NCZ and UCZ development will be discussed in following sections.

In this work we analyzed the temperature and salinity distribution in the pond in an attempt to develop a suitable method for establishing the salinity gradient and maintaining its stability.

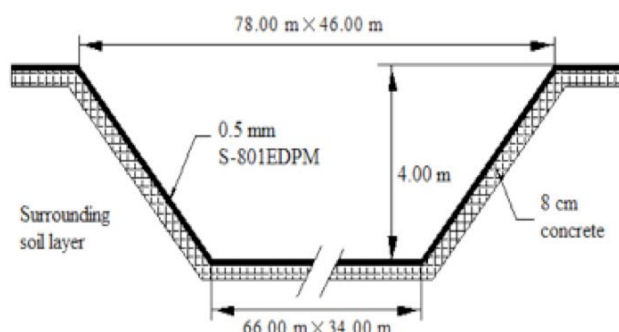


Figure 2—Dimensions of the Zabuye Salt Lake solar pond.

Construction of the Solar Pond

A barren tract of hilly land was available for construction of the solar pond on the southwest of Zabuye Salt Lake. The configuration of the “Zabuye Salt Lake solar pond” is trapezoid as shown in Figure 2. The dimensions of the pond are: bottom 66 x 34 m; surface 78 x 46 m, area 3588 m², and depth is 4 m. The slope of the side wall is 1.5:1. The soil was properly compacted after excavation according to the designed size. The pond bottom and the side surface were covered with an 8 cm thick layer of concrete. To reinforce the concrete surface of the pond, a steel grid was added in the corners and in the middle part of the pond bottom. The concrete surface had to be very smooth to affix a 0.5 mm thick sheet of S-801EPDM (Ethylene-Propylene-Diene-Monomer), applied to prevent brine leakage.

Experimental Design and Methods

To monitor the operation characteristics of Zabuye Salt Lake solar pond, an automatic temperature measurement system was installed prior to the start of operation of the pond. It included two digital thermometers, 32 thermistors, and a computer with a suite of control software. Every minute the system recorded 32 channels of the temperature profile as well as the soil temperature below the pond (Figure 3). The temperature at the surface of the pond was measured by a floating thermistor, and the surface level was recorded manually. The salinity of samples collected near the location of the temperature sensors was calculated from the specific gravity at 25°C. Specific gravity was measured with an accuracy of ± 0.001 , corresponding to an accuracy of approximately $\pm 0.5\%$ by weight for the salinity values. A weather observation station was established near Zabuye Salt Lake in November 1991. The station provides meteorological data including incident light, hours of sunshine, air temperature, ground temperature, relative humidity, wind speed, rainfall, fresh water evaporation rate, etc.

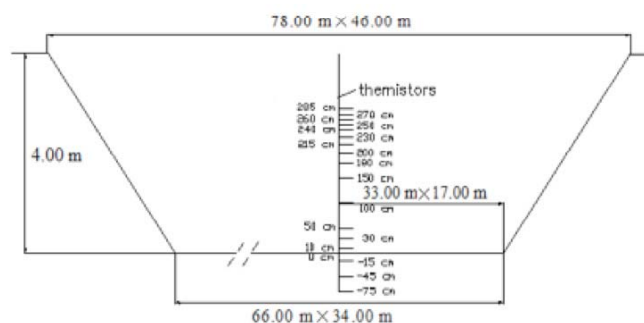


Figure 3—Location of the thermistors for measuring temperature profiles in solar pond.

The Salinity Gradient

Thirty ponds of similar size would be used to produce lithium carbonate at the same time in Zabuye. If the UCZ and NCZ were developed by using fresh water, a large amount of fresh water would be needed. As fresh water is rare in this area, a special method was developed to establish the salinity gradient in the Zabuye Salt Lake solar pond. The gradient has to be composed of three zones. The LCZ brine layer near the bottom is used as the brine for crystallization at increased temperature. To prevent heat loss from the pond, an NCZ has to be established in the pond. To maintain the salinity gradient in the NCZ, there has to be a UCZ layer. To achieve this, the bottom of the pond was filled with saturated lithium carbonate brine (the typical density of the brine is 1.267~1.288 g cm⁻³) to form

an LCZ of about 1.5-2.5 m depth. Then, fresh water was added directly on top of the LCZ layer to form a layer of about 5-20 cm. When the salinity of the surface of the fresh water layer started to increase, an additional similar amount of fresh water was added. Thus, a salinity gradient was gradually established in the NCZ layer. No salinity should be present at the surface of the pond. The final thickness of the UCZ depends on wind mixing, on the heating effect of the pond walls, and on the temperature distribution along the depth of the pond. The thickness of the NCZ layer was about 40-60 cm. It took about a month to establish the gradient. A typical salinity distribution for a perfectly established salinity gradient is shown in Figure 4, with a salt gradient zone (NCZ) of about 40 cm depth, an UCZ of about 25 cm, and an LCZ of about 2.1 m depth

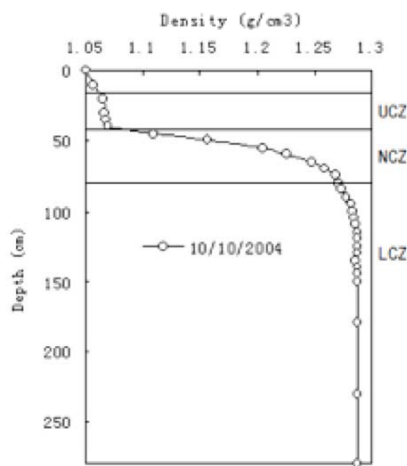


Figure 4–The density profile measured on October 10, 2004 in the pond after establishment of the salinity gradient.

Temperature Distribution

Information on the temperature distribution is important for the production of lithium carbonate. To control the production process, we measured the temperature changes with time and with depth. As climatic conditions changed dramatically over the seasons, the temperature distribution in the pond was also measured in an annual cycle. A typical temperature profile distribution during the operation period is given in Figure 5. The temperatures in the LCZ layer at different times of the year are shown in Figure 6. Figure 5 shows that the temperature increased with time. The lowest temperature is found on the pond surface, and the highest temperature occurs in the LCZ layer. This temperature distribution is very favorable for the production of lithium carbonate. The highest temperatures in the LCZ were observed in August and the lowest values were found in February. Thus, the summer season is more suitable for lithium carbonate production because the winter temperatures are too low for the process.

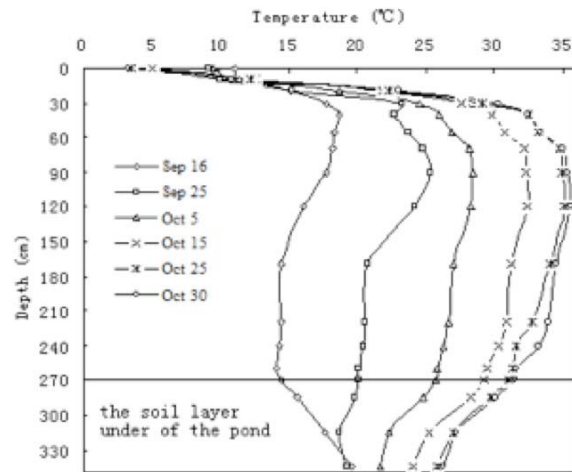


Figure 5–The temperature profile distribution in the Zabuye Salt Lake solar pond during 2004.

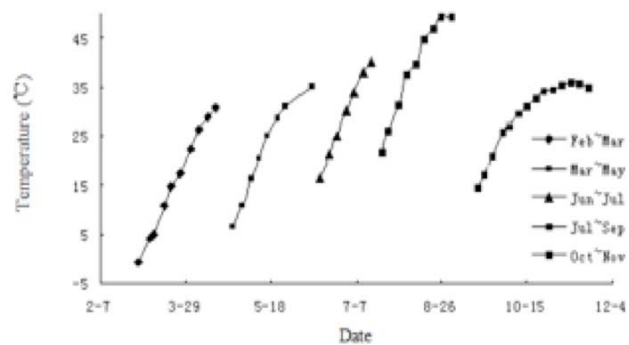


Figure 6–The temperature of LCZ distribution in Zabuye Salt Lake solar pond from February to November 2004.

Operation of the Solar Pond

Zabuye Salt Lake is exposed to strong winds from winter to spring, with wind speed up to 28 m s^{-1} and an average annual wind speed of 6 m s^{-1} . The wind can affect the establishment of the NCZ layer. When wind speed is lower than 10 m s^{-1} , the wind increases mixing of the UCZ and NCZ layers. This shortens the time needed to establish the NCZ layer, saving operation time. On the other hand, at wind speeds exceeding 10 m s^{-1} , the salinity gradient is destroyed and the NCZ layer cannot be established. To prevent destruction of the NCZ layer by wind, the size of the pond should not be too large. On the other hand, the “freeboard”, which is the distance between the solution surface in the pond and the top of the pond, reduces the wind speed reaching the solution surface (Atkinson & Harleman 1987). The “freeboard” was controlled at 1.5 m in winter and spring and 1 m in summer and autumn. When the level of the brine in the pond was low, fresh water was added. This mode of operation provided good conditions for establishing the NCZ and maintaining a stable NCZ layer.

Zabuye Salt Lake contains high concentrations of sodium and potassium ions. When the temperature in the solar pond is close to 35-40°C, the β -carotene-rich *Dunaliella salina* (Zheng et al. 1989) will develop in large numbers. This alga produces a red pigment that colors the brine, reducing penetration of solar radiation, decreasing the production rate and lowering the quality of the product. After the solar pond had been in operation for two months, the LCZ brine became red, and the temperature did not increase further. To avoid the effect of the carotene-rich *Dunaliella salina* growth on the production of lithium carbonate, the operation period was restricted to one and half months to two months.

The amount of heat stored in the solar pond is influenced by the depth of the LCZ. The deeper this layer is, the lower the temperature that can be reached in the pond, and the smaller the heat loss. The depth of the LCZ also affects the rate of temperature increase. To examine the effect of LCZ depth on the temperature changes in the pond, we experimentally varied the depth of the LCZ. It was found that the same temperature can be obtained for different depths of the LCZ, but the pond has to be operated for a sufficiently long time when the LCZ is deeper (Figure 7). Under optimal conditions, the brine depth (LCZ) is controlled at 2 m from February to May, and at 2.5 m in all other seasons. The depth of UCZ and NCZ are kept the same for different season.

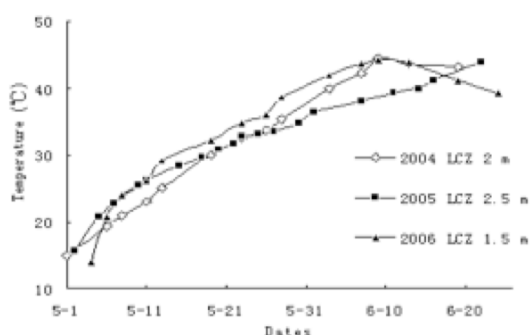


Figure 7—The temperature changes with time of the lower convective zone for different depths in the Zabuye Salt Lake solar pond from 2004 to 2006.

Theoretically, Zabuye Salt Lake solar pond can be operated throughout the year. However, during the winter the pond temperature does not increase sufficiently. Therefore lithium carbonate can only be produced from February until October. The operational period of the solar pond can be divided into four sections: (1) from February to March, for an operational time of 50 days, and temperature rising to 30-32°C; (2) from April to May, for a period of about

45 days, with temperatures increasing to 34-38°C; (3) from May to June, for an operational time of 40 days, temperatures reaching up to 40-45°C; (4) from July to September, for a period of 55 days, with temperature maxima of 45-50°C. The solar pond can be used for sodium carbonate production between October and February because the temperature is very low for producing lithium carbonate.

CONCLUSIONS

In this paper we described the basic construction of the Zabuye Salt Lake solar pond. The salinity gradient in the pond was determined for different operating conditions and methods. We have developed a suitable method for establishing the salinity and temperature gradients. The results prove that the method developed in this work can save large amounts of fresh water when building-up the temperature and salinity gradient in the solar pond. The technology to control the solar pond operation, based on our experimental results, is now being used in routine operation of the pond.

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Crystallization Path of Salts from Brine in Zabuye Salt Lake, Tibet, During Isothermal Evaporation

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ABSTRACT

Zabuye Salt Lake in Tibet, China is a carbonate-type lake, rich in Li, B, K and other useful trace elements that are of great economic value. We studied the concentration behavior of these elements and the crystallization paths of salts in the brine at 25°C, based on an isothermal evaporation experiment. The crystallization sequence of the primary salts from the brine at 25°C is halite (NaCl) → aphthitalite ($3\text{K}_2\text{SO}_4 \cdot \text{Na}_2\text{SO}_4$) → zabuyelite (Li_2CO_3) → sylvite (KCl) → trona ($\text{Na}_2\text{CO}_3 \cdot \text{NaHCO}_3 \cdot 2\text{H}_2\text{O}$) and thermonatrite ($\text{Na}_2\text{CO}_3 \cdot \text{H}_2\text{O}$), in accordance with the metastable phase diagram of the Na^+ , K^+/Cl^- , CO_3^{2-} , SO_4^{2-} - H_2O quinary system at 25°C, except for sodium carbonate heptahydrate ($\text{Na}_2\text{CO}_3 \cdot 7\text{H}_2\text{O}$) which is replaced by trona and thermonatrite. In the experiment, zabuyelite (Li_2CO_3) was precipitated in the early stage because Li_2CO_3 was supersaturated in the brine at 25°C. Potassium was precipitated as aphthitalite in the intermediate stage and as sylvite in the late stage, while boron was concentrated in the early and intermediate stages and precipitated as borax ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) in the late stage.

INTRODUCTION

Zabuye Salt Lake, a carbonate-type saline lake, is located in the interior of the Tibetan Plateau, China, ~1000 km west of Lhasa, capital of Tibet (Figure 1). The lake's brine is supersaturated with NaCl and other salts. Millions of metric tons of halite, potash, trona, and other minerals are deposited on the bottom of the lake (Zheng et al. 1988, 1989). The salt lake is of great economic value as a new type of large sedimentary deposit containing dominantly lithium and borate salts in addition to potash, halite, natron and Glauber's salt. The lake also contains cesium, rubidium and bromine (Zheng et al. 1989; Zheng 1999; Zhao et al. 2003). The level of the lake, which has been subject to historical variations, is currently (2008) at ~4422 m above sea level. At this level, the lake's area is approximately 247 km². The salinity varies from 36 to 44%, depending on periodical differences in water input and evaporation rate (Qi & Zheng 2007).

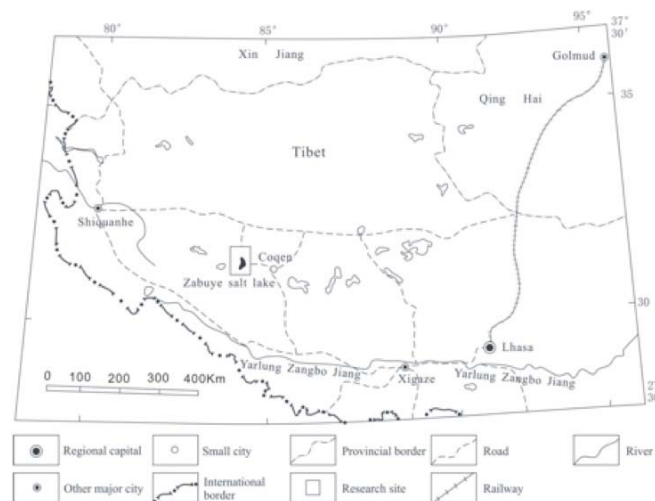


Figure 1– Location of Zabuye Salt Lake.

Zabuye Salt Lake was first recognized in 1961 to be a deposit of borax (Zheng et al. 1989). Since the 1980s, substantial scientific research has been performed in order to exploit and utilize its mineral resources (Yang et al. 1996; Nie & Zheng 2001). Here M. Zheng first found zabuyelite, a mineral of natural lithium carbonate (Zheng & Liu 1987). In 1990, the Research & Development Center for Saline Lakes and Epithermal Deposits at the Chinese Academy of Geological Sciences, established a long-term scientific observation station near Zabuye Salt Lake to monitor the physical-chemical data of the lake. This station is still in operation (Zhang et al. 2001; Nie et al. 2005).

Mineral exploitation in Zabuye Salt Lake is now conducted exclusively by a mining company, the Tibet Zabuye High-Tech Lithium Industry Company Ltd. This company has built a production plant and produced nearly 2000 metric tons of lithium carbonate per year since 2005 (Huang et al. 2008). Lithium carbonate is the company's only product; though lithium ranks only eighth in abundance in the lake, out of every 100 metric tons of minerals contained in the lake, only ~1.25 metric tons are lithium carbonate. Therefore, many tons of mineral salts have been stockpiled near the solar ponds during the process of extracting lithium carbonate from Zabuye Salt Lake. This stockpiled material may be harmful to the environment. This paper reports a

test designed to study the element concentration characteristics and mineral crystallization paths of Zabuye Salt Lake brine through an isothermal evaporation test at 25°C. This study will help us to devise ways to extract halite, potash, borax and lithium carbonate together, leading to an improved use of the total mineral resources of Zabuye Salt Lake and to enhance the protection of the environment.

MATERIALS AND METHODS

A brine sample for the experiment was collected from Zabuye Salt Lake in October 2001. The temperature of the lake's brines generally ranges between -15°C in January to 25°C in late summer. The experiments with the brine were conducted at 25°C.

The main chemical composition of a Zabuye Salt Lake brine sample is shown in Table 1. Na and Cl ions account for to up to 80% of the total chemicals in the lake, while Na, Cl, K, CO_3^{2-} and SO_4^{2-} ions together make up 95% of the total. The quinary water-salt system Na^+ , $\text{K}^+/\text{CO}_3^{2-}$, SO_4^{2-} , Cl^- -H₂O can be used to explain the concentration and crystallization behavior of the various elements in brine (Fang et al. 1991). From the metastable phase diagram of the quinary water-salt system Na^+ , K^+ , CO_3^{2-} , SO_4^{2-} , Cl^- -H₂O at 25°C (Figure 2) we may conclude that the sequence of mineral precipitation at 25°C is aphthitalite, $\text{Na}_2\text{CO}_3 \cdot 7\text{H}_2\text{O}$ and sylvite.

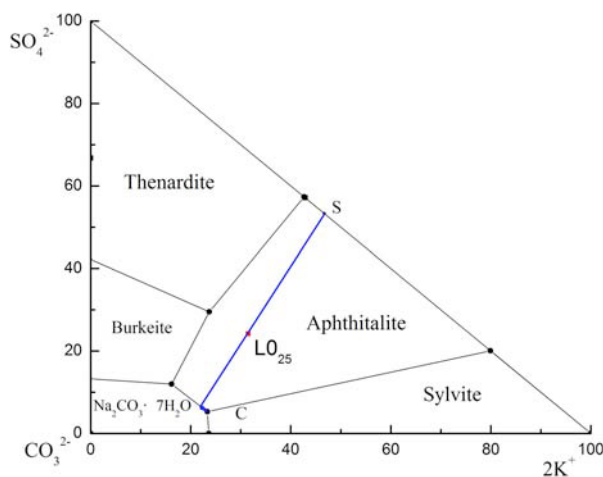


Figure 2—Metastable phase diagram and crystallization path of the quinary water-salt system Na^+ , K^+ , CO_3^{2-} , SO_4^{2-} , Cl^- -H₂O (at 25°C). L0₂₅, the compositional point of the original brine for the experiment; S, the solid compositional point of Aphthitalite; C, the steady equilibrium point of the metastable phase diagram; Thenardite, Na_2SO_4 ; Burkeite, $\text{Na}_2\text{CO}_3 \cdot 2\text{Na}_2\text{SO}_4$.

For the isothermal evaporation experiment the brine was placed on a glass evaporating dish and the experiment was conducted in a temperature-controlled environment at 25±0.5°C, regulated by a mercury contact thermometer. An

exhaust fan was used to supply a discontinuous air draft and an incandescent lamp was used as the heat source. Newly precipitated material was removed three times per day, chemically analyzed, and examined under the microscope to identify the precipitated minerals. The solid and liquid phases were separated when a new mineral was observed (Zhang et al. 2005; Zheng et al. 2007), and the relevant parameters of the solid and liquid phases were then determined, including complete chemical analysis of all the samples. The evaporation experiment was continued until the brine had dried up.

The following analytical methods were used (Analysis Group 1988): K, Li, and Na ions were quantified by atomic absorption spectrometry; Cl ion by silver nitrate volumetric analysis; the SO_4 ion was assayed gravimetrically as barium sulfate, and B_2O_3 and CO_3 ions were measured by mannitol volumetric analysis.

Table 1—The main chemical composition of Zabuye Salt Lake brines (g/l).

Sample no.	Na^+	K^+	Li^+	Cl^-	SO_4^{2-}	B_2O_3	CO_3^{2-}
L0 ₂₅	129.6	40.9	1.05	156.2	38.6	11.0	44.2

RESULTS

In the course of the isothermal evaporation experiment we analyzed the chemical composition of brines and precipitated (solid phase) mineral salts. Table 2 shows the chemical composition of the liquid phases formed by isothermal evaporation of autumn brines at 25°C, while Table 3 presents the composition of the minerals (solid phase) precipitated during this process.

There are five solid-phase regions saturated with NaCl in the metastable phase diagram of the quinary water-salt system Na^+ , $\text{K}^+/\text{CO}_3^{2-}$, SO_4^{2-} , Cl^- -H₂O at 25°C; these are sylvite (KCl), aphthitalite ($3\text{K}_2\text{SO}_4 \cdot \text{Na}_2\text{SO}_4$), sodium carbonate heptahydrate ($\text{Na}_2\text{CO}_3 \cdot 7\text{H}_2\text{O}$), thenardite (Na_2SO_4), and burkeite ($2\text{Na}_2\text{SO}_4 \cdot \text{Na}_2\text{CO}_3$). The compositional point of the original brine for the experiment is located in the aphthitalite region. Because the brine is saturated with NaCl, halite is precipitated immediately after the isothermal evaporation experiment begins. Then, aphthitalite becomes saturated as the experiment proceeds. The liquid-phase compositional points basically move along the extension of the tie line of the original brine compositional point and the aphthitalite solid phase point. The precipitated salts are mainly halite and aphthitalite.

Then, Li_2CO_3 becomes saturated and zabuyelite is precipitated together with halite and apththalite. After that, KCl saturation is reached, and sylvite is precipitated. In the late stage of the experiment, trona and thermonatrite start to precipitate in large amounts. The sequence of mineral precipitation from the brine is thus halite \rightarrow apththalite \rightarrow zabuyelite \rightarrow sylvite \rightarrow trona and thermonatrite. The whole 25°C isothermal evaporation and salt precipitation path essentially coincides with the metastable phase diagram of the Na^+ , $\text{K}^+//\text{Cl}^-$, CO_3^{2-} , SO_4^{2-} - H_2O quinary system at 25°C (Figure 3).

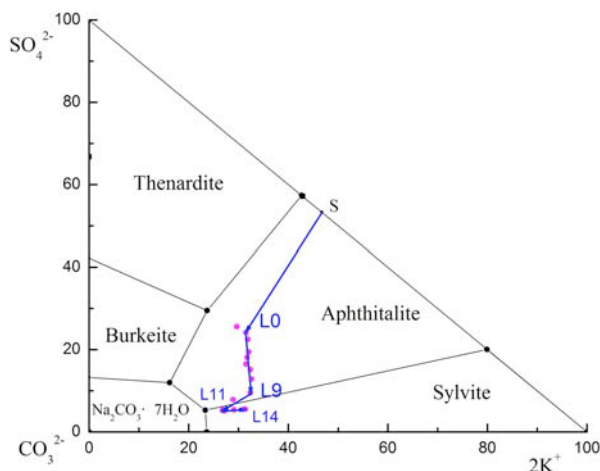


Figure 3—Crystallization path of autumn brine in Zabuye Salt Lake at 25°C.

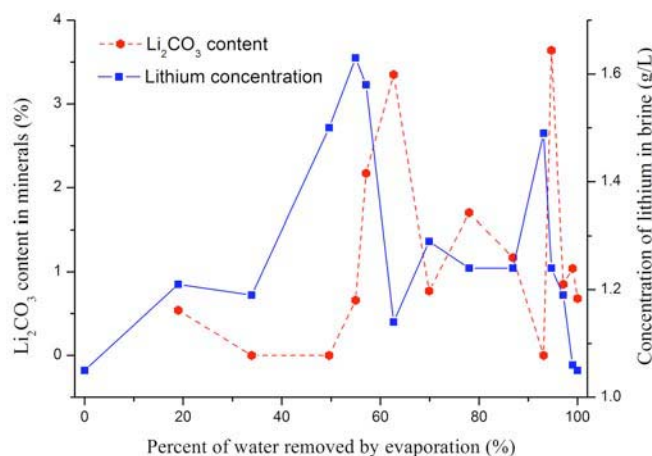


Figure 4—Content of lithium and its minerals during isothermal evaporation at 25°C. The relation of the weight percentages of minerals in the solid phase and ion concentration is shown as a function of the percentage of water removed by evaporation.

DISCUSSION

The main ions in Zabuye Salt Lake brine are Na and Cl, which account for 80% of the brine chemical composition. NaCl in the brine is in a saturated state during evaporation

at 25°C. Therefore, halite is precipitated throughout the entire isothermal evaporation process. In the early and middle stages of the experiment, halite was the dominant solid phase component. Only in the late stage did the amount of halite decrease.

The concentration and precipitation behavior of lithium in Zabuye Salt Lake brine at the 25°C temperature and its relation to the evaporation rate are shown in Figures 4. In the early stage of evaporation, lithium is concentrated in the brine. However, lithium attains saturation fast and a large amount is precipitated as lithium carbonate. The peak content of zabuyelite in the solid phase corresponds to the lowest lithium concentration in brine, exhibiting an inverse relation. Two main peaks of lithium precipitation were observed during the experiment, but overall, lithium precipitation is a continuous process that occurs throughout the experiment. During the experiment it was not easy to obtain a high-grade lithium salt through evaporation and separation.

During the early and middle stages of evaporation, potassium is mainly precipitated as apththalite. In the late stage it is precipitated as sylvite, accompanied by minor amounts of apththalite. In the whole evaporation process of the experiment, potash started to precipitate as soon as potassium became saturated in the brine. High-grade potash can be obtained from Zabuye Salt Lake brine through proper operation. The relation between the potassium mineral content in the solid phases and the evaporation rate is shown in Figure 5. The potassium concentration in the liquid phases went up progressively during the experiment.

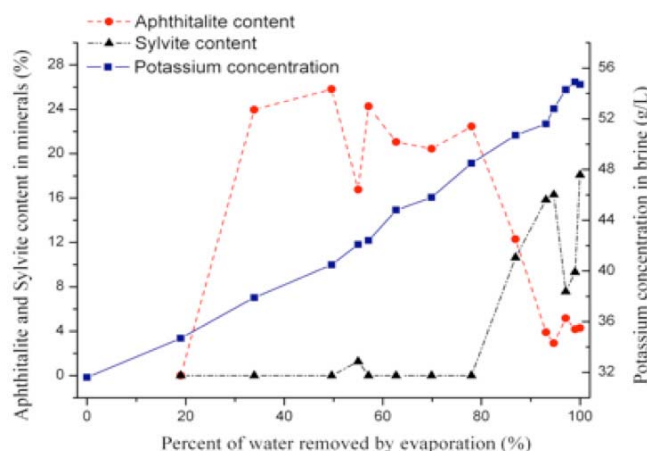


Figure 5—Content of potassium and its minerals during isothermal evaporation at 25°C.

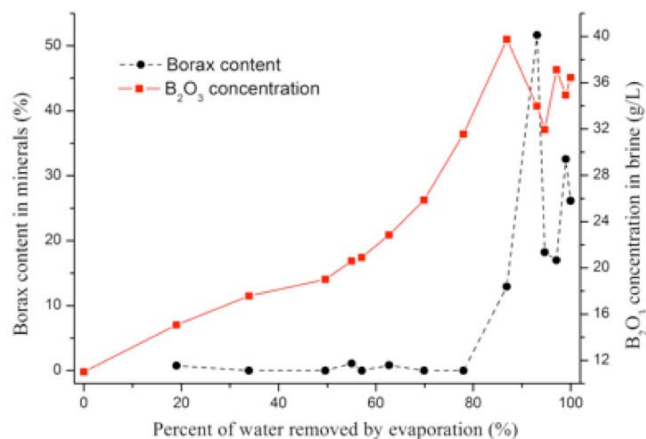


Figure 6–Content of boron and its minerals during isothermal evaporation at 25°C.

Boron becomes highly concentrated during evaporation (Figure 6). The concentration of boron in the liquid phases increased progressively with evaporation, especially in the late stage of the experiment. When the CWR (percent of water removed during evaporation) reached 85%, boron became saturated in the brine and borax began to precipitate. As a result, the boron concentration in the brine decreased. In the evaporation experiment, hardly any borax precipitated in the early stage of the experiment, and only when the CWR reached 90% was a large amount of boron precipitated as borax with a grade of almost 50%.

Therefore, the bittern remaining in the intermediate and late stages of evaporation can be used to extract borax.

After performing the isothermal evaporation experiment at 25°C with the brine of Zabuye Salt Lake, we have drawn the following conclusions.

1. The crystallization path of salts from brine in Zabuye Salt Lake is in accordance with that of the metastable phase diagram of the Na^+ , $\text{K}^+//\text{Cl}^-$, CO_3^{2-} , SO_4^{2-} - H_2O quinary system at 25°C.
2. Trona and thermonatrite precipitate earlier in the experiment compared to the metastable phase diagram.
3. Lithium precipitation is a continuous process that occurred throughout the experiment. During the experiment it was difficult to obtain high-grade lithium salt.
4. Potash is precipitated as apthitalite and sylvite. Apthitalite is precipitated first, then, sylvite and apthitalite precipitate together. High-grade potash can be obtained from Zabuye Salt Lake brine.
5. Boron is enriched in brine in the early and middle stage of the experiment and borax is precipitated in the late stage. The bittern remaining after evaporation can be used to extract borax.

Table 2–The chemical composition of the liquid phase formed by 25 °C-isothermal evaporation of autumn brines of Zabuye Salt Lake.

Sample No.	CWR %	Na^+ g/l	K^+ g/l	Li^+ g/l	Cl^- g/l	SO_4^{2-} g/l	B_2O_3 g/l	CO_3^{2-} g/l
L0	0.00	129.63	40.92	1.05	156.18	38.59	11.01	44.16
L1	19.00	136.90	45.46	1.21	153.14	48.21	15.07	52.53
L2	33.90	133.32	50.03	1.19	151.27	43.43	17.56	54.78
L3	49.60	133.85	53.46	1.50	149.95	39.86	19.01	62.04
L4	55.00	134.29	54.52	1.63	148.02	38.33	20.59	66.05
L5	57.10	136.01	56.82	1.58	146.73	36.58	20.90	71.82
L6	62.70	135.96	59.14	1.14	145.46	34.06	22.84	73.39
L7	69.90	135.34	61.37	1.29	142.98	29.75	25.86	78.66
L8	78.00	136.95	65.96	1.24	136.54	23.94	31.55	90.58
L9	86.90	148.04	70.47	1.24	121.21	23.63	39.75	118.15
L10	93.10	156.79	72.76	1.49	118.02	18.05	33.98	141.00
L11	94.70	150.66	74.98	1.24	107.64	17.47	31.95	143.70
L12	97.10	146.00	74.93	1.19	114.68	17.11	37.12	129.44
L13	99.00	136.64	74.94	1.06	117.94	16.24	34.94	115.48
L14	100.00	137.41	74.94	1.05	115.35	16.30	36.44	117.96

Table 3—Compositions of solid phases precipitated by 25 °C-isothermal evaporation of autumn brines of Zabuye Salt Lake.

Sample No.	CWR wt%	Zb wt%	Tr wt%	Th wt%	Bo wt%	Ap wt%	Sy wt%	H wt%
S1	19.00	0.54	0.00	0.17	0.74	0.00	0.00	98.55
S2	33.90	0.00	7.35	0.00	0.00	23.97	0.00	68.68
S3	49.60	0.00	10.93	0.00	0.00	25.81	0.00	63.26
S4	55.00	0.66	0.81	0.00	1.09	16.76	1.27	79.41
S5	57.10	2.17	0.00	0.00	0.00	24.26	0.00	73.57
S6	62.70	3.35	4.78	0.00	0.82	21.06	0.00	69.98
S7	69.90	0.77	18.43	0.00	0.00	20.43	0.00	60.37
S8	78.00	1.70	5.42	0.25	0.00	22.45	0.00	70.19
S9	86.90	1.17	2.84	7.62	12.92	12.29	10.64	52.52
S10	93.10	0.00	0.00	15.26	51.64	3.90	15.84	13.35
S11	94.70	3.64	0.40	17.10	18.21	2.92	16.31	41.42
S12	97.10	0.85	0.00	65.43	17.00	5.17	7.58	3.96
S13	99.00	1.04	0.00	44.48	32.55	4.17	9.33	8.43
S14	100.00	0.68	0.00	37.47	26.13	4.28	18.09	13.36

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Solubility and Supersaturation of Lithium Carbonate in Zabuye Salt Lake Brine, Tibet

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The highest abundance of lithium has been found in brine of salt lakes in China, such as Zabuye Salt Lake in Tibet and Dongtaijinaier and Xitaijinaier salt lake in Qinghai. Lithium has been produced from such brines since 1959. The alkaline Zabuye Salt Lake is hydrochemically unique, containing a comparatively complex and special mineral assemblage rich in Li, B, K, and sodium carbonate. The amount of the mineral zabuyelite (Li_2CO_3) is particularly large. The solar pond technology was developed based on the evidence that the solubility of Li_2CO_3 decreased with increasing temperature, as documented by Zheng Mian-ping between 1995 and 2002. The first industrial scale production line for Li_2CO_3 was founded in Zabuye Salt Lake in 2004. However, detailed solubility data for Li_2CO_3 in the presence of other salts in Zabuye Salt Lake brine have not been reported. Solubility data for pure Li_2CO_3 in fresh water cannot be used for the accurate prediction of zabuyelite production. Therefore, the solubility of Li_2CO_3 in Zabuye brine has to be determined. Because lithium production in solar ponds was not in chemical equilibrium because of the increasing temperature of the solar pond, the degree of supersaturation of Li_2CO_3 in the brine is very important in estimating the production rate and quality of the product. We have investigated the solubility of Li_2CO_3 and its degree of supersaturation in Zabuye brine. Solubility was studied using the isothermal method. The effect of sodium carbonate on Li_2CO_3 solubility was also studied. Based on the crystallization theory, salt supersaturation in solution depends on many factors such as the mixing intensity, cooling (or heating) rate, and the presence of solid surfaces. We studied lithium carbonate supersaturation at different heating rates because the solubility of lithium carbonate is decreased with increasing temperature. Lithium carbonate has limited solubility and its concentration change in solution is difficult to measure in supersaturation experiments. Therefore, supersaturation of lithium carbonate was expressed by a temperature difference calculated from (a) the directly measured temperature of the solution in the experiments. The lithium concentration is considered to be saturated at this temperature. (b), the temperature calculated based on the measured concentration of lithium carbonate in the solution. There is a saturation temperature which corresponds to the

measured concentration of lithium carbonate, and this temperature is used to express the degree of supersaturation of the solution. The difference between these two temperatures can then be used to express the level of solution supersaturation. The solubility of Li_2CO_3 in Zabuye brine was found to be quite different from that in fresh water, and to be strongly affected by the Na_2CO_3 concentration as shown in Figure 1. The solubility of the salt was expressed by weight percent, defined as the amount of salt in the solution divided by the total weight of the solution. At low temperature, Na_2CO_3 has a complex effect on the solubility of Li_2CO_3 . At low Na_2CO_3 concentration the solubility of Li_2CO_3 decreases with increasing Na_2CO_3 . However, when the Na_2CO_3 concentration is high, the solubility of Li_2CO_3 is increased with increasing Na_2CO_3 . At high temperature, the solubility of Li_2CO_3 decreased with increasing Na_2CO_3 concentration. With increasing heating rate, the supersaturation level was increased (Figure 1).

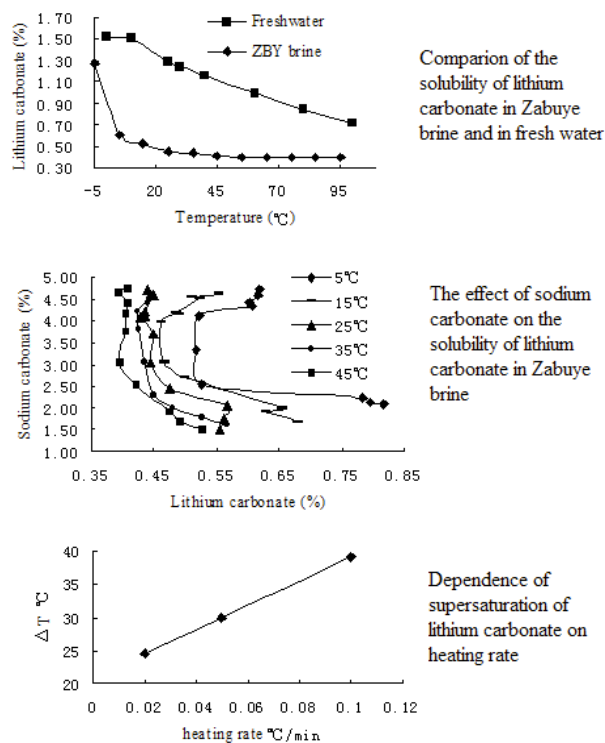


Figure 1–Solubility of Li_2CO_3 in Zabuye brine and the dependence of Li_2CO_3 supersaturation on heating rates.

Separation and Economic Recovery of Strontium from Nanyishan Oil-field Water, China

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ABSTRACT

The mass ratio of Ca to Sr is greater than 10 in Nanyishan oil-field water, which causes significant problems during the economic extraction and recovery of selected trace elements in the oil-field water. The oilfield water was isothermally evaporated and various salts such as Li, K, Mg, Ca, Na, Sr, Rb, Cs, Br, and I were obtained from the solution. The Sr content of each phase was determined by ICP-AES, the Sr distribution rule in this process was obtained, and the best separation stage for Sr was identified, to optimize the separation of Sr from Nanyishan oil-field water.

INTRODUCTION

With a content of 0.02-0.04% in the crust and 0.048% on the Earth's surface, Sr is the least abundant of all alkaline earth metal elements. It rarely appears as a high-grade ore body because of its geochemical nature. The most commonly occurring Sr minerals are celestite (SrSO₄) and strontianite (SrCO₃). Presently the Sr-producing industry mainly processes solid Sr ore. Presently most of the world's Sr is produced from mining and processing Sr ore; however, oil-field brines and other naturally occurring aqueous solutions may present a more economical Sr source. Many reports exist on Sr in aqueous solution. We present the first documentation of Sr production from a brine system rich in Ca.

Sr, Ca, Mg and Ba are all alkaline earth metal elements. Because of their high chemical activity they can only exist in the form of compounds in nature and not in their elemental state. This high chemical activity makes it very difficult to separate these alkaline earth elements, particularly the separation of Sr from Ca. Separation methods currently in use include extraction, ion exchange chromatography, and liquid membrane separation.

Among the most effective and widely used extraction agents are crown ethers such as dicyclohexyl-18-crown-6 (DCH18C6)-octanol (He et al. 1995; Ju 2002), 15-crown-5

(B15C5) (Vanura & Makrlik 2002; Vanura et al. 2002) and others. Although crown ethers are superior extractants for Cs(I), they are too expensive and need to be diluted by halogenated hydrocarbons, which upon irradiation produce hydrochloric acid, which can corrode equipment. This restricts their application in industrial operations.

The ion exchange technique focuses on a separation system for the decontamination of radioactive waste containing low Sr content (Cheadfield 1998). According to the separation mechanism, liquid chromatography can be divided into liquid-liquid distribution chromatography, liquid-solid adsorption chromatography, and others. Some widely used liquid stationary phases are 1,2-bis-(2-cyanoethoxy)propane (ODPN), polyethylene glycol (PEG) (Herbst & Law 2002a, 2002b), octadecylsilyl (ODS), squalane, and bis-2-ethylhexylphosphinic acid. Wang & Nagaosa (2003) separated Ca, Sr, Mg and Ba from aqueous solutions within the pH region of 5.3-6.5 units by using bis-2-ethylhexylphosphinic acid as the stationary liquid, 0.1 mol l⁻¹ chloroacetic acid containing 5% ethyl alcohol as the mobile phase, and this enabled effective separation of these four alkaline earth metal elements.

The liquid membrane technique is a separation operation with a membrane as the separation medium and the concentration gradient as the driving force. A liquid membrane is a very thin layer of emulsion particles suspended in liquid, which can separate two mutually soluble solutions due to the infiltration phenomenon. Based on their structure, such membranes can be divided into many types, of which three have found practical application: the emulsion membrane, the supported liquid membrane (Lee & Hong 2000) and the fluid membrane (Shamsipur & Raoufi 2002). Wang & Li (1997) studied a liquid membrane for the separation and enrichment of Sr. The system included a coordination flowing carrier (PMBP TBP), a surfactant (SPAN 80), an intensifier (glycerol), a solvent (hexylene) and an internal phase (1.2 mol l⁻¹ hydrochloric acid). The efficiency of Sr enrichment proved to be above 99.5% under external phase (pH = 7-8).

Table 1—Composition (in weight percent) of the brine raw material.

Ion	Li ⁺	Na ⁺	K ⁺	NH ₄ ⁺	Ca ²⁺	Mg ²⁺	Sr ²⁺	B ₂ O ₃	Cl ⁻	SO ₄ ²⁻	HCO ₃ ⁻	I ⁻
%	0.021	7.83	0.78	0.092	1.43	0.082	0.13	0.27	15.73	0.022	0.011	0.0031

Table 2—Test results of the evaporation concentration process of oil-field brine.

Sequence number	Density (g/ml)	Initial material mass (kg)	End material mass (kg)	Solid mass (kg)	Liquid mass (kg)	Rate of salt crystallization (%)	Comments
0	1.2019	56.50	56.50	0.00	56.50	0.00	Initial material
1	1.2560	56.50	27.50	9.90	17.60	17.52	NaCl
2	1.2890	17.60	13.64	1.38	12.26	2.44	NaCl
3	1.3375	12.26	9.54	1.12	8.42	1.98	Sylvite
4	1.3856	8.42	7.04	0.64	6.40	1.13	Carnallite complex salt
5	1.4239	6.40	5.29	0.79	4.48	1.40	Precipitated Antarcticacite
6	—	4.48	4.48	0.22	4.26	0.39	Antarcticacite
7	1.4431	4.26	4.26	0.86	3.40	1.52	Antarcticacite

Rich in Sr resources, China has the largest Sr reserves and is the biggest Sr supplier of the world, supplying two thirds of the global market. Due to the many years of exploitation of solid Sr mines (chiefly in the form of celestite), domestic exploitation of mines has now reached the final stage. Fortunately, there are abundant underground brine resources in the Qaidam basin. Their contents of K, Ca, Li, B, Br, I, Sr, Rb and Cs are large, with especially large concentrations in the oil-field water (Lu 1978; Li et al. 2001). Thus, these brines have great potential for large-scale industrial exploitation. Brine utilization can relieve the condition of insufficiency of the relevant resources. The exploitation of oil-field water resources can also actively impact the national economy, as well as aid the development of the local economy of Qinghai. Sr concentration is high in the oil-field water. For example, in the Nanyishan oil-field brine of Qaidam basin the average Sr concentration reaches 5364 mg l⁻¹, which generally exceeds the minimum concentration for industrial exploitation (Qinghai Geological Survey Institute 2003). The Nanyishan oil-field brine can be classified as a calcium chloride type brine. To rationally utilize these local resources, their existence and transformation characteristics need to be studied.

MATERIALS AND METHODS

The raw material for the experiment is the highly saline brine present in the third stratum of the west Qaidam basin. The brine was roughly divided into the Nanyishan K, B, Li, I bonanza area and the prospective enrichment area of Xiaoliangshan, Youquanzi, Kaitemilike, and Youdunzi. The Nanyishan tectofacies is located in a grade-3 tectonic belt in the west Qaidam basin discovered during a geological survey in 1955. The Nanyishan oil-water lake lies in the center of the tectofacies in a low-lying alluvial landscape. The latitude of the lake is 37°50'-38°35' north, and the longitude is 91°07'-92°10' east. It is a surface water field that spreads NW-SE, from the Sheng 202 well in the east to the Qian 8-1 well in the west. The oil-field water was released to the surface by an out of control oil-gas well. The area of the lake is 0.5 km² with a maximum depth of 1.75 m and an average depth of 0.97 m. The lake water is greenish blue, smells of oil and is greasy to the touch. Chemical analysis of water samples indicates that the concentration of Cl⁻ is much higher than that of Na⁺. The oil-field water should therefore be classified as a CaCl₂ type in Sulin's classification (Zhang 1979), characterized by high Ca²⁺, high Cl⁻ and low SO₄²⁻ and Mg²⁺ concentrations.

There is little vertical variation in ionic concentrations, and salinity variations from the edge to the center are slight. The shallower the lake water, the higher the salt concentration. The main elements of economic value are K, B, Li, I, Rb, Cs, and Sr, and they are homogeneously distributed (Duan & Yuan 1988; Fu et al. 2005; Xiong et al. 2005).

Using Nanyishan oil-field brine as raw material (composition listed in Table 1), an indoor evaporation experiment was performed in plastic evaporation containers using an IR lamp and an electric fan. The amount of water evaporated was calculated using a phase diagram of NaCl-KCl-MgCl₂ system. The distribution rules of each mineral and the extent of Sr enrichment were determined by analysis of the brine and the deposited solid minerals. In the process of the evaporation experiment, a densimeter was used to measure the solution density.

RESULTS AND DISCUSSION

The crystallization of salts during the evaporation concentration process of oil-field brine, the process data, and the change in concentration of Ca and Sr during the different stages of the process are shown in Figure 1, Table 2, and Table 3, respectively. The oil-field brine evaporated at room temperature until NaCl precipitated first. After separation of the solid from the liquid, the liquid evaporated until KCl saturated, when NaCl and KCl precipitated. After separation, evaporation was continued with subsequent precipitation of B, NaCl and KCl. By controlling the evaporites, three minerals containing B, K, Na and Mg were separated from the liquid. The remaining liquid was enriched in the rare dispersive elements. Evaporation rates of the remaining liquid were reduced when compared with earlier stage evaporation rates.

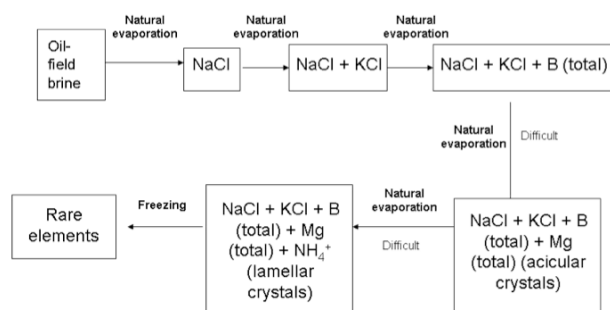


Figure 1—Salt crystallization during the evaporation condensation process of oil-field brine.

The oil-field brine contains different useful components. For the development of a comprehensive production process, the elucidation of the distribution laws of these compounds is essential. The evaporation experiment at room temperature provided useful information on the distribution of Sr in the oil-field brine. The precipitation process of the elements in oil-field brine follows the phase diagram law of the NaCl-KCl-MgCl₂-H₂O system in the initial phase of the evaporation condensation process. NaCl precipitated first, followed by precipitation of carnallite from the saturated brine. During further condensation of the liquid, the brine reached the co-saturation point of K-Mg, and a mass of acicular crystals precipitated from the solution (Cui et al. 2008).

Table 3—The concentration variation of Ca and Sr during the evaporation condensation process.

Density (g/ml)	Liquid Ca (%)	Liquid Sr (%)	Solid Ca (%)	Solid Sr (%)
1.2019	1.43	0.129	—	—
1.2560	4.46	0.400	0.32	0.034
1.2890	6.16	0.577	0.56	0.024
1.3375	8.88	0.827	1.04	0.099
1.3856	11.30	1.073	2.76	0.280
1.4239	12.93	1.256	6.31	4.070
—	—	—	14.69	4.360
1.4431	14.02	0.990	15.93	1.340

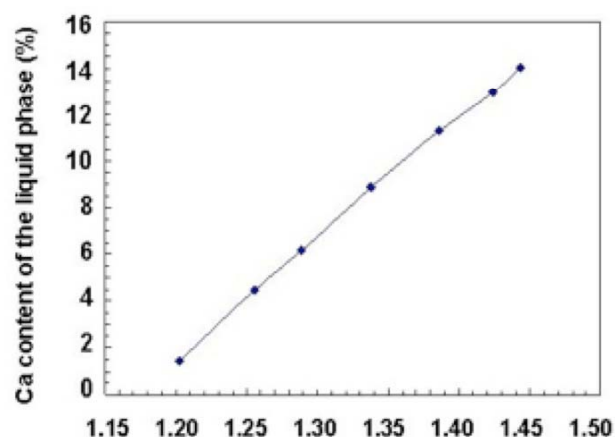


Figure 2—The enrichment trend of Ca in the liquid-phase during the room temperature evaporation condensation process.

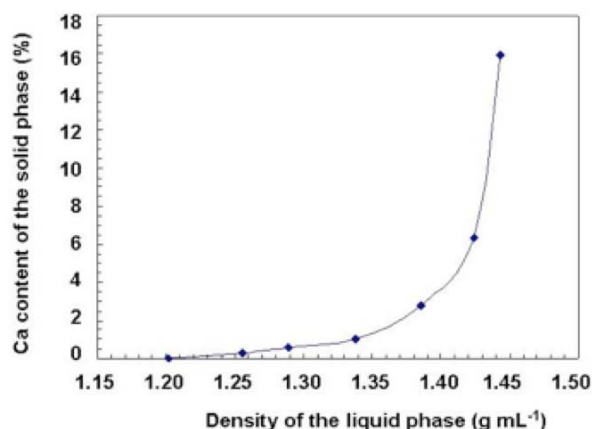


Figure 3—The enrichment trend of Ca in the solid-phase during the room temperature evaporation condensation process.

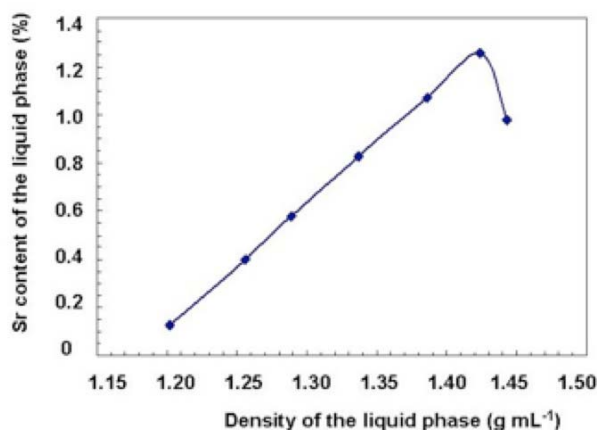


Figure 4—The enrichment trend of Sr in the liquid-phase during the room temperature evaporation condensation process.

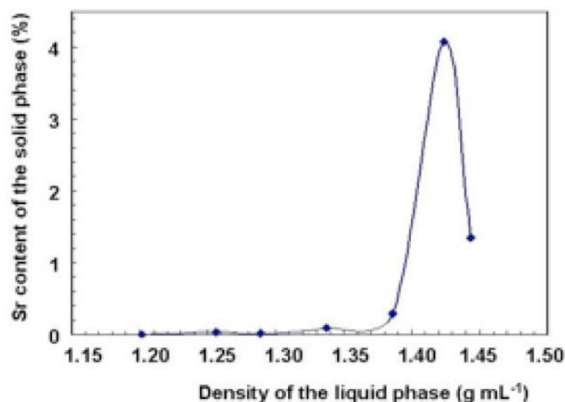


Figure 5—The enrichment trend of Sr in the solid-phase during the room temperature evaporation condensation process.

The density of the brine increased during the condensation process, the rate of change in density decreasing during mineral precipitation. After separation of the solids from the liquid, we studied the enrichment of Sr and of Ca in the solid and the liquid phase by separately analyzing the concentrations of these elements in the different phases (Figure 2–5).

The Sr content of each phase was determined by ICP-AES. The Ca content was analyzed by mass titration using EDTA standard solution as titrant. Analysis of the solids showed that Ca and Sr remained in the liquid phase, and that little was found in the solid-phase at the stage of NaCl and carnallite precipitation. During carnallite precipitation, Ca and Sr were further enriched in the liquid phase, and the concentration of these elements in the solid phase increased, mainly due to entrapment of mother solution. With the continuation of evaporation and condensation, the Ca and Sr contents in the solid-phase further increased while they approached saturation in the liquid. Compounds of Ca and Sr could then be obtained from the solid phase following further evaporation. The Ca content in the solid-phase increased in a linear fashion (Figure 2 and 3). When the density of the solution reached 1.4239 g mL^{-1} , the Ca content of the solid phase increased dramatically to a maximum of 15.93%. This shows that Ca precipitated instantly from the liquid phase, and that at the same time its concentration in the liquid phase kept increasing. The changes in Sr concentration in the liquid and the solid phase are shown in Figure 4 and 5, respectively. The Sr content of the liquid-phase increased with the density of the solution, and reached its maximum when the density reached 1.4239 g mL^{-1} . During further evaporation, the Sr content in the liquid phase began to decrease. Initially the Sr content in the solid phase increased at a low rate. When the brine density reached 1.4239 g mL^{-1} , Sr largely precipitated from the liquid phase, and its content in the solid phase reached more than 4%. This concentration is appropriate for Sr extraction. At the same time, massive amounts of Ca salts precipitated, and Ca and Sr were found in the solid phase at a ratio of 1.5:1, a value that increased further with the continuation of the process. To monitor the precipitation process in detail, we refined the brine evaporation experiment by adding Sr compounds and by increasing sampling times. When this stage was well controlled, we could obtain about 20% Sr compounds from oil brine that initially contained only about 0.037% Sr. Extraction of Sr did not affect recovery of potassium. A study of the relationship between the separation of Sr, Rb and Cs is

underway. Figure 6 shows the principal process that is to be adopted, based on our experimental results (Wu 2007).

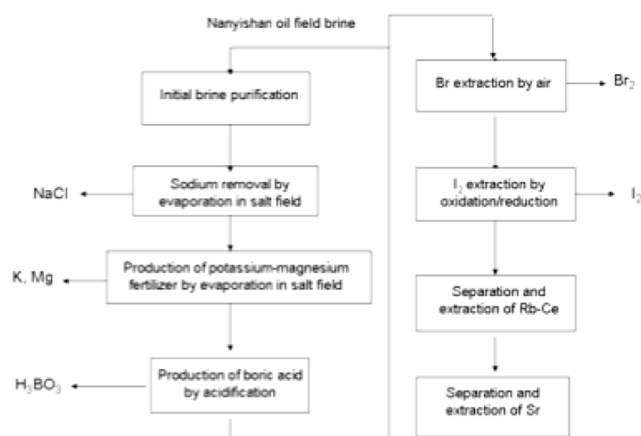


Figure 6—Schematic diagram showing the process of separation and extraction of strontium and other chemical elements from oil-field brine in Nanyishan.

CONCLUSIONS

Four conclusions can be drawn from the results of the Nanyishan oil-field brine evaporation experiment at room temperature:

1. Separation and extraction of valuable components from oil-field water is a complex process. Different interactions exist between the different components.
2. As oil-field brine mainly consists of Ca chloride, Ca must first be separated from Sr to enable the effective recovery of Sr.
3. Ca and Sr are concentrated in the liquid phase at the stage of potash precipitation due to evaporation. After many types of potassium salts have precipitated, Ca and Sr become saturated in the liquid phase. When evaporation continues and the liquid phase becomes further concentrated, Ca compounds co-precipitate with Sr salts.
4. Using different technological processes, Sr can be extracted either from the liquid or from the solid phase.

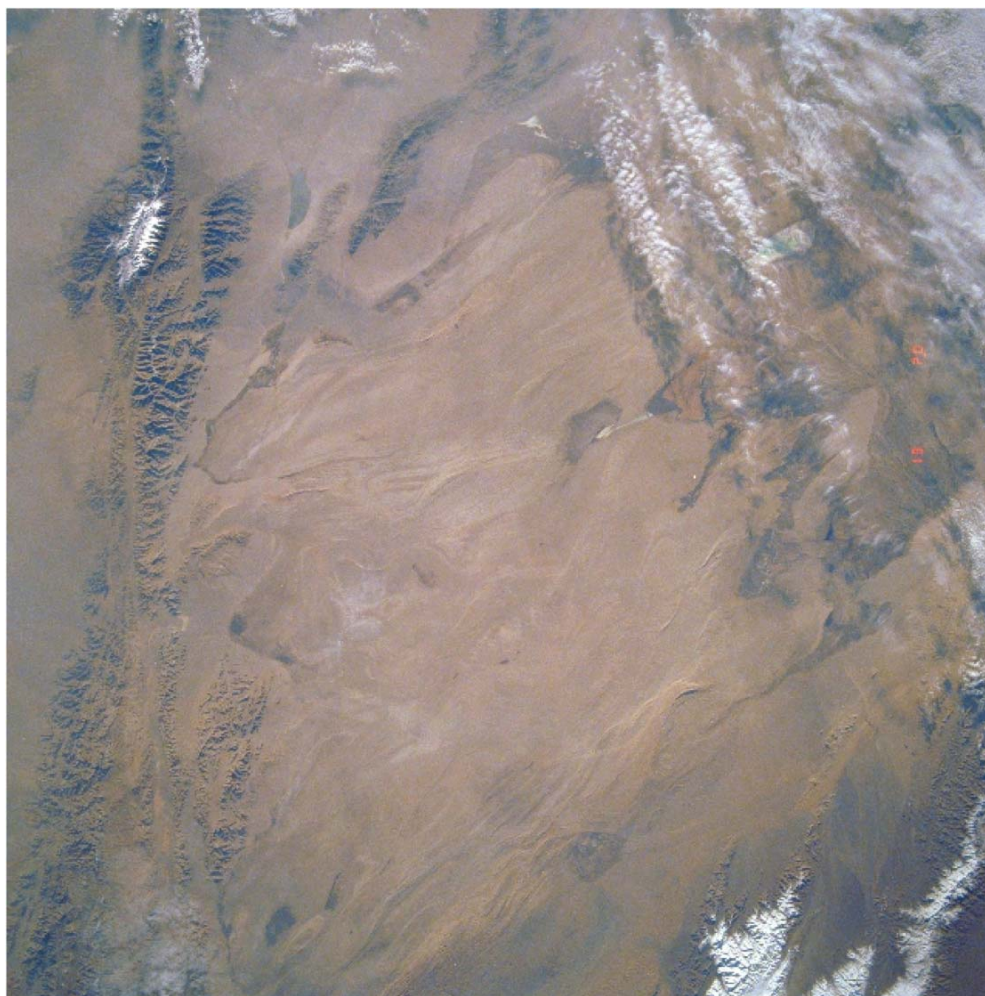
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Solubility of MgO in Chloride Melts Containing NdCl₃ or LaCl₃

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ABSTRACT

Due to extremely low solubility in conventional magnesium electrolyte, MgO tends to attach to cathode and causes cathodic passivation, which will dramatically lower current efficiency of magnesium electrolysis. In this paper, NdCl₃ or LaCl₃ was added to conventional magnesium electrolyte to increase MgO solubility. We investigated the effects of NdCl₃ or LaCl₃ content, the form of MgO, and temperature on the dissolution rate of MgO and on MgO solubility. MgO solubility increased with increasing MgCl₂ content or CaF₂ content in MgCl₂-KCl-NaCl-CaCl₂-CaF₂ melt, the maximum being only 0.145% by weight in the above melt with 30% MgCl₂ by weight. Both MgO solubility and dissolution rate increased with increasing NdCl₃ concentration in MgCl₂-NaCl-KCl-NdCl₃ melts. The MgO solubility in MgCl₂-NaCl-KCl-NdCl₃ (15% by weight) melts can reach about 3.0 wt % at 780°C. Raising temperature from 700 to 800°C can slightly increase the MgO solubility and dissolution rate. Addition of LaCl₃ to the MgCl₂-NaCl-KCl-CaCl₂-LaCl₃ melt also improved MgO solubility, but it took more time (about 60 minutes) to achieve equilibrium between MgO and LaCl₃ in the melt. Formation of the stable NdOCl compound examined by XRD analysis indicated that MgO can react with NdCl₃ in chloride melts.

INTRODUCTION

The electrolytic process will most likely be the method for future large scale magnesium production projects. The electrolyte system for magnesium production is usually composed of multiple metal chlorides including anhydrous MgCl₂. The salt lakes in western China contain large amounts of bischofite (MgCl₂·6H₂O), which is a suitable raw material for electrolysis of magnesium following complete dehydration. Anhydrous MgCl₂ readily adsorbs water, both in air and as a component of a molten electrolyte in an electrolytic cell, and reacts with water to form oxygen-bearing compounds such as MgO and MgOHCl. The presence of oxygen-bearing compounds in the electrolyte is deleterious to the operation of the

magnesium electrolysis cell (Strelets 1977; Kipouros & Sadoway 1987; Mediaas et al. 1996). MgO may sink to the bottom of the electrolysis cell and deposit as a sludge, or dissolve in the electrolyte and be involved in an anodic electrochemical reaction to form carbon oxide or carbon monoxide. MgOH⁺ and Mg₂(OH)₂²⁺ ions existing in MgOHCl, as suggested in the literature (Boghossian et al. 1991), will migrate towards the cathode and can be reduced to H₂(g) and MgO(s), and due to extremely low solubility, the MgO(s) will cover the cathodic surface to cause cathodic passivation and disperse the magnesium droplets produced at cathode which dramatically reduces current efficiency. Magnesium oxide also may increase the electrical resistance of the electrolyte which increases the power requirements for electrolytic separation of magnesium and chlorine. Therefore, MgO is regarded as one of the most harmful impurities in magnesium electrolysis.

How to solve the adverse effects of MgO on magnesium electrolysis has been a problem of the utmost concern for a long time. The ideal way is to transform the MgO into a soluble material, among which the oxygen ions or complex oxygen will not be involved in the electrochemical reaction and therefore the anode will not be consumed. However, thus far it is difficult to meet both the above requirements. In fact, MgO has limited solubility both in chloride melts such as the conventional magnesium electrolyte MgCl₂, NaCl, KCl and CaCl₂, and in fluoride melts (Bauxitbanya 1949). A mechanism for the dissolution of MgO in pure MgCl₂ was suggested according equation (1) (Combes et al. 1980; Boghossian et al. 1991). The solubility of MgO reaches up to 0.15% by weight (0.36 mol %) at 730°C.



Sharma (1994) found that a rare-earth chloride of NdCl₃ can greatly increase MgO solubility in NdCl₃-MgCl₂ melts by the suggested reaction:



During the electrolysis from MgO-NdCl₃-MgCl₂ melts, it was observed that only chlorine with no CO or CO₂ was evolved at the anode at a higher current density at 700°C (Cathro et al. 1997), indicating that oxychloride species were not involved in the anodic electrochemical reaction at a temperature of 700°C. Therefore it was assumed that a small amount of NdCl₃ or LaCl₃ can be used in the MgCl₂-NaCl-KCl-CaCl₂ melt to dissolve impurities of MgO, and oxychloride species will not be oxidized at the anode at a higher current density at about 700°C. The purpose for the present work is to examine the solubility of MgO in a MgCl₂-NaCl-KCl-CaCl₂ melt with NdCl₃ or LaCl₃.

EXPERIMENTAL METHODS

Anhydrous magnesium chloride (0.0098 wt % MgO impurity) was used as the active component of magnesium electrolysis. Sodium chloride (> 99.5 wt %), potassium chloride (> 99.5 wt %), and anhydrous calcium chloride (> 96.0 wt %) served as supporting electrolyte for magnesium electrolysis, and MgO (> 98 wt %) was added to the above melt as an impurity during the experiments. Lanthanum chloride was prepared from LaCl₃·7H₂O as follows: (1) an aqueous solution was prepared by mixing NH₄Cl and LaCl₃·7H₂O (8:1 molar ratio); (2) the solution was heated to 120°C and maintained for 3 hours to remove water; (3) after water removal the solution was slowly heated to 200°C; and (4) the solution was held for 3 hours in an electric furnace heated to 340°C, and kept for 3 hours under vacuum. Anhydrous NdCl₃ was prepared similar to the above process for anhydrous LaCl₃. All chemicals were dried under vacuum for 4-5 hours at 200°C before use.

For the purposes of the experiment, an excess of MgO flake that had been compacted under 10⁴ MPa pressure was placed at the bottom of an alumina crucible covered with chloride melts. Unless otherwise stated, MgO flake was used in the experiment. After melting and mixing uniformly at high temperature, about 2 g of molten salt was taken out from the crucible and dissolved in 10 ml hydrochloric acid solution (0.05 N) to measure MgO solubility in the melt. An acid consumption method was used to measure the MgO concentration in the melt. The dissolved MgO will react with part of the acid, and the remaining acid was then titrated with sodium hydroxide. The solubility of MgO can be calculated according to the amount of sodium hydroxide consumed. The experiment was carried out in an atmosphere of argon gas to avoid absorption of water from the air by the chloride melt.

All experiments were performed in an alumina crucible inside an electric furnace under an argon gas atmosphere. For each experiment, between 100 and 120 g of electrolyte was added to the crucible. Samples were quickly withdrawn from the melt using an iron rod to avoid water absorption from the air. The samples were immediately quenched and then placed into a hydrochloric acid solution (0.05 N) to determine MgO solubility.

EDTA volumetric analysis method was used to analyze the NdCl₃ and LaCl₃ concentration in the melt, among which xylenol orange was used as an indicator in order to avoid Mg²⁺ interference (China technical supervision bureau, GB/T 14635.2—1993).

Table 1—The dependence of amounts of dissolved MgO (g/100 g melt) with time.

Time (min)	0	0.5	1	1.5	2	3	5	10	15	30
Dissolved MgO (%)	0.0518	0.57	1.03	1.41	1.6	1.702	1.72	1.75	1.746	1.77
NdCl ₃ concentration in the melt	10%	8%	6.15%	4.40%	3.60%	3.25%	3.10%	2.65%	2.49%	2.50%

Table 2—Amounts of dissolved MgO (g/100 g melt) change over time at different temperatures (initial NdCl₃ concentration 10 wt %).

Time (min)	0	0.5	1	1.5	2	3	5	10	15
700 °C	0.0518	0.57	1.03	1.41	1.60	1.702	1.720	1.75	1.746
780 °C	0.0518	0.67	1.40	1.74	1.76	1.770	1.765	1.78	1.780
850 °C	0.0518	0.75	1.56	1.75	1.77	1.770	1.790	1.78	1.790

RESULTS AND DISCUSSION

MgO Solubility in $\text{MgCl}_2 - \text{NaCl} - \text{KCl} - \text{CaCl}_2 - \text{CaF}_2/\text{NaF/MgF}_2$ Melts

Figure 1 shows the effects of MgCl_2 on MgO solubility in $\text{NaCl-KCl-CaCl}_2\text{-CaF}_2$ melts (60:20:15:5% by weight) (total 100 g) at 810°C under an argon atmosphere. This treatment gave a rapid build up for MgO solubility in the melt with increasing MgCl_2 content. The dissolution mechanism for MgO in the above melt can be described by equations (3-5) (Mediaas 2000), indicating that an increasing MgCl_2 content favored the reaction in the right hand direction, thus increasing MgO solubility. Addition of fluorides such as NaF , MgF_2 , or CaF_2 to the $\text{MgCl}_2\text{-NaCl-KCl-CaCl}_2$ melts increased MgO solubility as well, and addition of CaF_2 led to a higher MgO solubility than addition of NaF or MgF_2 , as shown in Figure 2.

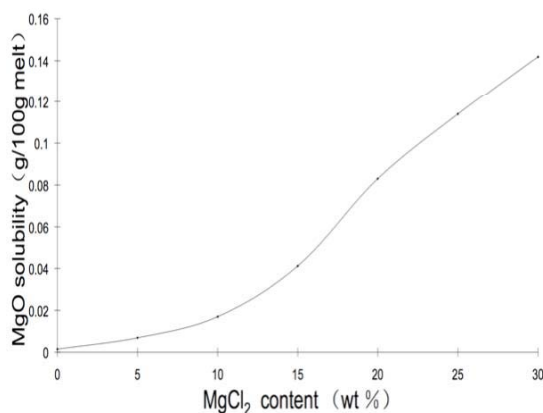
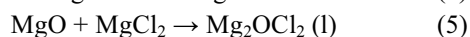
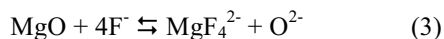


Figure 1—The effect of MgCl_2 content on MgO solubility.

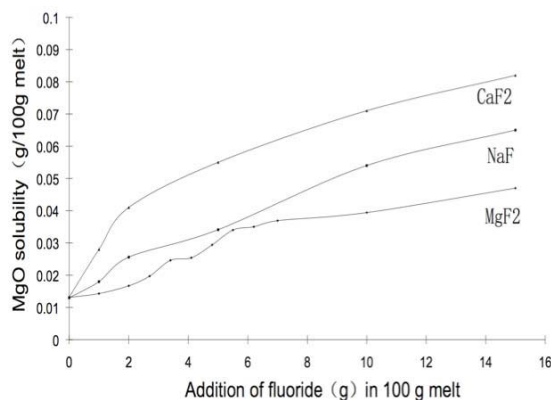


Figure 2—The effect of fluoride on MgO solubility.

MgO solubility in $\text{MgCl}_2 - \text{NaCl-KCl} - \text{NdCl}_3$ melts

However, the MgO solubility in the $\text{KCl-CaCl}_2\text{-CaF}_2\text{-NaCl-MgCl}_2$ melt is still quite low, the maximum value reaching only 0.145 g per 100 g (0.145 wt %) in the above melt with 30 wt % MgCl_2 . Furthermore, during the routine magnesium electrolysis process, the melt usually contains 10-20% by weight of MgCl_2 with up to 2% by weight of CaF_2 , which leads to a very low solubility of MgO (usually < 0.1 wt %).

Figure 3 shows the dissolved amount of MgO after different times in a $\text{NaCl-KCl-CaCl}_2\text{-MgCl}_2$ (15 wt %) melt containing between 5 and 15 weight percent of NdCl_3 at 780°C . The dissolved amount of MgO reached its maximum after approximately 5 minutes for all three concentrations of NdCl_3 . MgO solubility increased for all three concentrations of NdCl_3 with increasing NdCl_3 content in the melt. After equilibrium with MgO in 30 minutes, 10 wt % NdCl_3 was not completely consumed, and 2.5 wt % NdCl_3 was still left in the melt. Probably this was NdCl_3 equilibrium concentration in the above melt due to the formation of a complicated complex with other chlorides.

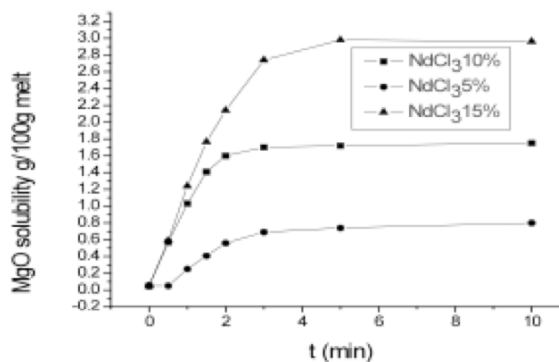


Figure 3—The relationship between the amount of dissolved MgO and time.

The dissolution rate of MgO also was dependent on the NdCl_3 concentration in the melt, as shown in Figure 4. The MgO dissolution rate gradually increased with increasing NdCl_3 concentration in the melt, and reached the maximum value at the initial stage with initial addition of 15 wt % NdCl_3 to the melt, but reduced the minimum value at the final stage with initial addition of 5 wt % NdCl_3 to the melt. A possible interpretation of this observation is that NdCl_3 first formed a kind of complicated complex with other chlorides, more NdCl_3 speeded up the reaction, and then the remaining NdCl_3 reacted with magnesium oxide. Powdered

MgO initially dissolved more rapidly than flakes because of the relatively larger surface area, but the initial form of MgO had no effect on the final MgO solubility.

The effects of temperature on MgO dissolution rate and MgO solubility in NaCl-KCl-CaCl₂-MgCl₂ (20 wt %) melt with 10 wt % NdCl₃ was investigated (Table 2). The MgO solubility (g/100 g melt) slightly increased with temperature in the range from 700-850°C in NaCl-KCl-CaCl₂-MgCl₂ (20 wt %) melt with 10 wt % NdCl₃, and the MgO dissolution rate also increased with increasing temperature.

The X-ray diffraction patterns for the melt before and after MgO dissolution (Figure 5, 6) showed that NdOCl was formed after dissolution of MgO in the melt containing NdCl₃, indicating that MgO reacted with NdCl₃ to form NdOCl according to equation (2) as previously documented by Sharma (1994). This reaction occurs spontaneously according to negative free energy ΔG_o (-20.2 kJ/mol at 700°C).

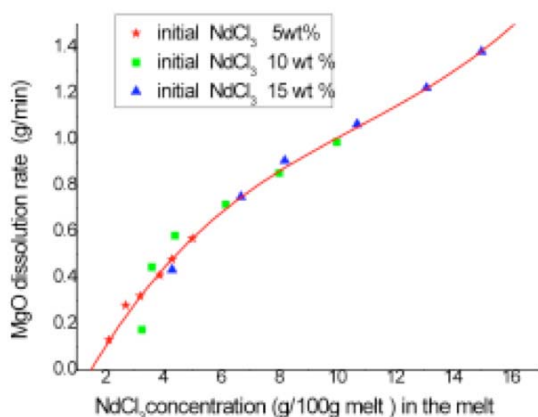


Figure 4–The relationship between the dissolution rate of MgO and NdCl₃ concentration.

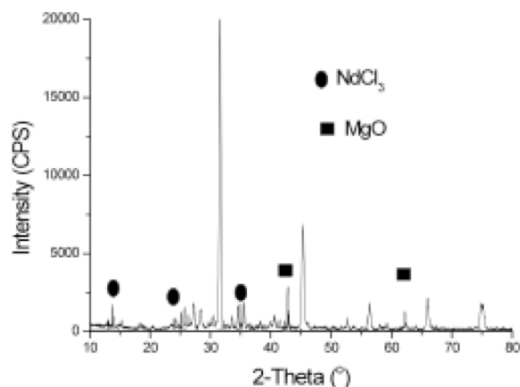


Figure 5–XRD patterns for a NaCl-KCl-MgCl₂-NdCl₃ (5:2:2:1 weight ratio) melt with MgO melts before dissolution.

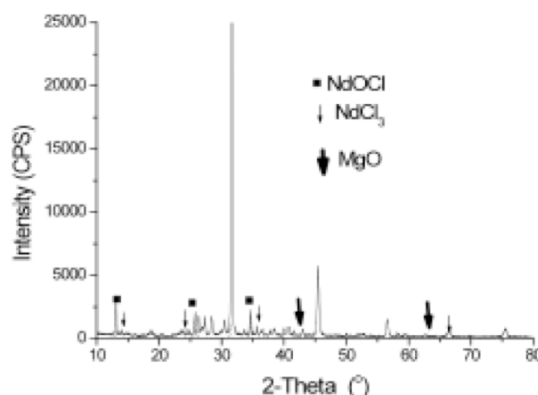


Figure 6–XRD patterns for NaCl:KCl:MgCl₂:NdCl₃ (5:2:2:1 weight ratio) melts after dissolution of MgO.

The MgO dissolution process is thought to proceed in two steps. In the first step, crystalline MgO is eroded by the melt ion and MgO solute is formed (the MgO dissolution and activation process). In the second step MgO solute reacts with NdCl₃ to form NdOCl and MgCl₂.

MgO Solubility in NaCl-KCl - LaCl₃ Melt

LaCl₃ has the similar properties as NdCl₃, so it was assumed that LaCl₃ would react with MgO according to the following equation:



In this experiment, one hundred grams of KCl-NaCl (1:1 weight ratio) was melted in a corundum crucible and maintained at 720°C for 1 h. Then LaCl₃ was added to the melt and stirred, and the La³⁺ concentration was monitored until the melt was uniform. A MgO flake pressurized at 10⁴ MPa was added to the melt, and the La³⁺ concentration was analyzed again when the melt was homogenous. MgO consumption can be estimated by the La³⁺ concentration difference, and the dependence of MgO consumption on time was shown in Figure 7. MgO consumption increased with time during the first 30 minutes and reached a stable value at 40 minutes. A time of 60 minutes was chosen for reaction equilibrium. Then MgO solubility in a KCl:NaCl (1:1 weight ratio, 100 g) melt with various LaCl₃ content was investigated at 720, 750, and 800°C. The La³⁺ concentration in the melt was analyzed at 60 minutes after LaCl₃ had been added to the melt.

Figure 8 shows that the MgO solubility has a linear relationship with LaCl₃ content in the melt, and increases with temperature from 720 to 800°C, as represented by the following equations:

$$Y_{\text{MgO}} = 7.82W_{\text{LaCl}_3} - 0.0428 \quad 720^\circ\text{C} \quad (7)$$

$$Y_{\text{MgO}} = 9.04W_{\text{LaCl}_3} - 0.0573 \quad 750^\circ\text{C} \quad (8)$$

$$Y_{\text{MgO}} = 10.59W_{\text{LaCl}_3} - 0.0448 \quad 800^\circ\text{C} \quad (9)$$

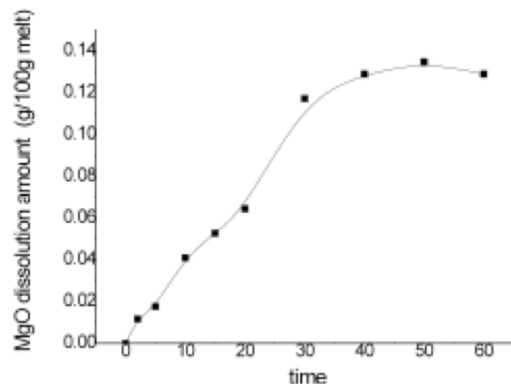


Figure 7—The dependence of the amount of dissolved MgO with time.

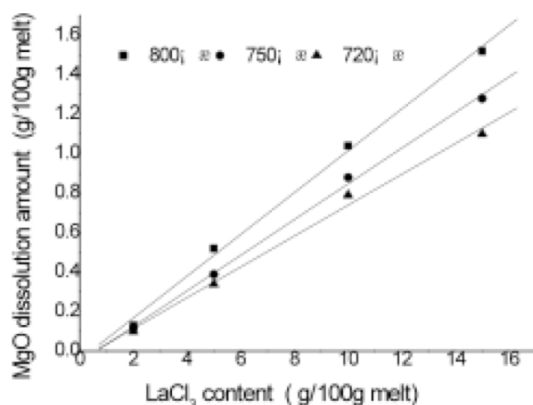


Figure 8—The effect of LaCl₃ content on the solubility of MgO (g/100 g melt).

CONCLUSIONS

The solubility of MgO increased with increasing MgCl₂ content in KCl-NaCl-CaCl₂-CaF₂, but it is still quite low, the maximum value reached being only 0.145 wt % in the above melt with 30 wt % MgCl₂. Addition of CaF₂ led to a higher MgO solubility than addition of NaF or MgF₂ in a MgCl₂-KCl-NaCl-CaCl₂ melt.

MgO solubility is clearly dependent on the NdCl₃ content in the melt. Both MgO solubility and dissolution rate increased with increasing NdCl₃ concentration in MgCl₂-NaCl-KCl-NdCl₃ melts. Raising the temperature from 700 to 850°C can slightly increase MgO solubility in MgCl₂-NaCl-KCl-NdCl₃ melts. The MgO solubility in MgCl₂-NaCl-KCl-NdCl₃ (15 wt %) melts can reach about 3.0 wt %

at 780°C. XRD pattern analysis clearly indicated that NdCl₃ reacted with MgO to form NdOCl in a melt containing NdCl₃.

Addition of LaCl₃ to a NaCl-KCl melt can also improve MgO solubility, but dissolution of MgO took more time (about 60 minutes) to reach equilibrium with a melt containing LaCl₃. The solubility of MgO increased linearly with increasing melt temperatures between 720 and 800°C.

ACKNOWLEDGEMENTS

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A Study of the Saline Lakes of the Esperance Hinterland, Western Australia, with Special Reference to the Roles of Acidity and Episodicity

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ABSTRACT

Most saline lakes are alkaline, but acid groundwaters in some southern areas in Western Australia cause some to have pHs as low as 3. Their fauna is severely restricted to an endemic brine shrimp (*Parartemia* sp.), a copepod *Calamoecia trilobata*, and two species of ostracods, including *Australocypris bennetti*. Nearby alkaline salt lakes show an attenuating fauna with increasing salinity with dominance by various crustaceans particularly *Parartemia* spp., various ostracods, copepods, *Daphnia* (*Daphniopsis*) *truncata*, *Haloniscus searlei* and snails including *Coxiella glauerti*, as is typical in salinas in southern Australia. When both types of lakes fill with episodic rain, their salinity is vastly reduced and pH approaches neutrality. Such lakes are colonized by insects and by large branchiopods. Many of the latter are new to science and occur only in these brief hyposaline stages. Such a unique assemblage is in danger of extinction due to hypersaline mining waste waters being dumped in saline lakes and to secondary salinization.

INTRODUCTION

The essential characteristics of Australia's numerous saline lakes are now relatively well-known (e.g. Williams 1984, 1998), including those in southwestern Australia (Pinder et al. 2002, 2004, 2005). This has been achieved by many broad scale studies (e.g. Geddes et al. 1981 on southwestern Australia) and by some more detailed studies of salt lake districts (e.g. Halse 1981 on the Marchagee lakes in Western Australia (WA)). While these studies have concentrated on the predictably seasonal lakes in southern Australia, some information is also available on typically episodic lakes of the inland (e.g. Timms et al. 2006 on the Carey system in the northern goldfields of WA). However there are few data showing the effect of unusual episodic events in the south, or on those saline lakes subject to rising acid groundwater due to anthropogenic influences.

There are thousands of small saline lakes inland of the town of Esperance in south-western Australia. Knowledge on them is restricted to one lake included in the Geddes et al. (1981) study, plus three in the Brock & Shiel (1983) study and three in the Pinder et al. (2004) study. These reveal typical alkaline lakes, seasonally filled and of low biotic diversity with dominance by crustaceans, including

Parartemia spp., and ostracods. However, within the Esperance hinterland there are numerous natural acid salinas (T. Massenbauer, pers. comm.) and long-term rainfall records reveal there are episodic summer periods of filling in addition to fillings during the normal winter-spring rainfall (A. Longbottom, pers. comm). In view of salinization in wheatbelt lakes which often involve acidification of saline lakes (Halse et al. 2003) and the unpredictable nature of episodic fills (Timms 2005), this study aims to elucidate the characteristics of naturally acid saline lakes and the effect of an episodic summer fill. This was done in a background of normal seasonal filling and the dominant alkaline lakes of the area.

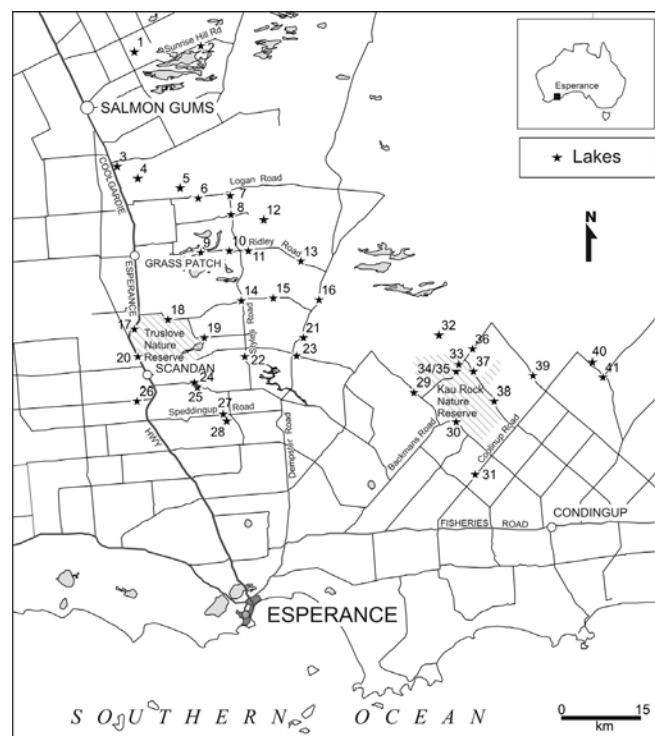


Figure 1—Map of the Esperance area showing towns and major roads and the position of the 41 lakes studied.

The Lakes

Thousands of small lakes lie on the Esperance sandplain within ca 100 km north and northeast of Esperance (Figure 1; Morgan & Peers 1973). Most are roundish to elongated, very shallow and saline and all lie in swales of old dunes most of which are aligned in a general east-west direction. They tend to fill in winter-spring and dry in summer-autumn, less reliably in the north where rainfall is less, and

also tending to be of higher salinity in the north. The greater majority lie in carbonate rich sands, but mainly in the north these deposits are very thin or absent so that granitic rocks or sandstones/siltstones lie near the surface (Morgan & Peers 1973). This fundamental difference in geology means that the latter group of lakes tends to be acidic from emerging acidic groundwaters while those on calcareous substrates are alkaline. Fifteen of the 41 lakes chosen were acidic, a much greater proportion than in nature. Even more were targeted, but they were so saline and/or so acidic that no macroscopic life was present; they are represented by Lake No. 1 in this study. The majority of lakes chosen were 1 to 10 ha in size and were < 30 cm deep (Appendix 1); this group likely over-represents the smaller lakes in the region. Many lakes are moving slowly westwards so that westward shores are steeper and eastern shores have spits and lunettes and may even be segmented (Timms 1992). The latter may mean the two parts can be different hydrologically and in salinity and hence have a somewhat different fauna. This was explored only in one basin (Lakes 34 & 35).

METHODS

The 41 chosen lakes were visited four times: August and September 2005 and January and March 2007. In addition some data are available on a few of the lakes (Nos. 5, 9, 18, 20, 22, 27, 28) from August 2006 to January 2008. The study was interrupted by drought during the normal wet season in 2006 and recommenced when episodic summer rain fell in early January 2007. Some sites were not sampled in September 2005 and March 2007 because they had already dried. Of the 41 sites studied (Table 1, Appendix 1), 8 were sampled twice, 24 three times and 9 four times. Surprisingly, cumulative species richness was not affected by sample number ($r = -0.0462$, not significant at 0.05%).

At each site a surface water sample was taken about 10 m from the shore and conductivity determined with a Hanna HI 8663 m. Conductivities were converted to TDS in g l^{-1} using the formula of Williams (1966). The word salinity is used in the general sense; it is roughly equivalent to TDS, but the latter includes dissolved organic matter as well as salts. Water clarity was measured with a Secchi tube calibrated in NTUs. The pH was determined with a Hanna HI 8924 m.

All biological sampling was done by wading, as most sites were shallow enough. Zooplankton was collected with a plankton net of mesh size 159 μm mounted on a pole and with an aperture 30 x 15 cm. It was trawled for 1 minute (sometimes longer when zooplankton was sparse) and the sample preserved in formalin. Species present were identified in the laboratory and their relative abundance was

determined by counting the first 200 organisms seen in a representative subsample and then by scanning the whole collection looking for rare species. Phytoplankton samples were collected in September 2005 and January 2007, using a 35 μm mesh net, also on a handle. Samples were preserved immediately in Lugol iodine.

Table 1—Summary of the physicochemical data in the study lakes.

no.	Site name*	mean pH	mean TDS g l^{-1}	mean turbidity (NTU)
1	McRea	2.7	171	110
2	Salmon Gums NE	5.2	146	217
3	Highway Circle Valley	8.3	99	117
4	Guest (West)	4.5	54	74
5	Guest (Southeast)	4.8	42	44
6	Logans	9.3	41	12
7	Styles & Logan	5.0	98	26
8	Styles & Kent	7.2	112	90
9	Big Ridley	5.8	122	8
10	Ridley W Little	5.2	131	42
11	Ridley Near E	3.4	194	60
12	Ridley Mid E	4.3	110	77
13	Ridley Far E	5.8	70	160
14	Styles & Lignite	8.1	116	127
15	Lignite East	8.0	86	170
16	Dempster Nth	3.2	122	41
17	West Truslove	5.0	60	20
18	Truslove Nat. Res.	4.5	136	173
19	Coxs Rd	4.5	110	135
20	Highway Scaddan	8.9	51	7
21	Dempster Mid	9.2	31	7
22	Styles South	7.8	79	325
23	Norwood Rd	7.8	54	42
24	unnamed Rd Scaddan	8.8	74	6
25	unnamed Rd Scaddan	8.7	66	40
26	Griffith	8.2	63	12
27	Speddingup Nth	7.4	170	42
28	Speddingup Sth	9.0	66	10
29	Burdett	7.1	103	22
30	Eld Rd	7.7	145	4
31	Coolingup	8.9	53	8
32	Kau Rock	8.5	70	46
33	Kau Nat Reserve	8.2	60	10
34	Kau Nat Res main	7.4	123	37
35	Kau Nat Res cutoff	7.2	68	32
36	Mt Nev	3.9	60	70
37	Avondale	8.3	37	15
38	Kau Rd	8.8	76	15
39	Howick	7.7	58	40
40	Heywood	8.9	59	7
41	Berg	8.2	106	30

* not one of the sites has an official name, these names refer to a nearby road or Nature Reserve.

Table 2—Crustaceans found in the inland Esperance lakes.

Species	Salinity Range g l ⁻¹	pH Range	Number of Records	Lakes From Which Recorded
Anostraca				
<i>Artemia parthenogenetica</i> Bowen & Stirling	97-102	7.4-7.6	2	39
<i>Parartemia cylindrifera</i> Linder	12-140	7.6-8.2	16	6,20,21-23,31-33,37,40
<i>Parartemia longicaudata</i> Linder	87-240	7.3-8.0	10	14,27,34
<i>Parartemia serventyi</i> Linder	119-258	7.5-8.2	4	3
<i>Parartemia</i> sp. A	20-235	7.4-9.2	41	15,22,24,25,26,28-32,34,38,41
<i>Parartemia</i> sp. F	35-210	3.4-7.4	42	2,4,5,7-13,16-19,36
<i>Branchinella affinis</i> Linder	2.8-9	7.8-8.2	2	5,23
Notostraca				
<i>Triops</i> sp. near <i>australiensis</i> (Spencer & Hall)	27-31	9.3-9.9	2	6
Spinicaudata				
<i>Limnadia</i> sp. near <i>cygnorum</i> (Dakin)	2.8-15	5.8-9.8	4	4,5,26
<i>Caenestheriella packardii</i> (Brady)	2.8-9	7.8-8.2	2	5,23
Cladocera				
<i>Alona</i> spp	31-37	5.4-5.8	2	4,5
<i>Daphnia carinata</i> s.l.King	2.8	7.8	1	5
<i>Daphnia</i> (<i>Daphniopsis</i>) <i>truncata</i> (Hebert & Wilson)	15-84	6.9-9.5	14	20,23,24,26,31,33,40
<i>Moina ?australiensis</i> Sars	2.8-11	5.8-8.2	4	4,5,21,23
Copepoda				
<i>Boeckella triarticulata</i> Thomson	2.8	7.8	1	5
<i>Calamoecia ampulla</i> (Searle)	2.8	7.8	1	5
<i>Calamoecia clitellata</i> Bayly	45-102	8.2-9.4	9	20,31,33,39,40
<i>Calamoecia salina</i> (Nicholls)	46-110	7.4-9.2	15	15,22,24-26,28,38
<i>Calamoecia trilobata</i> Halse & McRae	54-63	3.8-3.9	1	36
<i>Apocyclops dengizicus</i> (Lepeschkin)	15-56	5.1-9.8	4	6,26,27,29
<i>Metacyclops laurentisae</i> Karanovic	3-123	3.8-9.2	22	4,5,13,18,20,22,23,32,33,36,37,38
<i>Metacyclops</i> sp.	24-118	7.4-9.5	13	3,15,21,27,28,31,34,35,39,40
Ostracoda				
<i>Australocypris beaumontii</i> Halse & McRae	45-81	8.2-9.4	12	20,24,26,31,32,38
<i>Australocypris bennetti</i> Halse & McRae acid form	63-164	3.4-6.1	24	4,5,7,9-13,16-19,36
<i>Australocypris bennetti</i> Halse & McRae alkaline form	62-124	7.4-9.2	14	8,14,15,22,23,25,28,32,34,35,41
<i>Australocypris insularis</i> (Chapman)	52-102	7.6-8.3	5	3,21,33,37,39
<i>Mytilocypris splendida</i> (Chapman)	15-85	6.9-9.8	5	26,37,39
<i>Cyprinotus edwardi</i> McKenzie	15-35	7.8-8.4	3	23,33,40
<i>Diacypris</i> spp. ¹	15-164	3.4-9.8	77	all except 1,21,27,29 and 39
<i>Platycypris baueri</i> Herbst	24-128	3.8-9.5	48	3,4,7,10-15,18-26,28,32-35,37,40,41
<i>Reticypris</i> spp.	23-120	7.4-9.5	15	23,24,28,31,34,40
Isopoda				
<i>Haloniscus searlei</i> Chilton	15-102	6.4-9.8	26	15,20-22,24-26,31,33,35,37,38,40

¹at least five species present: *D. dictyota* De Deckker, *D. spinosa* De Deckker, *D. compacta* (Herbst), *D. whitei* (Herbst) and *Diacypris* sp.

Littoral invertebrates were collected with a 30 x 20 cm D-shaped net with 1 mm mesh. At each site, 15 minutes was spent collecting invertebrates on each trip, ample time for the species accumulation curve to plateau. Littoral samples contained animals from a variety of microhabitats, including nektonic, epiphytic, and epibenthic, but some eubenthic species such as polychaetes, chironomids and ceratopogonids were probably incompletely sampled. All collections were sorted on site and preliminary

identifications made. For each species an estimate was made of abundance using a coarse logarithmic scale (0.1, 0.5, 1, 1.5, 2, 2.5, 3, 4 for 1, 3, 10, 30, 100, 300, 1000, 10000 individuals per 15 min collection).

Some taxa could not be identified to the species level, either because of inadequate taxonomic knowledge (e.g. *Coxiella* spp., many dipteran larvae) or because unknown variability meant it was too time consuming a task (e.g. *Diacypris*

–De Deckker 1981). Fortunately, work by Halse & McRae (2004) allowed acid-alkaline forms of *Australocypris bennettii* to be separated and hence distinctions made between the fauna of acid and alkaline lakes. Possible similar distinctions in *Diacypris* have been missed, probably dulling the difference between lakes.

Relationships between the 40 sites (site 1 had no animals) were investigated using PRIMER (v5) software (Clarke and Gorley 2001). Littoral and planktonic invertebrates were analysed together. The abundance data of the invertebrates was log (N+1) transformed prior to multivariate analysis. To elucidate the influence of the episodic filling, data for the most affected sites (numbers 4, 5, 23, 26 and 39) were separated into seasonal filling collections and episodic collections and a few acid (numbers 7, 9, 13, 18, 36) and alkaline sites (numbers 22, 31, 37, 38) of similar salinity added for comparisons. Non-metric multidimensional scaling, based on the Bray-Curtis similarity matrix, was used to represent assemblage composition or physical-chemical conditions in two-dimensional space. Relative distances apart in ordinations represent relative dissimilarity. In the ordination on physicochemical features, only pH, salinity and turbidity were used, as there was no relationship with size or depth.

RESULTS

The 41 lakes ranged in conductivity from 5–250 mS/cm, i.e. from 3.5–198 g l⁻¹ salinity, and from pH 2.1 to 9.8 (Table 1, Appendix 1). Even higher salinities and lower pHs were encountered in exploration of lakes, mainly to the northeast of Grass Patch and Salmon Gums (author's unpublished data and K. Benison, pers. comm.). The highest pH values were recorded on warm summer afternoons, suggesting photosynthesis had increased apparent alkalinities. Turbidities ranged from 2–450 NTU (Appendix 1) and in alkaline lakes were haphazardly influenced by resuspension of the fine calcareous deposits if wind had occurred shortly before or during the sampling.

Only two species of aquatic plants were found in the lakes and then only in alkaline waters: *Lepilaena preissii* (Lehm.) F. Muell in lower salinities (to 54 g l⁻¹) and *Ruppia tuberosa* Davis & Tomlinson at higher salinities (to 95 g l⁻¹). Both are species typical of seasonal ephemeral systems (Brock & Lane 1983).

From limited collections, approximately 33 taxa of phytoplankton were identified in the lakes over the two sampling periods, 27 taxa in 2005 and 31 taxa in 2007 (Joan Powling, Melbourne University, pers. comm.). The list comprises 15 diatoms, the most common of which were halophile species of *Nitzschia* and *Chaetoceros*, two unidentified dinoflagellates, 10 green algae including the

halophile flagellate *Dunaliella salina*, and six filamentous cyanobacteria including *Nodularia spumigena*. Diatoms were more diverse in the winter collection of 2005 but were rare in the low pH sites in both years. *Dunaliella* was very common in three sites in 2007 with high conductivity and very low pH. A filamentous green alga, cf. *Oedogonium* sp. was common in three sites in 2005 and two sites in 2007, which had a low pH and moderately low conductivity. *Nodularia* was found only in the summer sampling of 2007 in sites of moderate conductivity.

Seventy taxa of invertebrates, representing about 75 species, were encountered in the 41 lakes (Tables 2 and 3). Widespread dominant species included crustaceans *Parartemia* sp. a and sp. f (see Timms 2004 for explanation of notation), *Australocypris bennettii*, *Diacypris* spp., *Platycypris baueri* and the snail *Coxiella glauerti*. A further nine crustacean taxa (*Parartemia cylindrifera*, *P. longicaudata*, *Daphnia* (*Daphniopsis*) *truncata*, *Calamoecia clitellata*, *C. salina*, *Metacyclops laurentisae*, *Australocypris beaumonti*, *Reticypris* spp., and *Haloniscus searlei*) were common, together with just three beetles (*Antiporus gilberti*, *Berosus* spp. and *Necterosoma penicillatum*) and a rotifer (*Hexarthra* nr *fennica*) (Table 2 & 3). Although similar numbers of insects and crustacean taxa were recorded, insects were far less common and moreover, restricted to much lower salinities (Tables 2 & 3). The dominant crustaceans together with the two common *Parartemia* species were by far the most salt tolerant (Table 2).

A seasonal study of seven of the lakes from August 2006 to December 2007 added an average of 1.4 species to each lake's list, resulting mainly from the unusual summer filling (Timms et al. 2008).

Tadpoles of the frog *Littoria cyclorhynchus* (Boulenger) grew in Lake 4 during January to March, 2007, after it had been markedly reduced in salinity and increased in pH by the episodic rainfall. It occurred over a salinity range of 3–11 g l⁻¹ and a pH range of 4.1–7.8. No fish were found in any of the lakes.

Cumulative species richness per lake ranged from 2 to 32. This parameter was influenced positively by increasing pH ($r = 0.3676$, significant at $P < 0.05$) and negatively by salinity ($r = -0.7869$, significant at $P < 0.001$). When the lakes (Nos. 4, 5, 23, 26, 39) which changed greatly by episodic in-fills were removed from the series, the correlation with pH increased ($r = 0.6022$, significant at $p < 0.001$) while that with salinity decreased a little ($r = -0.6895$, significant at $p < 0.001$). Within both alkaline and acid lakes series, correlations with species richness were not significant ($p > 0.05$; $r = 0.2581$ and $r = 0.4611$ respectively).

Table 3—Insects and miscellaneous animals of the inland Esperance lakes.

Species	Salinity Range g l ⁻¹	pH Range	Number of Records	Lakes from which Recorded
Odonata				
<i>Hemianax papuensis</i> (Burmeister)	10	4.1	1	5
<i>Hemicordulia tau</i> (Selys)	9-49	3.7-8.2	5	4,5,23,26,39
<i>Austrolestes annulosus</i> (Selys)	9-49	3.7-8.5	7	4,5,23,26,37,39
<i>Xanthoagrion erythroneurum</i> Selys	24	7.6	1	39
Hemiptera				
<i>Agraptocorixa</i> spp.	3-36	3.7-8.2	6	4,5,23,26,29,39
<i>Micronecta</i> sp.	3-67	3.7-8.5	7	4,5,27,39
<i>Anisops</i> spp.	3-56	4.1-8.2	8	5,23,26,29,37,39
Coleoptera				
<i>Allodesses bistrigatus</i> (Clark)	3-10	5.1-7.8	4	4,5,29
<i>Antiporus gilberti</i> Clark	7-94	3.7-9.8	11	4,5,7,26-28,32,39
<i>Berosus</i> spp. ¹	3-94	3.7-9.8	13	4,5,7,15,20,21,23,29,37,38
<i>Cybister tripunctatus</i> Olivier	9	8.2	1	23
<i>Enochrus elongatus</i> (W. MacLeay)	10-25	4.1-8.2	2	5,39
<i>Eretes australis</i> (Erichson)	2.5-70	4.1-9.9	7	4-6, 24,26,32,
<i>Limnoxenus zealandicus</i> (Broun)	9-24	4.1-8.2	4	4,5,23,39
<i>Megaporus howitti</i> Clark	10	4.1	1	5
<i>Necterosoma penicillatum</i> (Clark)	9-85	4.1-9.8	11	5,6,21,23,26,29,31,32,37,38
<i>Rhantus suturalis</i> MacLeay	9-10	8.2	2	23,29
unidentified Curculionidae	9-6	3.7-4.1	2	3,4
Diptera				
<i>Chironomus cloacalis</i> Atchley & Martin	22-54	8-9.8	3	20,28,29
<i>Cryptochironomus</i> sp.	10-49	4.1-9.8	4	5,26,31,37
<i>Polypedilum nubifer</i> (Skuse)	10-32	4.1-9.8	4	5,26,39
<i>Procladius paludicola</i> Skuse	9-40	6.7-9.8	3	23,26,29
<i>Tanytarsus barbitarsus</i> Freeman	24-96	5.1-9.1	5	3,29,35,39
<i>Tanytarsus semibarbitarsus</i> Glover	9-50	3.7-9.8	7	4,5,17,23,29,32,38
<i>Aedes camptorhynchus</i> (Thomson)	10-54	8.2-9.2	2	20,39
unidentified ceratopogonid larva	24	7.4	1	39
unidentified dolichopodid larva	24	7.4	1	39
unidentified stratiomyid larva	10-75	3.9-5.7	3	5,9
unidentified tabanid larva	25-126	5.2-9.5	4	13,14,32,39
Lepidoptera				
unidentified pyralid larva	15-31	9.5-9.8	2	6, 21, 26
Platyhelminthes				
unidentified rhabdocoel	63	8.4	1	35
Rotifera				
<i>Brachionus plicatilis</i> s.l. (Müller)	9-50	6.7-9.9	9	6,7,21,23,26,29,32,37
<i>Brachionus rotundiformis</i> Tschugunoff	40	6.7	1	29
<i>Hexarthra cf brandorffii</i> Koste	36	3.7	1	4
<i>Hexarthra cf fennica</i> (Levander)	9-68	3.7-9.9	14	4-6,13,23-26,32,38
<i>Hexarthra</i> sp.	54-62	3.8-3.9	1	36
Mollusca				
<i>Coxiella glauerti</i> Macpherson	15-118	7.4-9.8	32	3,15,20-24,26-28,32-34,37
<i>Coxiella</i> spp.	9-92	7.7-9.5	5	23,32,40

¹ *B. nutans* (W. MacLeay), *B. reardoni* Watts

In many lakes spits have grown across the original dune hollow to segment it into two or more sub-basins (Timms 1992). Despite being less than 2 m apart at their closest point and in the same greater basin, such twin lakes can be very different. In the only example studied, the two sub-basins had different physicochemical features, with the main circular central basin (Lake 33) more saline and of higher pH than the cut-off bay (Lake 34) (Appendix 1). The central basin had a lower species richness (6 species) compared to that of the cut off bay (9 species). Of the differences in species composition, the most obvious were the presence of *Parartemia* n.sp. and *Reticypris* sp. in Lake 33 and the presence of *P. cylindrifera*, *Daphnia truncata* and *Haloniscus searlei* in Lake 34. Of the shared species, some were much more common in one or the other lake (e.g. *Diacypris* spp. in Lake 33, *Coxiella glauerti* in Lake 34). Reconnaissance of other lakes with subbasins suggested differences between such divided lakes were common.

Multivariate analysis using just the physiochemical features of the lakes showed a clear distinction between acid and alkaline lakes, with many alkaline lakes variously turbid (haphazardly due to wind — see earlier) and both sets of lakes traversing a wide salinity range. In the ordination plot (Figure 2), the most acid lakes (1, 11, and 16) are on the far left and the least acid lakes (9, 13) nearest the least alkaline lakes (8, 29). This acid-alkaline division is reflected in the ordination plot of invertebrate communities in the lakes (Figure 3), in which the acid lakes cluster together, with the alkaline lakes scattered in an gentle arc away from the acid lakes. Among the acid lakes, 4 and 5 are somewhat separate from the main group which is explained by their episodic filling (see later). One alkaline lake, No. 8, grouped with the acid lakes, which is not surprising given its fluctuation between pH 6.5 to 7.7. Among the alkaline lakes, numbers 29, 30, 6, 21, 35 39 and 27 are separated from the main group on different axes (Figure 3) for different apparent reasons. Lake 27 is highly saline and more persistent than most (T. Massenbauer of WA Department of Environment and Conservation, pers. comm.), lakes 6 and 21 are of persistent relatively low salinity and lakes 35 and 39 are of seasonally low salinity.

Acid lakes have low diversity and are dominated by *Parartemia* sp. f and have the acid form of *Australocypris bennetti* (Table 4). On the other hand, alkaline lakes are less dominated by one species and have *Diacypris* spp. and *Parartemia* sp. a common and an array of other species, all crustaceans, differentiating them (Table 4).

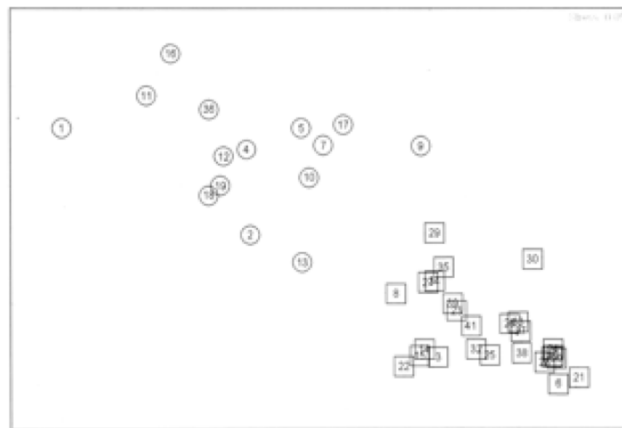


Figure 2—Ordination of the lakes based on their physicochemical features. Acid lakes are circled and alkaline lakes are in squares. Numbers in symbols refer to lakes described in Table 4. A stress factor of 0.05 was used in the analysis.

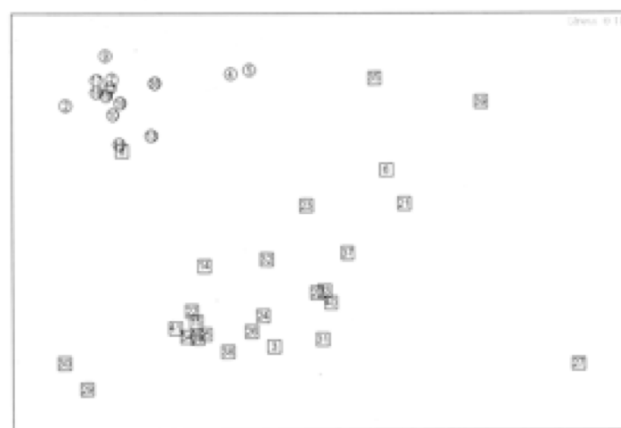


Figure 3—Ordination of the lakes based on their invertebrates. Acid lakes are circled and alkaline lakes are in squares. Numbers in symbols refer to lakes described in Table 4. Stress 0.17.

The unusual ± 200 mm of rain that fell on January 4, 2007 put water in most lakes, but generally at depths not much different from a normal winter-spring filling. Some lakes, however, received much water from overland flow and filled to far greater depths than normal. Two of these were from the acid series (Nos. 4 and 5) and three from the alkaline series (Nos. 23, 26 and 39). All five became almost fresh and near neutral in pH. Species normally found in them were replaced by many insects, a few species of branchiopods and a few others. These episodic changes in biota are plotted in an ordination diagram (Figure 4) in which data for each were divided into normal winter-spring features ('a' notation) and episodic conditions ('b' notation) and then compared with a few other lakes from each group of similar salinity. Clearly this approach recognized three types of lakes (see Figure 4): acid to the lower left, alkaline to upper middle and a new group of low salinity lakes to the lower right. Four lakes (Nos. 4, 5, 23, 39) changed markedly as a result of their episodic fill and one changed position in the alkaline group (No. 26).

Characteristic species in the acid and alkaline groups were similar between the 'a' sites and their respective groups, but a new low salinity episodic group ('b' sites) had a new array of characteristic species, dominated by an array of insects (Table 4).

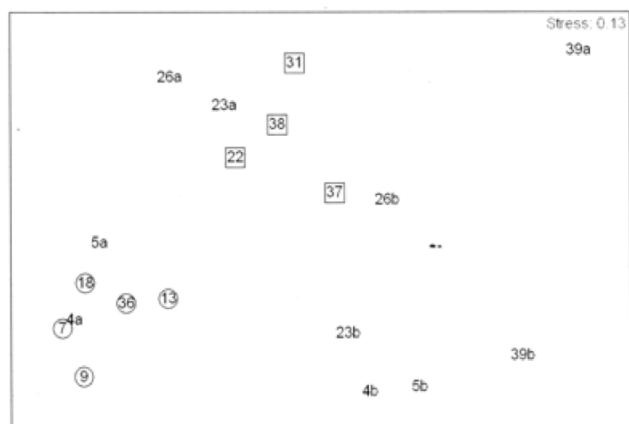


Figure 4—Ordination of episodic lakes based on their invertebrates. Lakes remaining acid are circled and lakes always alkaline are in squares. Numbers in symbols refer to lakes described in Table 4. The episodic lakes in normal condition are labelled 'a' and after the episodic rain are labelled 'b.' Stress 0.13.

DISCUSSION

Differing characteristics among these lakes are due largely to their position with reference to surface rocks and their derived soils. Although some lakes are subject to occasional large overland flows, most are groundwater "windows". As such, acidity/alkalinity of groundwater influences their pH. Throughout much of the southwest on the Yilgarn Block groundwaters tend to be acid, often very much so (pH < 2.8) (Mann 1983; McArthur et al. 1991). The reason for this is contentious, but David Gray of CSIRO, Perth (pers. comm.) thinks the acidity is generated through soil pedological processes, resulting in the accumulation of carbonates in the soil profile and recharge of iron-rich, sometimes acidic waters, to shallow aquifers. On the other hand, Kathy Benison of Central Michigan University (pers. comm.) hypothesises that sulfide oxidation in host rocks results in acid groundwaters, which may be further acidified by ferrololysis, microbial action and/or evaporative concentration in lakes without carbonates to buffer the acidity. Where carbonates are scarce or isolated from lakes, as in most lakes north of Ridley Rd (east of Grass Patch) and a few others mainly in the Truslove Nature Reserve area (Morgan & Peers 1973), lakes are acid. Elsewhere in deep carbonates (particularly Lakes 14, 15, 20-35, 37-41), lakes are alkaline. At least for one lake in shallow carbonates (No. 3), groundwaters are acid (pH 3.5) though

lake waters are alkaline (pH 8-8.5) (K. Benison, pers. comm.). This may be at least a partial explanation for differing pHs in main and cut-off parts of a basin (as in Lakes 33 & 34), the later being more active hydrologically. The cut-off part may also be more influenced by overland flows which would not be as acid as the groundwater and certainly would be less saline. The lakes subject to large overland flows (Nos 4, 5, 23, 26, 39) all had low salinities and neutral pHs following episodic rainfall events.

Table 4—Characteristic species of each group of lakes.

Higher category	Genus and species	% *
Acid lakes		
Anostraca	<i>Parartemia</i> n.sp. f	51.6
Ostracoda	<i>Australocypris bennetti</i> acid	28.0
Ostracoda	<i>Diacypris</i> spp.	13.6
Alkaline lakes		
Ostracoda	<i>Diacypris</i> spp.	37.7
Anostraca	<i>Parartemia</i> n.sp. a	24.8
Ostracoda	<i>Platycypris baueri</i>	6.8
Ostracoda	<i>Australocypris bennetti</i> alkaline	6.0
Anostraca	<i>Parartemia cylindrifera</i>	4.7
Isopoda	<i>Haloniscus searlei</i>	4.3
Ostracoda	<i>Australocypris beaumonti</i>	4.0
Episodic change –standard acid lakes		
Anostraca	<i>Parartemia</i> n.sp. f	54.9
Ostracoda	<i>Australocypris bennetti</i> acid	31.5
Ostracoda	<i>Diacypris</i> spp.	9.8
Episodic change – standard alkaline lakes		
Ostracoda	<i>Diacypris</i> spp.	37.1
Ostracoda	<i>Australocypris bennetti</i> alkaline	10.5
Isopoda	<i>Haloniscus searlei</i>	9.2
Anostraca	<i>Parartemia</i> n.sp. a	8.8
Ostracoda	<i>Platycypris baueri</i>	8.2
Copepoda	<i>Metacyclops laurentiisae</i>	5.8
Ostracoda	<i>Mytilocypris splendida</i>	4.8
Episodic change – new low salinity lakes		
Insecta	<i>Anisops</i> spp.	13.8
Insecta	<i>Agraptocorixa</i> spp.	13.7
Anostraca	<i>Branchinella affinis</i>	10.2
Copopoda	<i>Metacyclops laurentiisae</i>	9.5
Rotifera	<i>Hexarthra</i> nr <i>fennica</i>	9.1
Insecta	<i>Austrolestes annulosus</i>	8.3
Insecta	<i>Micronecta</i> sp.	8.2
Insecta	<i>Hemicordulia tau</i>	7.0
Insecta	<i>Limnoxenus zealandicus</i>	6.9
Spinicaudata	<i>Caenestheriella packardi</i>	6.6

* The percentages in the table refer to the % of the total difference between the groups that is contributed by the different species.

Groundwater-controlled lake pHs are known for lakes north of the study area (Lakes Swann, Gilmore, Lefroy) (McArthur et al. 1991; Clarke 1994) but with unknown implications for lake invertebrates. However, in the Lake Hope area, 150 km to the northwest of Grass Patch, saline lakes adjacent to laterite are acid and have a different fauna from that of alkaline lakes on the sand plain (Timms 2006). Conte & Geddes (1988) first noted the restrictive role of pH in Western Australian salt lakes, noting the presence of just *Parartemia contracta* in acid lakes. These Esperance acid lakes provide a more intensive example of the role of low pH in restricting fauna. Secondly salinized waters are often acidic as well and Halse et al. (2003) have noted the absence of most halophilic/halobiont species in them.

In addition to the influence of pH, salinity, not surprisingly exerts a strong control over species composition and species richness as it does in most inland saline waters, including those in Western Australia (Pinder et al. 2002). On the other hand, the apparent effects of seasonality on community composition were more easily explained by differences in pH and/or salinity (Timms et al. 2008). Two exceptions were many insects which were more abundant in summer and *Daphnia* (*Daphniopsis*) *truncata* which was most abundant in winter-spring.

The alkaline saline lakes of the Esperance inland support a fauna that is broadly similar throughout southern Australia (Geddes 1976; De Deckker & Geddes 1980; Pinder et al. 2002, 2004, 2005; Timms 2009), though species differ regionally (Williams 1984). The Esperance hinterland lakes have a species of *Parartemia*, many species of ostracods, including a mytilocyprinid species, *Diacypris*, *Platycypris* and *Reticypis*, and perhaps a species or two of rotifers and copepods, a halophilic *Daphnia* (*Daphniopsis*), the isopod *Haloniscus searlei*, and a *Coxiella* snail. Of these, *Parartemia* n.sp. a and *Australocypris beaumonti* are apparently endemic to the Esperance area, and *Parartemia cylindrifera*, *P. longicaudata*, *P. serventyi*, *Australocypris bennetti* and *Coxiella glauerti* are shared with a few others. Previous studies have found fewer species in the area (Geddes et al. 1981; Brock & Shiel 1983) or a few more, mainly a polychaete and some chironomids (Pinder et al. 2002).

The fauna of the acid salinas is more restricted with just *Parartemia* n.sp. f, *Australocypris bennetti* (acid form), *Diacypris* spp. and the copepod *Calamoecia trilobata*, with the first two apparently restricted to the area. This restricted fauna is less diverse than that in acid lakes in the wheatbelt

further west in Western Australia which have these species, except *Parartemia* sp. f., and also perhaps a variety of dipterans, a few beetles, more ostracods and *Parartemia contracta* (Adrian Pinder, pers. comm.). This fauna is more restricted than occurs in alkaline lakes in the wheatbelt (Pinder et al. 2004, 2005). At least for *Parartemia* spp this is probably due to the necessity for a different method of osmoregulation in acid conditions where there are no bicarbonate/carbonate ions (Conte & Geddes 1988).

Many saline lakes of the Australian inland are dominated by episodic events so that they fill and dry over years, vary widely in salinity and have a corresponding array of characteristic species as salinity changes widely (Timms 2008). Most lakes in the south fill and dry regularly each year and, though varying widely in salinity, lack a fresh/hyposaline stage and so have a more limited fauna that changes little during the wet phase (e.g. Geddes 1976; De Deckker & Geddes 1980). This is explained by a rapid change in a richer array of species as salinity slowly increases in the hyposaline range but wide tolerances of the limited array of species in the much wider mesosaline-hypersaline range, or in other words a 'question of scale' (Williams et al. 1990). The Esperance hinterland lakes are of the second type based on their latitude and some seasonal evidence. However, they are subject to occasional episodic filling, and in some lakes where the hydrology is favourable, there can be vast changes in their characteristics (Figure 4). Their fauna, when fresh or hyposaline, is uncharacteristic of their normal composition and more akin to widespread faunal elements in hyposaline filling stages of lakes further inland. Particularly noticeable in both these unusual Esperance lakes and the episodic inland lakes is the abundance of insects tolerant to lower salinities and the absence of brine shrimp and ostracods tolerant to high salinities.

Of even more interest is the presence in these episodic fillings of animals not seen in typical saline lakes. These are large branchiopods either of tolerant freshwater forms or of specialist hyposaline forms. The Esperance hinterland lakes subject to the episodic filling of January 2007 had the slightly tolerant *Branchinella affinis* and *Caenestheria packardii* (Timms in press) and two nearby lakes had a new species of *Branchinella*, and the clam shrimps *Limnadia* near *cygnorum* and *Eocyclus parooensis* (Timms 2009). Their presence in appreciable numbers shows these lakes have sufficiently frequent episodic fillings to have an egg bank capable of responding to the unusual conditions of being fresh or hyposaline and around neutral pH. Should

episodic fills be too spaced temporally or averted due to increased salt loads in a basin due to mining inputs or salinization, then this specialized fauna will be lost (Timms 2005). It is not known how much of this fauna is catalogued, but in Western Australia it includes the fairy shrimps *Branchinella compacta*, *B. nicholli*, *B. nana*, and *Branchinella papillata*, the clam shrimps, *Caenestheria dictyon*, *Caenestheriella packardii*, *Eocyclus parooensis* and *Limnadia* near *cygnorum* and a form of the shield shrimp *Triops* near *australiensis* (Timms 2009).

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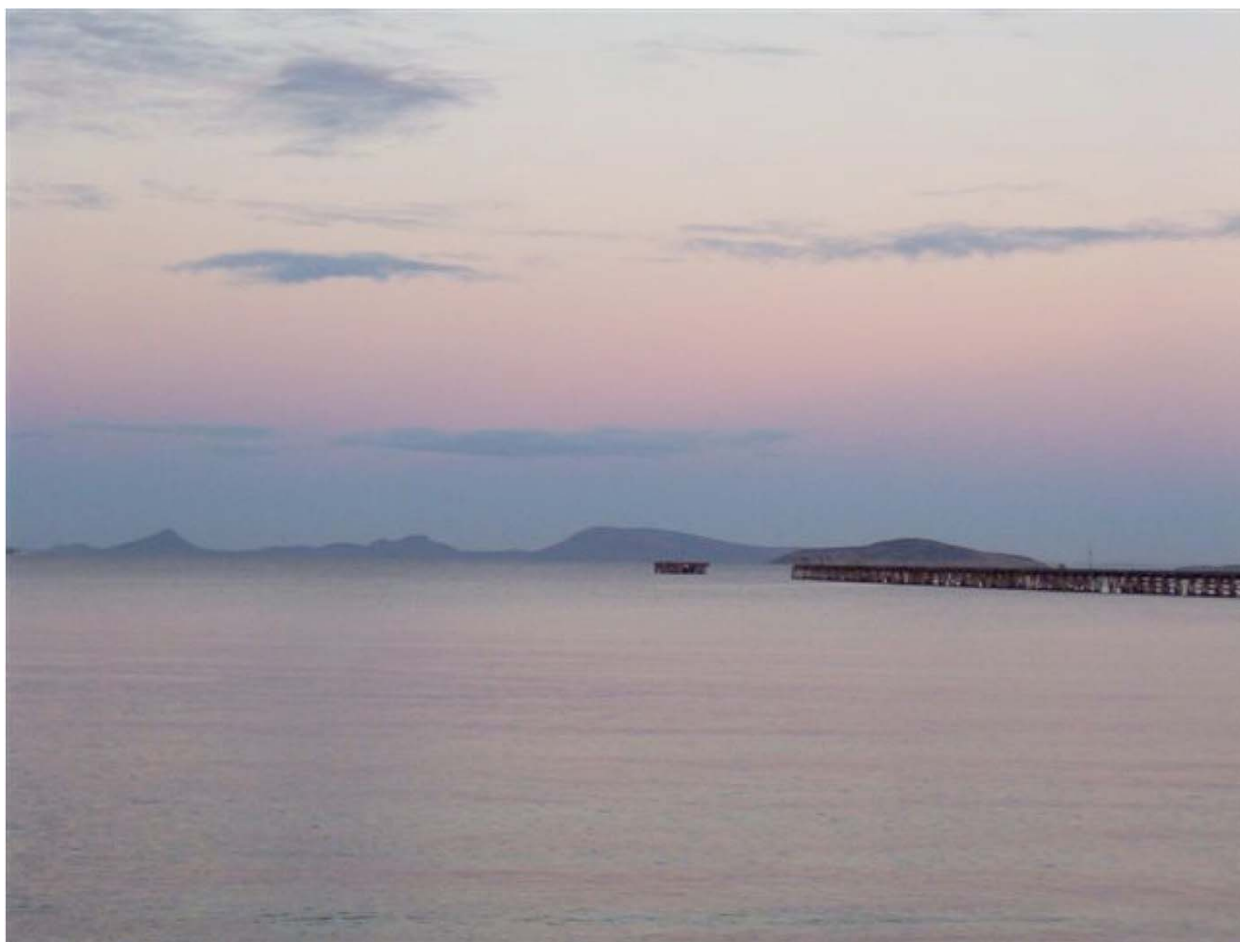
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Esperance Bay featuring the Tanker Jetty. December 2005. Wikimedia Commons at en.wikipedia.org. Accessed February 2009.

Appendix 1—Geomorphic and physicochemical data on the lakes.

No	Name	Latitude	Longitude	Area when full (ha)	Lake depths (cm)		Number of visits	pH range	Salinity Range g l ⁻¹	Turbidity (NTU) range
					mean	Max.				
1	McRea	32° 53'	121° 45'	4.0	6	10	3	2.1-3.0	148-195	60-160
2	Salmon Gums NE	32° 53'	121° 53'	33.5	7	15	3	4.9-5.5	88-187	200-250
3	Highway Circle Valley	33° 05'	121° 42'	8.2	13	30	3	7.5-9.1	92-109	45-240
4	Guest (West)	33° 06'	121° 44'	3.2	22	ca 50	4	3.7-5.8	11-95	10-250
5	Guest (Southeast)	33° 08'	121° 49'	2.6	49	ca 150	4	3.7-7.8	4-182	10-130
6	Logans	33° 09'	121° 51'	1.4	30	35	2	8.8-9.9	31-51	10-15
7	Styles & Logan	33° 09'	121° 55'	2.8	16	40	4	3.7-7.7	27-144	5-50
8	Styles & Kent	33° 11'	121° 55'	13.4	5	10	3	6.5-7.7	87-133	10-200
9	Big Ridley	33° 14'	121° 51'	365	7	12	3	5.1-6.3	117-132	5-10
10	Ridley W Little	33° 14'	121° 54'	4.1	40	ca 70	3	4.5-6.3	129-131	10-70
11	Ridley Near E	33° 15'	121° 57'	3.7	23	25	3	2.5-4.4	111-148	5-150
12	Ridley Mid E	33° 15'	121° 59'	23.7	12	15	3	4.1-4.7	101-124	10-160
13	Ridley Far E	33° 16'	122° 03'	27.5	7	10	3	5.2-6.2	46-88	60-330
14	Styles & Lignite	33° 19'	121° 55'	38.4	5	10	3	7.9-8.5	99-126	40-250
15	Lignite East	33° 19'	121° 59'	6.6	4	5	3	7.4-8.7	44-116	30-300
16	Dempspter Nth	33° 20'	122° 05'	5.5	13	20	3	2.7-3.6	110-149	18-60
17	West Truslove	33° 22'	121° 42'	5.4	7	10	3	4.3-5.5	40-84	10-40
18	Truslove Nat. Reserve	33° 20'	121° 46'	7.3	11	12	3	4.3-4.7	123-159	50-320
19	Coxs	33° 23'	121° 51'	7.6	7	7	3	4.4-4.6	104-128	35-320
20	Highway Scaddan	33° 24'	121° 42'	6.0	26	30	3	8.4-9.2	45-55	5-10
21	Dempster Mid	33° 24'	122° 03'	1.5	33	45	2	8.9-9.5	24-38	5-10
22	Styles South	33° 25'	121° 55'	1.8	4	10	2	7.7-7.9	56-101	200-450
23	Norwood	33° 25'	122° 02'	13.2	18	ca 50	4	7.4-8.2	9-92	5-180
24	unnamed Rd Scaddan	33° 28'	121° 49'	3.7	15	25	3	8.5-9.2	66-88	2-10
25	unnamed Rd Scaddan	33° 28'	121° 49'	3.1	15	20	3	8.3-9.0	61-70	5-80
26	Griffiths	33° 29'	121° 42'	2.2	27	40	4	6.9-9.8	15-84	5-25
27	Speddingup Nth	33° 31'	121° 52'	1.8	37	ca 50	4	7.0-8.0	118-195	15-95
28	Speddingup Sth	33° 32'	121° 53'	2.0	22	30	3	8.4-9.3	62-67	5-20
29	Burdett	33° 30'	122° 16'	2.2	55	ca 100	4	6.1-7.8	40-56	20-30
30	Eld	33° 33'	122° 21'	2.4	28	30	2	7.6-7.9	121-169	2-5
31	Coolingup	33° 39'	122° 23'	5.3	28	35	3	8.5-9.4	44-68	5-10
32	Kau Rock	33° 25'	122° 20'	10.9	9	12	3	7.8-9.5	28-105	8-120
33	Kau Nat Reserve	33° 28'	122° 21'	6.1	12	15	3	8.0-8.4	52-68	8-12
34	Kau Nat Res main	33° 28'	122° 22'	4.2	20	25	2	7.3-7.5	120-128	20-55
35	Kau Nat Re cutoff	33° 28'	122° 22'	0.5	8	12	2	6.4-8.0	63-74	20-45
36	Mt Nev	33° 26'	122° 23'	6.1	15	20	3	3.8-3.9	56-62	50-80
37	Kau Rd south	33° 29'	122° 23'	0.6	8	12	2	8.1-8.5	24-49	10-20
38	Kau Rd north	33° 32'	122° 26'	3.3	35	40	4	7.8-9.2	46-147	5-30
39	Howick	33° 29'	122° 31'	6.0	63	ca 100	4	7.4-8.2	10-102	10-60
40	Heywood	33° 29'	122° 37'	6.9	11	15	3	8.5-9.2	52-66	5-10
41	Berg	33° 30'	122° 39'	1.1	32	40	2	8.0-8.4	95-116	20-40

The Effects of Mining on a Salt Lake in the Western Australian Goldfields

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Lake Carey is a large ephemeral lake of an approximate area of 750 km² in the arid Eastern Goldfields of Western Australia. It is one lake in a chain that makes up the Carey palaeodrainage system, formed during the Tertiary period, about 65 million years ago. Lake Carey has recently (within the last 10 years) become the focus of interest of a number of mining companies, most of them operating gold mines. There is concern from a number of people that the joint activities of several mining operations may have an impact on the biological functions of the lake.

Mining activities with the potential to affect the natural lake functions include:

- Hypersaline groundwater discharge from mine dewatering—extra salt load and extended hydroperiod.
- Drawdown effect from dewatering bores—destabilization and mobilization of lake sediments.
- Sediments in hypersaline discharge—fine sediments altering makeup of lake sediments.

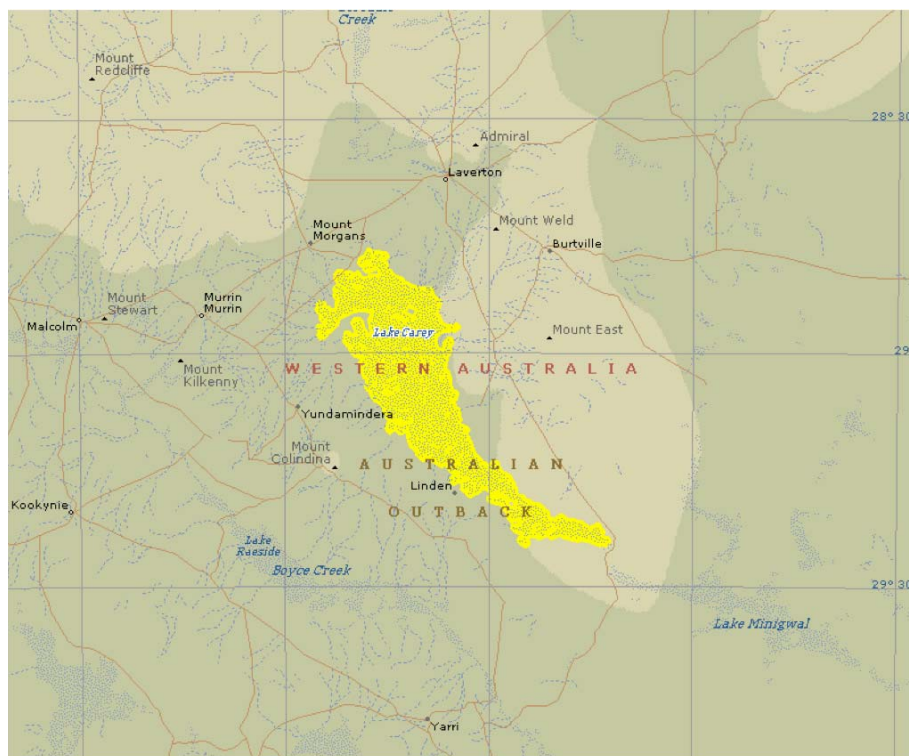
- Interception of palaeodrainage system—increased evaporation from open pits, local drawdown effect.

- Mine infrastructure—pits, overburden, roads, and exploration vehicles.

After ten years of monitoring the lake, both physical and biological changes have been observed that have a high likelihood of being caused by hypersaline groundwater dewatering discharge, dewatering drawdown effects, sedimentation or a combination of some or all of these factors. As climatic conditions in the Goldfields are cyclic, biological changes may also be due to natural variations.

Environmental monitoring has indicated changes in fringing vegetation with little or no recruitment, and reduction in numbers of species. Fringing vegetation zonation has changed in areas affected by dewatering drawdown.

It has been thirteen years since the last major lake fill—there have been lake fills but not as large as the aftermath of Cyclone Bobby in 1995. Until there will be another similar event it is not certain if changes to the fringing vegetation have been caused by mining activities, natural fluctuations or a combination of the two.



Map of Lake Carey, Western Australia. Copyright © 1988-2004 Microsoft Corp. <http://www.microsoft.com/virtualearth>. Accessed January 2009.

Waterbirds of the Saline Lakes of the Paroo, Arid-Zone Australia: A Review with Special Reference to Diversity and Conservation

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ABSTRACT

About sixty species of waterbird live in the Paroo area of Australia and most of these have been recorded on its 150+ saline lakes of various salinities and sizes. Unlike water plants, invertebrates and fish, avian species richness is hardly influenced by salinity in the Paroo, although the data suggest that richness might be lower in hypersaline lakes. Common species at salinities < 30 g/l include Eurasian coot, Black swan, Pink-eared duck, Grey teal, and Australian pelican while at salinities > 100 g/l Red-necked avocets and stilts are common. Because saline lakes generally have more abundant food than freshwater lakes, waterbirds tend to be more abundant on them. However, most species do not utilize them for breeding, unless islands are present as in Lake Wyara. Bird numbers fluctuate widely in response to many factors, including food availability and state of wetlands elsewhere in the inland. Birds move freely between fresh and saline waters and are nomadic between wetlands across the vast Australian inland. Some Paroo salinas and their birds are threatened by localized siltation and local freshwater lakes could be destroyed by water harvesting for desert irrigation. The effect of water harvesting on other inland wetlands could also affect the Paroo and its salinas.

INTRODUCTION

The interconnected Paroo-Warrego catchment, the westernmost of the Murray-Darling Basin, contains the largest concentration of wetlands in Australia, and indeed is significant on a world scale (Kingsford & Porter 1999; Kingsford et al. 2004). In wet years 12% of the landscape is inundated, meaning there are 1.8 million ha of wetlands of various types (Kingsford & Porter 1999; Kingsford et al. 2004). Of these, saline lakes comprise 1.5×10^4 ha (0.8%) organized into 170 discrete salinas, the great majority of which lie in the Paroo catchment (155) and in NSW (118). These wetlands are saline because they are located in endorheic basins away from the main flood channels and are rarely, if ever, flushed of any accumulated salt. This region of Australia experiences short ‘boom’ periods in wet (La Nina) years, interspersed with long, irregular and unpredictable periods of ‘bust’ levels of productivity during dry (El Nino) years (Kingsford et al. 1999).



Figure 1—Map of the Paroo showing many of the study sites mentioned in the text.

The arid inland of Australia was once thought to be of little value to waterbirds (Frith 1982), but perspectives and focus changed in the 1980s due to the work of Braithwaite, Briggs, Halse, Kingsford and Maher, among others (see Kingsford & Porter 1999). In particular, Kingsford and colleagues have shown that the Paroo wetlands episodically support a relatively diverse and an abundant waterbird fauna. It is the aim of this review to record the importance of Paroo saline lakes for waterbirds. This needs to be done in the context of the unique environment in inland Australia (Stafford Smith & Morton 1990) and of the interconnectivity of the waterbirds of all inland saline and freshwater lakes (Kingsford & Porter 1994, 1999; Kingsford 2000).

Table 1—Some saline lakes of Paroo. The other 135 lakes are omitted as no data available for them, or they were too small (< 20 ha).

Lake	State	Coordinates	Area (ha)	Known salinity range (g/l)	Average salinity type	References
Wyara	Qld	28° 42' S 144° 14' E	3400	2.8-350	mesosaline	Kingsford & Potter 1994; Timms 1998a, 2007
Yumberarra	Qld	28° 53' S 144° 19' E	151	0.3-114	subsaline	Timms & McDougall 2004; Timms 2007
North Blue	Qld	28° 50' S 144° 57' E	205	0.3-31	hyposaline	Timms 2007, 2008
Mid Blue	Qld	28° 52' S 144° 57' E	210	0.7-103	hyposaline	Timms 2007, 2008
Bulla	Qld	28° 54' S 144° 55' E	420	1.8-262	mesosaline	Timms 2007, 2008
Lower Bell	NSW	29° 30' S 144° 48' E	160	7-123	mesosaline	Kingsford et al. 1994; Timms 1993, 1998b, 2007
"Bells Bore SL"	NSW	29° 31' S 144° 50' E	24	29-187	hypersaline	Timms 1993, 1998b, 2007
Horseshoe	NSW	29° 32' S 144° 46' E	746	13-310	hypersaline	Kingsford et al. 1994; Timms 1993, 1998b, 2007
Gidgee	NSW	29° 33' S 144° 50' E	185	5-52	hyposaline	Timms 1993, 1998b, 2007
Burkanoko	NSW	29° 46' S 144° 49' E	280	6-37	hyposaline	Timms 1993, 1998b, 2007
Nichebulka	NSW	29° 49' S 144° 48' E	322	54-360	hypersaline	Kingsford et al. 1994; Timms 1993, 1998b, 2007
"Barrakee"	NSW	30° 05' S 145° 02' E	90	23-218	hypersaline	Timms 1993, 1998b, 2007
"Kings Bore"	NSW	30° 06' S 144° 55' E	151	22-330	mesosaline	Timms 1993, 1998b
Taylors	NSW	30° 07' S 145° 04' E	62	0.7-9.1	subsaline	Timms 1993, 1998b, 2007
"Avondale Salt"	NSW	30° 09' S 144° 39' E	520	9-69	mesosaline	Timms 1993, 1998b
Mere	NSW	30° 11' S 144° 59' E	68	4-82	hyposaline	Timms 1993, 1998b
Utah	NSW	30° 15' S 144° 38' E	1900	46-264	hypersaline	Timms 1993, 1998b
Mullawoolka	NSW	30° 31' S 143° 50' E	2026	unknown	subsaline	Kingsford et al. 1994.

The Lakes

Only a few of the saline lakes of the Paroo are known scientifically. Limnological data are available on 25 salinas in the middle Paroo (Timms 1993, 1997, 1998a), two saline lakes in the southern Paroo (Kingsford et al. 1994; Timms & Boulton 2001) and on seven saline lakes in the Queensland Paroo (Kingsford & Porter 1994, Timms 1998b, 2008; Timms & McDougall 2004). Censuses of waterbirds are available for 14 of these sites (Kingsford & Porter 1994; Kingsford et al. 1994; Timms 1997, 2008; Timms & McDougall 2004). Fortunately these censused lakes include the largest and many of the intermediate-sized ones and most of the 14 are listed in Table 1.

All of the well-studied lakes (i.e. those in Table 1) of the Paroo system are episodic, rather than seasonal or permanent. As a rule, they fill in La Nina years when rainfall is above average and dry in El Nino years when rainfall is scarce. Some contain water for only a few months every few years, while others may have water for many years and then sometimes dry for years also (Timms 2007). All are shallow, rarely exceeding 2 m in depth and often much less. While exhibiting wide ranges in salinity, each has a characteristic 'average' salinity which ranges through the salinity spectrum from subsaline to hypersaline. During droughts when saline lakes have dried or have become hypersaline, most adjacent freshwater lakes salinize naturally before they too dry (e.g. Lake Numalla, Timms 2007). In most cases ionic dominance is markedly by Na

and Cl ions, but some hyposaline lakes have significant amounts of Ca and SO₄ (Timms 1993).

Bird Censuses

Recording the diversity and numbers of waterbirds on these saline lakes is challenging. Two basic methods were used in the studies reviewed here: estimates from aerial surveys and direct counts from the ground. In aerial surveys counts were made from a low flying aircraft either using transects across the wetland or proportional counts 150 m away from the shore, where most birds were concentrated (Kingsford & Porter 1994; Kingsford et al. 1994; Kingsford 1999). Each lake was surveyed up to four times on each visit so as to gain a mean and standard deviation for each species on each lake. For species with fewer than 50 individuals sighted, the highest number seen on the four counts was taken as the number present on that visit. Such methods are known to underestimate bird numbers by > 50% for large concentrations and to miss species present in small numbers (Kingsford 1999). However, direct counts from the lake edge ideally record all individuals and species present at the time, but were not repeated due to time constraints (Timms & McDougall 2004; Timms 1997, 2008). Also on larger lakes, only a proportion (often half) of the lake was scanned, introducing another error. Given the mobility of waterbirds, all counts are subject to large random errors, so that single counts are not as reliable as replicated counts.

Table 2–Waterbirds on saline lakes of the Paroo.

Species		Feeding group#	Relative numbers in:					
Common name	Latin name		fresh L.Numalla ¹	hyposaline Yumberarra ²	hyposaline Nth Blue ³	mesosaline Wyara ¹	mesosaline Bulla ³	hypersaline Horseshoe ⁴
Duck, Blue-billed	<i>Oxyura australis</i>	d		xx	xx	x	x	
Duck, Musk	<i>Biziura lobata</i>	pw	x	x		x	x	
Duck, Freckled	<i>Stictonetta naevosa</i>	d	xxx	xx	x	xx	x	
Swan, Black	<i>Cygnus atratus</i>	h	xx	xxx	xxx	xxx	xxx	x
Duck, Australian Wood	<i>Chenonetta jubata</i>	h	x	xx	xx	x	x	
Duck, Pacific Black	<i>Anas superciliosa</i>	d	xx	xx	xx	x	x	xx
Duck, Australian Shoveller	<i>Anas rhynchotis</i>	d	x	xx	xx	xx	xx	x
Duck, Grey Teal	<i>Anas gibberifrons</i>	d	xx	xxx	xxxx	xxxx	xxx	xxx
Duck, Chestnut Teal	<i>Anas castanea</i>	d		x	x		x	
Duck, Pink-eared	<i>Malacorhynchus membranaceus</i>	d	xx	xxx	xxx	xxxx	xxx	xxx
Duck, Hardhead	<i>Aythya australis</i>	d	xxx	xxx	xx	xxx	xx	x
Grebe, Australasian	<i>Tachybaptis novaehollandiae</i>	d	x	xx	xx	x*	xx	x
Grebe, Hoary-headed	<i>Poliiocephalus poliocephalis</i>	d		xxx	xx		xx	
Grebe, Great crested	<i>Podiceps cristatus</i>	pw	x	xx	xx	x	xx	
Darter	<i>Anhinga melanogaster</i>	pw	x	xx	x	x	x	
Cormorant, Little Pied	<i>Phalacrocarax melanoleucos</i>	pw	x	xx	xx		x	
Cormorant, Pied	<i>Phalacrocarax varius</i>	pw	xx	x	x	x	x	
Cormorant, Little Black	<i>Phalacrocarax sulcirostris</i>	pw	x	xx	xx	x	xx	
Cormorant, Great	<i>Phalacrocarax carbo</i>	pw	xx	x		x		
Pelican, Australian	<i>Pelecanus conspicillatus</i>	pw	xx	xx	xx	xxx	xx	
Heron, White-faced	<i>Ardea novaehollandiae</i>	pw	x	x	x	x	x	
Heron, White-necked	<i>Ardea pacifica</i>	pw	x	x			x	
Egret, Great	<i>Egretta alba</i>	pw	x	x	x		x	
Egret, Intermediate	<i>Egretta intermedia</i>	pw		x				
Egret, Little	<i>Egretta garzetta</i>	pw	x*	x			x	
Night Heron, Rufous	<i>Nycticorax caledonicus</i>	pw		x				
Ibis, Glossy	<i>Plegadis falcinellus</i>	pw	x	xx	x		x	
Ibis, Australian White	<i>Threskiornis molucca</i>	pw	x	x	x		x	
Ibis, Straw-necked	<i>Threskiornis spinicollis</i>	pw	x	x	x		x	
Spoonbill, Royal	<i>Platalea regia</i>	pw	x	x	x		x	
Spoonbill, Yellow-billed	<i>Platalea flavipes</i>	pw	xx	xx	x	x	x	
Sea Eagle, White-bellied	<i>Haliaeetus leucogaster</i>	pw		x				
Brolga	<i>Grus rubicundus</i>	h	x	x	x	x	x	
Crake, Australian Spotted	<i>Porzana fluminea</i>	h		x				
Swamphe, Purple	<i>Porphyrio porphyrio</i>	h		x				
Native Hen, Black-tailed	<i>Gallinula ventralis</i>	h	x	xx	x			
Moorhen, Dusky	<i>Gallinula tenebrosa</i>	h		x				
Coot, Eurasian	<i>Fulica atra</i>	h	x	xxxx	xxxx	xx	xxxx	x
Snipe, Latham's	<i>Gallinago hardwickii</i>	w		x				
Godwit, Black-tailed	<i>Limosa limosa</i>	w		x	x			
Sandpiper, Marsh	<i>Tringa stagnalis</i>	w		x				
Sandpiper, Common	<i>Tringa hypoleucos</i>	w			x		x	x

Greenshank, Common	<i>Tringa nebularia</i>	w	x*	x	x	x*	x	x
Sandpiper, Sharp-tailed	<i>Calidris acuminata</i>	w		x				
Stint, Red-necked	<i>Calidris ruficollis</i>	w		x				
Stilt, Black-winged	<i>Himantopus himantopus</i>	w	x	xx	xx	xx	x	xx
Stilt, Banded	<i>Cladocrhynchus leucocephalus</i>	w		x		xx	x	x
Avocet, Red-necked	<i>Recurvirostris novaehollandiae</i>	w	x	xx	xx	xx	x	xx
Plover, Red-capped	<i>Charadrius ruficapillus</i>	w	x*	xx	x	xx*	x	xx
Dotterel, Black-fronted	<i>Elseyaornis melanops</i>	w		xx			x	x
Dotterel, Red-kneed	<i>Erythronyx cinctus</i>	w		x			x	x
Lapwing, Masked	<i>Vanellus miles</i>	w	x	xx	x	x	x	
Lapwing, Banded	<i>Vanellus tricolor</i>	w		x				
Gull, Silver	<i>Larus novaehollandiae</i>	w	x	xx	xx	xx	x	x
Tern, Gull-billed	<i>Sterna nilotica</i>	pw	x	x		x	x	
Tern, Caspian	<i>Sterna caspia</i>	pw	x	x	x	x	x	
Tern, Whiskered	<i>Chlidonias hybridus</i>	pw	x	xx	xx	x	x	x

¹ from Kingsford & Porter 1994, averages of 12 visits; ² from Timms & McDougall 2002, average of 23 visits; ³ from Timms 2008 and unpublished data, averages of 23 visits; ⁴ from Timms 1997 and unpublished data, averages of 6 visits; ^ code x = < 3/km², xx = 3.1 - 30/km², xxx = 31 - 300/km², average, xxxx = > 301/km², all on average, but note that many figures vary widely between visits (see references above); * some species lumped together, see Kingsford & Porter 1994; # from Kingsford & Porter 1994, code d = ducks and small grebes, h = herbivores, pw = piscivores and large invertebrate feeders and w = small wading birds.

Diversity

As a whole, the Paroo supports about 60 species of waterbirds, with at least 31 of these known to breed in the area (Maher 1991; Maher & Braithwaite 1992; Kingsford et al. 1994). However, species richness on individual salt lakes is lower (Table 2), ranging from 18-43 species on many important lakes (Kingsford & Porter 1994, Timms 2008, Timms 1997 and my unpublished data). On lower salinity (i.e. subsaline, hyposaline) lakes, the dominant species generally are herbivorous Eurasian coot and Black swan, the ducks Pink-eared duck, and Grey teal, and the piscivorous Australian pelican, Little Black and Little Pied cormorants. By contrast, hypersaline lakes are dominated by waders such as Red-necked avocet and stilts (mainly Black-winged), with large waders such as herons, egrets and spoonbills and herbivores such as swans and coots notably absent. Mesosaline lakes like Lake Wyara can have all of these dominants. Other abundant species include Little and Hoary-headed grebes, Freckled duck, Hardhead, Black duck and Australian shoveler mainly in lower salinity lakes, and Silver gulls, terns and small waders such as the Red-capped plover in all saline lakes.

None of these species or others found in saline lakes is restricted to such habitats, though some, such as Pink-eared duck and the Avocet are more common in saline sites (Kingsford et al. 1994). Almost all species in Paroo saline lakes are nomadic throughout the inland (Briggs 1992; Kingsford 1996; Kingsford & Norman 2002) though for

few of the uncommon species, such as the Musk duck and Chestnut teal, the Paroo is at the northern edges of their distribution. Notwithstanding the ubiquity of waterbirds in the inland, Paroo saline lakes, together with associated freshwater lakes, harbor a significant proportion of at least two threatened species — Freckled duck and Blue-billed duck (Kingsford & Porter 1994).

While studies often show an inverse relationship between salinity and bird species richness (Halse et al. 2003), studies in the Paroo have not shown this trend. For example Timms (2008) on the Rockwell Lakes found no correlation between salinity and species richness, possibly because other factors such as habitat heterogeneity overrode the influence of salinity (Timms 2001a). Furthermore, Paroo lists seem to be inflated by rare occurrences of atypical species and by visitors temporarily passing through. Certainly food resources are determined by salinity, with most water plants restricted to < ca. 70 g/l (Porter 2005), fish dying beyond ca 30 g/l in the Paroo (Timms 2008) and invertebrate diversity inversely determined by salinity (Timms 1993). Lower salinity lakes with their resources of aquatic plants, diverse invertebrates and fish, can support wide array of birds (but may not for various reasons—see later), but hypersaline lakes with their limited array of crustaceans, largely brine shrimp and large ostracods, attract mainly avocets, stilts and small waders, with few resources for ducks (and hence occasional records contributing to richness and diversity) and nothing for herbivores and piscivores. Consequently, more intensive field surveys of bird use of lakes in the Paroo might show a decrease in diversity in hypersaline lakes.

Abundance

Salt lakes support greater numbers of waterbirds per unit area than freshwater lakes. In a year-long study at Bloodwood station salt lakes supported more individuals per ha than all other wetland types combined, except for clear freshwaters as they dried (Timms 1997). However, the best example is provided by similar-sized Lakes Wyara and nearby Numalla (Kingsford & Porter 1994). Between 1987 and 1989, Lake Wyara's salinity (actually TDS) varied between 5 and 30 g/l and averaged 19 g/l, while Lake Numalla's waters were always < 2 g/l (Timms 1998b and my unpublished data). In the same period Wyara averaged nearly 4.2×10^4 birds and Numalla 7533 birds (from Table 2, Kingsford & Porter 1994). The difference is explained by greater food resources in Wyara. In Wyara, salt precipitates colloidal clays so that its clear water encourages plant growth. This not only supplies a direct resource to herbivorous birds, but habitat and food for invertebrates (Kingsford & Porter 1994). Zooplankton was six times more abundant in Wyara than in Numalla, and aerial invertebrates caught on sticky traps were seven to eight times more abundant in Wyara (Kingsford & Porter 1994).

Among the various salinity types, hyposaline waters often support the most birds followed by mesosaline waters and finally by hypersaline sites. This phenomenon is often seen in the same lake as it changes salinity, e.g. in Lake Horseshoe bird numbers plummeted from 3100 to 465 as salinity increased from 40 g/l to 110 g/l in four months. On the other hand, in the Rockwell lakes there was no correlation between bird species richness or numbers with lake salinity (Timms 2008). This may be due to unquantified fluctuating habitat heterogeneity overriding any salinity effect in these lakes (see earlier), or to loafing birds, which were not distinguished from feeding birds. Freshwater lakes such as Numalla naturally salinize as they dry (Timms 2007) and in doing so attract more waterbirds (Kingsford & Porter 1999). This may not be related to salinity per se, as field observations suggest most of these extra birds are loafing rather than feeding and using such lakes because other sites had dried. This was certainly the case in the Bloodwood study, where numbers in drying and salinizing freshwater lakes increased from ca 6 to 127 ha^{-1} as the other sites dried, with most of the extra birds apparently loafing rather than feeding (Timms 1997).

At times saline lakes support huge numbers of waterbirds. Lakes Wyara (and Numalla) had about 2.8×10^5 (ca 42 ha^{-1}) birds in March 1988 when Wyara was hyposaline (Kingsford & Porter 1994) and even hypersaline Lake Nichebulka has supported more than 1.10×10^4 (ca 33 ha^{-1})

birds (Kingsford et al. 1994). The Lower Bells Creek Lakes (Lower Bell, Mid Bell and Gidgee) have been known to have 1.3×10^4 birds (ca 34 ha^{-1}) (Kingsford 1995). Maximum numbers in the Rockwell Lakes have reached 1.3×10^4 (33.5 ha^{-1}) birds in Lake Bulla and 1.2×10^4 (57.6 ha^{-1}) in Mid Blue Lake (Timms 2008). Lake Altiboulka (area 300 ha) on one occasion had 102.9 birds per ha (Kingsford 1999). However, even larger numbers have been recorded on some larger lakes inland Australia. The Lake Gregory system in northwestern Australia on one occasion had at least 6.5×10^5 birds (Halse et al. 1998), Lake Eyre North (northern SA) 3.25×10^5 , Lake Blanche (northeastern SA) 1.48×10^5 and Lake Galilee (north Qld), 2.54×10^4 (Kingsford 1995). These lakes are all much bigger than the Paroo salinas, but it seems that densities may be greater in the Paroo as none of the above exceed 20 birds ha^{-1} . I suggest the reason for the relatively higher densities in the Paroo, is the small size of its lakes compared to these huge lakes, because small lakes have proportionally more near-shore areas where waterbirds tend to congregate (80% of Wyara's birds occur within 300 m of the shore, Kingsford & Porter 1994).

Fluctuations and Connectivity

Given the episodic nature of the Paroo salinas and their wide changes in salinity when water is present, it is no surprise that bird numbers fluctuate markedly. In Wyara during 1987–89, numbers ranged from < 2600 to > 100000 (Kingsford & Porter 1994). In the Bloodwood study, numbers during 1995 on Gidgee Lake varied between 85 and 3910, and in Horseshoe Lake from 28 to 3050 (Timms 1997 and my unpublished data). Even greater proportional fluctuations have been recorded in the Rockwell lakes, e.g. Lake Bulla (22 to 12850; Timms 2008) and on Lake Altiboulka (517 to 38686; Kingsford 1999). Fluctuations in Lake Gregory have been explained in terms of the amount of water in the lake, the amount of water elsewhere, the taxonomic range of the available food items, and the extent of trees and shrubs inundated (Halse et al. 1998). The latter factor is largely inapplicable to treeless Paroo lakes, but the others are important. The effect of the amount of water and its salinity in Horseshoe Lake has already been noted, and taxonomic range of food available has been noted in Lake Wyara's case (Kingsford & Porter 1994). A specific example is provided by the relationship between the numbers of Pink-eared duck and its food, the cladoceran *Daphnia* (*Daphniopsis*) *queenslandensis* in Gidgee Lake (Timms 1997). Finally, low numbers in the Lake Yumberarra and the Rockwell lakes in 2000 are thought to be associated with better conditions in the Lake Eyre Basin at the time (Timms 2008).

These fluctuations in bird numbers are enabled by the birds of the inland being mobile and nomadic (Lawler & Briggs 1991; Briggs 1992; Kingsford & Norman 2002). Exactly what guides them to come and go is unknown, but in effect it means that episodic fillings of remote lakes are generally colonized and their resources harvested by one species or another. When a wetland system dries and enters a 'bust' period, their waterbirds either move to a system in 'boom,' or go to the coast, or occasionally crash catastrophically (Kingsford et al. 1999). Because inland wetlands are episodic, some lakes are utilized only occasionally (Kingsford 1996).

In these movements between lakes, there is full connectivity for waterbirds between fresh and saline waters locally and continentally (Kingsford & Porter 1994; Kingsford et al. 1999). Avian diversity is not much different between the fresh and saline wetlands, though, as seen above, saline sites usually have more individuals because of their greater food resources. No species in the Paroo is restricted to saline waters, but many species, mainly large waders, are rarely seen in salt lakes (see Table 2). This means that bird populations on salt lakes should be considered as an integral part of the population of the entire region, with easy interchange between various water types.

Not only must waterbirds on saline lakes of an area be considered part of the whole population of that area, strong nomadism means that all inland wetland areas of the continent are connected by interchange of populations. Usually there is at least one wetland system in arid Australia with abundant water (Roshier et al. 2002), so waterbirds congregate there. Should saline sites be part of that system, then birds will spread to them. Paroo's waterbirds, including those on the saline wetlands, could be from anywhere in inland Australia, and indeed could move onto any wetland system, fresh or saline.

BREEDING

Most Australian waterbirds do not breed in saline waters (Goodsell 1990; Halse et al. 1993). Of the 31 species known to breed in the Paroo, nine breed in the saline wetlands (Maher 1991), though many utilize the food resources of saline lakes in the process, mainly in the building of body reserves before breeding (Kingsford et al. 1999). It is not known what factors contribute to this avoidance of saline lakes for breeding, but the lack of trees could be adverse for many ducks, cormorants, and large waders. When fringing trees are partially drowned cormorants are known to breed in Lake Gregory (Halse et al. 1998) and also in Lake Wyara (author, unpublished data). Briggs (1992) has noted that breeding is more

common on long lasting wetlands of any type. Given that most saline wetlands are shallow and ephemeral, this may contribute to their avoidance, though it should be noted that in wet La Nina years some salinas such as Lake Gidgee persist for 2 -3 years (Timms 2007) and little breeding occurs. Perhaps the young dehydrate more than the adults (Hannan et al. 2003). Few salt lakes, and most fresh waters for that matter, have persistent islands which provide safe sites for ground-nesting species. In this respect the islands of Lake Wyara are of paramount importance. The most common breeders in Paroo's salinas are Black swans, as well as in some freshwater lakes throughout the Paroo (Kingsford et al. 1994). Considered separately, Paroo lakes are not significant sites on a continental scale, but as a group, Paroo lakes are important breeding sites. Swans have been recorded breeding in Lake Wyara (Kingsford & Porter 1994), Gidgee and Lower Bell Lakes on Bloodwood lakes (Timms 1997) and in Lake Bulla and the Blue Lakes on Rockwell (Timms 2008) as well as in Lake Burkanoko, Mere and Taylors Lakes in the middle Paroo (author, unpublished data). All of these sites were hyposaline or mesosaline at the time and grew abundant aquatic plants, mainly *Chara*, *Lepilaena* and *Ruppia* spp. Other breeding species include Australian pelicans, Little Pied cormorants, Red-necked avocets, Black-winged stilts, Red-capped plovers, Silver gulls, Whiskered terns and Caspian terns. Most of these breed on islands in Lake Wyara (Kingsford & Porter 1994; Timms 1998b). One such island supported ca 1500 nests of Australian pelicans during 1987-1989 and they bred again in 1990-91, 1995 and 1998-2001 (R.T. Kingsford, pers. comm.; A. McDougall, pers. comm.). These pelicans feed mostly in the freshwater Lake Numalla (and hence are an exception to the general movement of resources from saline to fresh waters for breeding), though they and cormorants fish in Wyara till rising salinity kills its fish. Although smaller than the huge colonies of Australian pelicans on Lakes Eyre and Cawndilla, this is an important breeding site as it is more regularly used (Kingsford & Porter 1994). These islands also provide the only breeding site in the Paroo for Silver gulls and Caspian terns. They also are used occasionally by Little Pied cormorants, Red-necked avocets, Red-capped plovers and Whiskered terns.

Breeding is directed by rainfall and river flows (Kingsford & Norman 2002). Black swans are usually the first to breed after a lake fills, because their food (aquatic plants) recolonizes before fish do (Kingsford et al. 1999). As an example, in Lake Wyara the number of nests of swans peaked July to September in 1988 and 1989, and most broods were recorded in September of each of these years, whereas piscivorous pelicans only formed their breeding colony in December of 1988 and their activity peaked in March 1989 (Kingsford & Porter 1994).

CONSERVATION

The saline lakes of the Paroo lie in a remote part of Australia with a low human population and few visitors. Generally the lakes are in pristine condition (Timms 2001b), and common problems elsewhere in Australia such as salinization, permanent flooding and eutrophication are of little concern (Briggs 1994; Kingsford & Norman 2002; Timms 2005). Given the permanent ban on hunting waterfowl in NSW, hardly any birds are shot or are disturbed by hunters. Minor problems in the Paroo include wallowing by feral pigs along shores and hence enhancing sedimentation and perhaps preying on young shorebirds. Furthermore, some of the lower salinity lakes have introduced fish (carp, Mosquito fish) and while they may adversely affect invertebrates, it is possible their presence is advantageous to piscivores. On the other hand, accelerated sedimentation and diversion of water for desert irrigation pose significant threats to these lakes (Kingsford 2000).

Considering sedimentation, Gidgee Lake has lost ca. 50% of its capacity due to recent siltation, meaning it probably holds water for shorter periods after filling (Timms 2007). The islands of Lake Wyara face connection to the coastline because of sedimentation at the mouths of Benangra and Youlainge Creeks, thus potentially giving access to foxes and cats to prey of the island rookeries (Timms 2001b). Despite this lake being a Ramsar site, no permanent solution to this problem is in sight, but temporary protection is provided by a baiting program against foxes. Of greatest concern, however, is the potential of water harvesting for desert irrigation. Though this does not affect Paroo saline waters directly because they are not filled by riverine water, its freshwater lakes could be deprived of water resulting in catastrophic reduction of waterbirds, which by connectivity would pass onto the saline wetlands. Fortunately for the Paroo, there is now an intergovernmental agreement between Queensland and New South Wales not to develop this last free-flowing tributary of the Murray Darling system (NSW National Parks & Wildlife Service 2003). However, with greater connectivity, any water withdrawals affecting other systems (e.g. Lake Eyre basin) could impact on the Paroo and on the birds of all inland wetlands.

CONCLUSIONS

The species composition of waterbirds of Paroo saline lakes is little different from those in nearby freshwater lakes, and indeed from water bodies across the vast inland of Australia. There is a suggestion of an inverse relationship between species richness and salinity, but certainly low salinity waters are more productive than most other fresh waters, so that abundances are often great in hyposaline/mesosaline lakes, including many in the Paroo.

However, populations fluctuate greatly according to ecological state of the lakes, and their nomadic movements between lakes and wetland regions. Some birds breed in Paroo salinas, but not on a significant continental scale, except perhaps Australian pelicans. Paroo salinas (and freshwater wetlands) are almost pristine, but some are adversely affected by accelerated sedimentation and threatened by water diversion for desert irrigation. The latter is particularly pernicious since the wetland habitats in other regions are similarly threatened.

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Oxygen Production and Use in Benthic Mats of Solar Salt Ponds

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ABSTRACT

The benthic mat in the ponds of solar salt producers is important because as a beneficial effect, the mat reduces loss of brine from the field, but it unfortunately also supports species which can have a serious detrimental effect on the halite crystallisation process. Anaerobic and aerobic activity of the mat which is thought to be a significant factor in the management of the salt field is not quantified by traditional monitoring methods. A method of measuring the generation of oxygen from benthic algal mats, tested in the north west of Western Australia at three solar salt fields has been developed to estimate the benthic primary production in solar salt fields. Net oxygen production peaks at approximately 1 g m^{-2} over a 24 hour period for saltfields in the north-west of Western Australia. There was a significant linear relationship between production and salinity with production decreasing with increasing salinity. Maximum production was $100 \text{ mmol O}_2 \text{ m}^{-2} \text{ day}^{-1}$ and the minimum was $-11 \text{ mmol O}_2 \text{ m}^{-2} \text{ day}^{-1}$. The average oxygen production in ponds with a normal salinity within the range of $115\text{-}250 \text{ g l}^{-1}$ was $13 \text{ mmol O}_2 \text{ m}^{-2} \text{ day}^{-1}$. The relationship between dissolved oxygen demand at night versus salinity was not significant.

INTRODUCTION

A solar salt field in Australia is typically made up of a series of large ponded areas up to 5000 ha in size, and totalling between nine and twelve thousand hectares. Seawater is pumped into the initial pond and the brine evaporates as it flows or is pumped into successive ponds. The process is carefully controlled so that the pond salinities are kept as constant as possible given meteorological conditions. Salinity at a particular point in a pond is normally controlled to within $\pm 5 \text{ g l}^{-1}$. As a result, the series of ponds is a constant flow system with ponds maintaining a stable hypersaline environment. At roughly five times sea water, gypsum starts to precipitate. At about ten times seawater concentration, sodium chloride (halite) begins to precipitate and the brine is then passed to specialized ponds that have been levelled and engineered to facilitate mechanical harvesting of the salt. The former ponds are normally called concentrators or condensers and

the latter crystallizers. At times this stable system is impacted by episodic events such as cyclones and monsoon rainfall which may reduce the salinity by 100 g l^{-1} .

Biological features in the series of ponds change as water is evaporated from the brine. Different plants and animals predominate according to salinity, temperature and nutritional variation (Figure 1). The most important biological factor in a salt field is the potential for the cyanobacterium *Synechococcus* (*Aphanothece*) to produce mucilage in large enough quantities to degrade the salt. It has been noted that the mucilage is produced at times of environmental disturbance. This mucilage changes the viscosity of the brine interfering with the formation of salt crystals, resulting in fragile crystals and also causing fine salt crystals to form on the surface of the brine (Coleman & White 1993; Roux 1996). These fine and fragile crystals are difficult and costly to harvest, and retain impurities in the final product. Biological attributes also benefit the production of salt by removing soluble metals, nutrients and total suspended solids from the brine and depositing them into the sediments. A saltfield with the biology in equilibrium will produce good-sized solid crystals with low impurities, exploiting evaporation to its greatest potential.

Benthic algae are very important in the stabilization of the pond sediments and in reducing seepage; however they also provide the greatest biomass of the detrimental cyanobacterium *Synechococcus* which is normally found in large numbers in the immediate pre- and post-gypsum ponds. The interaction between the benthic algal mat and overlying brine is difficult to study *in situ*, and more particularly to monitor on a regular repeatable basis. Salt field technologists have traditionally focused on the planktonic biology as it has been difficult to monitor the large biological biomass within the mat. One method of understanding the mat activity is to study the use and production of oxygen as a measure of its productivity. During sunlight hours the cyanobacteria use sunlight as an energy source to produce carbohydrates by which CO_2 is consumed and O_2 is produced. These same organisms use O_2 in their metabolism but the production in the day time far exceeds their use of O_2 (Figure 2).

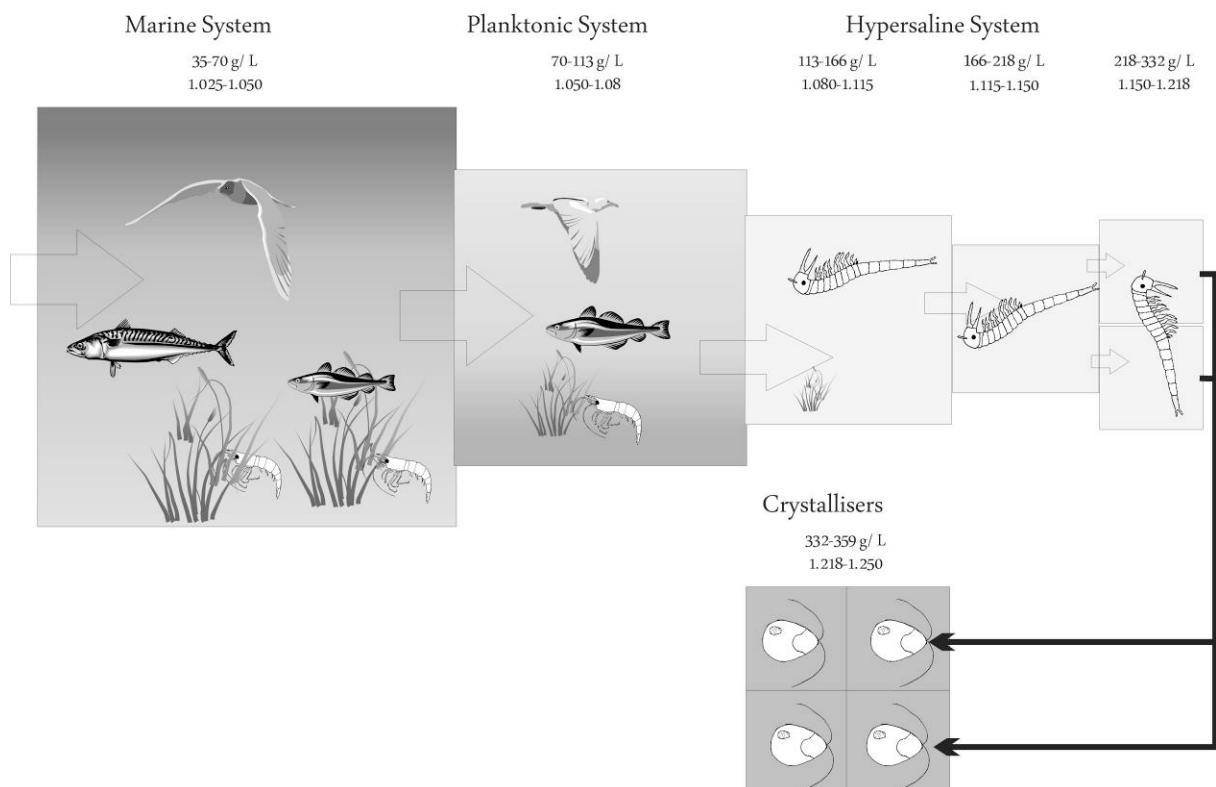


Figure 1–Biology of a salt field.

A matrix of possible changes has been compiled and presented in Table 1. These are not meant to be definitive and all dissolved oxygen (DO_2) production and use scenarios must be interpreted within the context of local events. It does give an understanding of what might be expected if all other factors were constant. The aim of the current work is to understand what changes can be measured in the field, and if those changes are real, and what conditions are likely to impact on the production of salt.

METHODS

Introduction

A technique was devised to study dissolved oxygen changes in situ. In situ studies provide more accurate estimates of changes in the field for management of a commercial field. For this purpose a technique was devised to isolate algal mats in the ponds of commercial saltfields. This technique is similar to that used by Segal et al. (2006) and others. The equipment used was a Perspex dome pressed into the benthic mat, with automatic physical chemistry probes measuring conductivity, DO_2 , temperature, pH and light intensity in the brine above the benthic mat enclosed by the dome. This technique isolates a portion of benthic mat and its composite biology under the mat's 'normal' conditions of light, temperature and salinity.

The DO_2 method has the additional advantage of estimating carbon sequestration in the ponds which is becoming of great interest as the concept of 'Carbon Credits' is introduced into Australia. In all but exceptional circumstances carbon would be sequestered on a mol by mol ratio with O_2 produced.

The dome technique (as with most sampling) is limited because:

1. Small areas are used to extrapolate over the entire pond.
2. It assumes the mat to be homogenous over the entire pond and sampling locations.
3. Isolation of an area is critical to monitoring changes but that in itself introduces changes.

LOCATION

The surveys were completed at three tropical solar salt fields in north-west Australia (Figure 3). The salt fields have an operational concentrator area of approximately 100 km^2 . The sampling sites were located in ponds which were normally within the operational salinity range of 115 to 215 g l^{-1} . Rainfall had sometimes temporarily reduced the salinity at the time of survey. Port Hedland and Onslow have three ponds within the above range while Dampier has four such ponds.

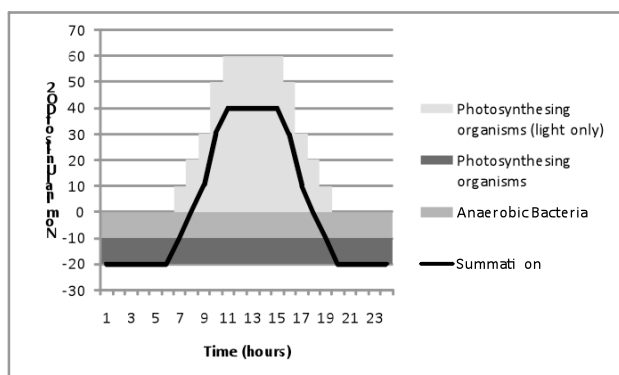
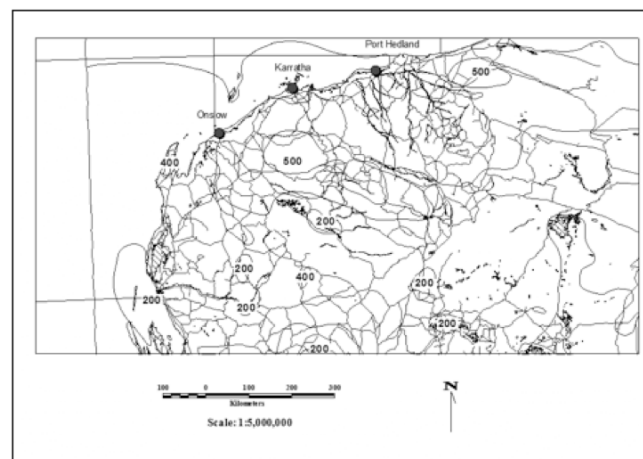
Table 1—Matrix of changes to varying oxygen production scenarios.

	O ₂ production increased	O ₂ production static	O ₂ production decreased
O ₂ use increased	Unsure if only cyanobacteria increased but likely all biological activity greater	Anaerobic activity greater	Anaerobic activity greater with reduced cyanobacteria activity
O ₂ use static	Likely that cyanobacteria biomass greater	No change	Cyanobacteria biomass reduced
O ₂ use reduced	Cyanobacteria biomass greater	Reduced biological activity	Reduced biological activity generally

Dome Construction

The Perspex domes were 700 mm in diameter with an approximate rise of 200 mm to the top of the dome. Each dome had a 100 mm PVC skirt that dropped from the rim of the dome. The dome also had several ports: a large port at the apex of the dome to allow air to be removed, a large port at roughly 45° to insert the probe and two small ports lower on the dome profile for recirculating brine within the dome, by a small pump. All ports could be sealed for water tightness. The Perspex dome was then placed in a stainless steel base that protected it and enabled the dome to be fixed to the substrate with iron stakes through four channels welded to the base. The assembled dome can be seen in Figure 4. The area of the dome was 0.385 m², and its volume was 0.0725 m³.

The loggers for the domes were Tyco CS304 loggers with multiple probes for temperature, pH, DO₂ and conductivity. An Odyssey PAR light meter was attached to each dome. The light meters and loggers were calibrated by Murdoch University Marine and Fresh Water Research Laboratory. Care was taken when assembling and placing the domes that the light meters and domes were not shaded by infrastructure or sediment disturbed on installation of the domes. A 12V pump was used to circulate the brine within the dome.

**Figure 2**—Hypothetical production and use of oxygen in the mat structure.**Figure 3**—North-west Australia showing field locations (contours of annual rainfall mm isohyets).

ANALYSIS OF DATA COLLECTED

The procedure for the analysis of data collected from the field is as follows. The data were downloaded from the logger and analysed at the close of each trial. Percent saturation was converted to DO₂ concentration using the following equation, derived by the author in previous unpublished work:

$$\text{DO}_2 \text{ at saturation (ppm)} = 10.79 - 0.113 \times \text{Temperature (}^{\circ}\text{C)} - 0.032 \times \text{Salinity (g l}^{-1}\text{)}$$

Ambient light was calibrated and expressed as accumulative incident light (PAR). Finally daytime *in situ* DO₂ versus the ambient accumulative light relationship was calculated and the slope of the linear regression determined. The slope of the curve gave the rate of DO₂ change per unit of light, an indication of the photosynthetic activity of the algae.

The DO₂ demand of the benthos at night was expressed as change of DO₂ per unit time. There was a slight discrepancy due to plankton activity in the entrapped brine within the dome, but as the majority of the biomass is within the benthic mat in these ponds it has been ignored as trivial. Excess oxygen during the day time was collected in an air trap at the top of the dome. This was measured and compared with the calculated production/respiration rates, but was not used other than as a check. The statistical analysis was completed using Mintab 14.



Figure 4—Assembled dome and probe/logger ready for use.

RESULTS

The production of DO_2 in the daytime and its use in the dark (night) for all sites at the three locations is shown in Figure 5 and Figure 6 respectively. The net use of oxygen per day is presented in Figure 7. The results are an accumulation of surveys over three years at a frequency of twice a year for each site. The results have been filtered to remove data sets used to evaluate unusual benthic locations in the ponds such as localized exposed sulfide mud and spring holes.

The highest production of oxygen for all sites in a 24 hour period peaked at approximately $1 \text{ g of O}_2 \text{ m}^{-2} \text{ day}^{-1}$. The average was much lower at approximately $0.2 \text{ g m}^{-2} \text{ day}^{-1}$. The maximum production was $100 \text{ mmol O}_2 \text{ m}^{-2} \text{ day}^{-1}$ recorded at a salinity of 125 g l^{-1} and the minimum was $-11 \text{ mmol O}_2 \text{ m}^{-2} \text{ day}^{-1}$ recorded at a salinity of 68 g l^{-1} in a pond that was normally at a salinity of 115 g l^{-1} . The average production in ponds with a normal operating salinity, each at a point in the range of $115\text{--}250 \text{ g l}^{-1}$, was $13 \text{ mmol O}_2 \text{ m}^{-2} \text{ day}^{-1}$.

DISCUSSION

There have been a number of papers (Canfield et al. 2004; Wieland et al. 2004; Wieland & Kühl 2005) on the productivity of benthic mats in saltfields in the salinity range between $100\text{--}200 \text{ g l}^{-1}$. Many found that with increasing salinity benthic primary productivity decreased while respiration remained relatively constant. Levels of DO_2 production found in this study are comparable to those found by Canfield et al. (2004).

It has also been suggested (Coleman & White 1993; Canfield et al. 2004) that cyanobacteria habituated to a constant salinity within this range will increase productivity if the salinity is decreased. It is thought that most species within this range are halotolerant and not truly halophilic and organisms typically have an optimum salinity less than found in the in a salt field.

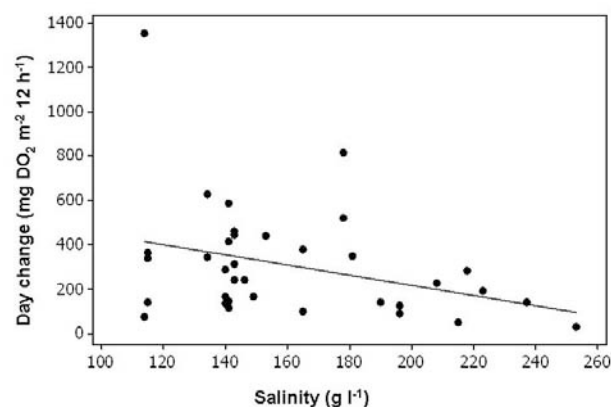


Figure 5—Dark period DO_2 flux.

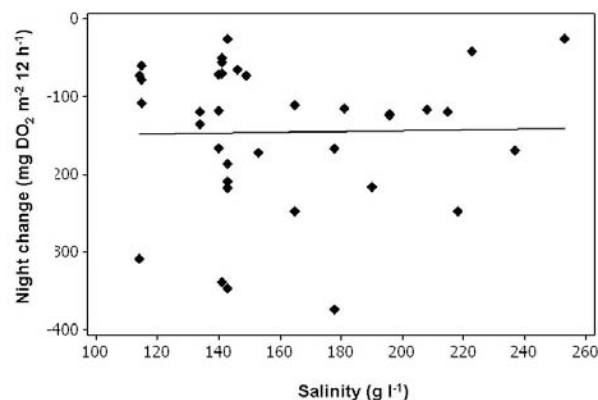


Figure 6—Light period DO_2 flux.

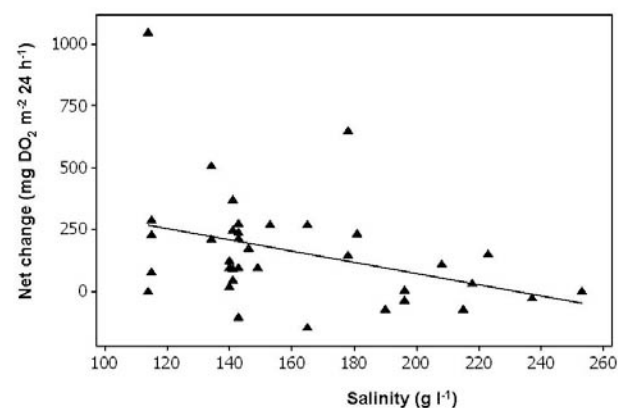


Figure 7—Net daily DO_2 flux.

Regression analysis of daytime production versus salinity showed that there was a significant linear relationship between production and salinity ($P = 0.045$) with production decreasing with increasing salinity (Figure 5). However the R^2 value was small (0.12), indicating that salinity is not a major factor in production. The relationship between DO_2 demand at night versus salinity as shown in Figure 6 was not significant at a probability of 0.91. Net production over a 24 hour period versus salinity analysis (Figure 7) showed that net production decreased with salinity at a significance of $P = 0.026$ but a relatively low R^2 of 0.14. This is consistent with the literature cited above.

The data were sorted under individual ponds normally maintained at targeted salinities. At times salinity of the brine in the pond was reduced due to large rain events. The purpose of this analysis was to determine if benthic mats that are normally maintained at a constant salinity would increase production of oxygen at times of radical salinity decrease. When the relationship between salinity and production/use was tested for individual ponds, the probability was much lower with most (five out the eight sites) having no significant relationship between oxygen production and salinity (see Table 3). This indicates that although there were significant relationships between DO_2 production and static salinity the relationship between DO_2 production and temporary reductions in salinity was much weaker.

Table 2—Probability (ANOVA) of a relationship between changing DO_2 and salinity.

Pond	Light	Dark	Net
D Pond 1A	NS	NS	NS
D Pond 1B	0.026		NS
D Pond 2	NS	NS	NS
O Pond 2	NS	NS	NS
O Pond 3	0.028	NS	0.036
P Pond 4	NS	NS	NS
P Pond 5	NS	NS	0.017
P Pond 6	0.019	NS	NS
D=Dampier; O=Onslow; P=Port Hedland; NS=not significant			

This was somewhat surprising as it did not fit the observed bloom of algae in the ponds after rain. One possible explanation is that there is often a significant time lag between rain events and sampling. Ideally the mat should be sampled immediately before a rain event and immediately after an event but access to the ponds immediately after rain is restricted for safety reasons. The

current program may have delays of up to three months after rain before the ponds are surveyed so the mats may have acclimated to the new salinity regime.

Most of the sites were net DO_2 producers. Under normal circumstances the generation of one mol of O_2 would signify that one mol of C was fixed. This has important implications for the commercial fields under the new 'carbon credit' scenario proposed by the Australian Government as most ponds were net producers of O_2 . Anecdotal evidence points to significant amounts of organic matter being deposited in the ponds over time.

In terms of biological management of salt fields the methodology is still being developed, and at this stage, still needs to demonstrate its use as a monitoring tool. The difficulty is that a biological disturbance is not a common occurrence and until one has been recorded it is difficult to know if this methodology will accurately predict such an event by the changes to the benthic activity. It is possible to state that if a biological event is heralded by a change in benthic mat activity over a period of months then this method would adequately quantify those changes. The method is adequate for quantifying the oxygen cycle of the benthic mat and a measure of carbon sequestration for the ponds.

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The Effect of Salt on Germination of Samphire Species

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ABSTRACT

Nine *Halosarcia** plant species from Lake Carey, Western Australia, were tested to determine the effect of salt on seed germination. These species were *Halosarcia* 'Angel Fish Island' (B. Davey 4)** *Halosarcia calyptrata* Paul G. Wilson, *Halosarcia halocnemoides* (Nees) Paul G. Wilson, *Halosarcia halocnemoides* (Nees) Paul G. Wilson subsp. *caudata* Paul G. Wilson, *Halosarcia indica* (Willd.) Paul G. Wilson subsp. *bidens* (Nees) Paul G. Wilson, *Halosarcia peltata* Paul G. Wilson, *Halosarcia pergranulata* (J. M. Black) Paul G. Wilson, *Halosarcia pruinosa* (Paulsen) Paul G. Wilson and *Halosarcia undulata* Paul G. Wilson. All species were subjected to salt concentrations of 0, 10, 20 and 30 g/l NaCl both in the laboratory in Petri dishes and under outdoor conditions, either buried or on the surface of soil from the lake margin. The nine species were also tested for their ability to recover after exposure to high NaCl concentrations. To test the ability to recover, the seeds were germinated in fresh water. For the majority of species, increased NaCl concentrations resulted in decreased germination percentages both in the laboratory and outdoors. In contrast, *H. halocnemoides* showed a slight increase in germination percentages at higher NaCl concentrations. In laboratory trials, the greatest reduction in germination percentage was 81% for *H. 'Angel Fish Island'*; the average reduction was 37% across all species. In outdoor experiments, germination only reached a maximum of 25%, which could have been due to lack of moisture, scarification of seeds, or temperature. Averaged across all the species, there was an increase of 58% in germination of the remaining seeds once salt was removed by flushing with fresh water.

INTRODUCTION

The effect of the saline environment can be the dominant factor that determines the ability of halophytes to reproduce and perpetuate their existence (Waisel 1972), whilst also influencing the zonation and inhabitation of samphire species. Salty environments can prevent the uptake of water by the seed due to the high osmotic potential of the medium, or the embryo may become poisoned due to the toxic effects of certain ions (Waisel 1972). Germination

inhibition is proportional to the external osmotic potential, which occurs at high salt concentrations (Waisel 1972).

Samphires belong to the family Chenopodiaceae and represent the group of succulent shrubs, which include the genus *Halosarcia*. These plants are generally associated with saline environments such as salt lakes and pans, salt marshes and samphire flats with the availability of water governing their life cycles (Datson 2002), hence samphires have adapted to survive in different zones of saline wetlands. Samphire seeds along with other flora and fauna of these saline environments only have a small window of opportunity to reproduce and germinate. Previous experiments have determined that seed germination is reduced at high NaCl concentrations for some *Halosarcia* species but that germination can increase once salt is flushed from the seeds (Barrett 2000). Malcolm (1964) found temperature was a vital factor for germination, with the highest germination recorded at a fluctuation of 5-35°C. However very little information is available on germination for the majority of *Halosarcia* species, except for *Halosarcia pergranulata*, which has been studied for use in revegetation programs. Barrett 2000 conducted germination testing and found that removing a small portion of the testa was beneficial for germination of *Halosarcia pergranulata*.

In Lake Carey, located in the eastern Goldfields region of Western Australia, saline groundwater is being released onto the lake playa on a continual basis. The groundwater is abstracted from a number of mine sites to ensure safe and dry working conditions. The volume of water is not enough to alter water levels of the lake but concerns have been raised that this flush of hypersaline groundwater is increasing the concentrations of salt within the soil and preventing germination of samphire species. We have observed that samphire species at Lake Carey germinate when rain flushes salt from the surface soil.

[*Since this paper was written the genus *Halosarcia* has been renamed *Tecticornia* after genetic testing (Shepherd & Wilson 2007). The species names remain the same. **Taxonomic synonym of *Tecticornia mellaria* (K.A. Sheph.).]

We conducted germination trials to establish the effect of salt on the germination of nine salt tolerant species from the genus *Halosarcia* present at Lake Carey. The results will determine whether the continued release of saline groundwater onto lake playas from surrounding mine sites has a detrimental affect on samphire germination. This trial was carried out within two environments to gain a more thorough understanding of expected germination rates. The laboratory trial was carried out within a controlled environment to observe germination rates absent of variables such as temperature, whilst the nursery trials provided a more realistic environment that would be expected within the natural habitat of the samphires.

MATERIALS AND METHODS

Seed Collection and Viability Testing

Trials were carried out in the laboratory trials for four weeks and six weeks for nursery trials, from September to November 2007. Seeds from nine species of *Halosarcia* (Table 1) were collected in October 2006 from Lake Carey and cleaned to remove any foreign material. Cleaning methods included hand rubbing over a screen, putting seed through a de-huller screen and seed air separator. *H. pergranulata* was scarified to increase germination (Barrett 2000). Seeds were subjected to salt concentrations of 0, 10, 20 and 30 g/l NaCl in either tap water (outdoor settings) or deionized water (laboratory trials). The upper saline concentration of 30 g/l NaCl was chosen because previous experiments by Barrett (2000), and English et al. (2002) demonstrated that germination success for *Halosarcia pergranulata* decreased above this concentration. To reduce the risk of contamination all seeds were surface sterilized by soaking for ten minutes in a 1% solution of sodium hypochlorite followed by rinsing with deionized water (Sauer 1986). This is a standard procedure also reflected within the International Rules for Seed

Testing (ISTA 1985). However, sodium hypochlorite treatment did not prevent mold, particularly at salinities of 20 and 30%.

Seed viability was assessed using the tetrazolium (TZ) salt biochemical test (Barrett 2000). Two replicates of 20 seeds were randomly selected and cut using a scalpel and placed in the TZ solution for 24 hours. Replicates of 20 seeds were used to represent the sample size of the replicates in the trials. After 24 hours the seeds were removed from the TZ solution and the embryos checked for pink staining under a microscope. The average of the two replicates was recorded as percentage viable.

Laboratory Trial

Five replicates of 20 seeds from each species were placed on filter paper (7 layers of Whatman No 1 filter paper) within Petri dishes. The treatment solution was added to each dish to ensure saturation of the filter paper without runoff. All dishes were secured in plastic bags and randomly positioned in a controlled temperature room at 20-25°C to represent the optimum temperature for germination (Shepherd 2007). Numbers of germinated seeds were recorded at weekly intervals for four weeks and removed together with contaminated seed. Contaminated seeds were those subject to mold, and were removed to avoid cross contamination to remaining healthy seeds. Saline water representing each concentration was added as required to maintain moisture using a dropper to avoid over saturation and seed movement. The seeds were scored as having germinated when the radicle emerged from the testa. The germination success per trial was expressed as a percentage of clean seed used in the trial.

Table 1—*Halosarcia* species, preferred habitat and viability (Datson 2002).

Species	Code	Habitat	Viability
<i>Halosarcia</i> 'Angelfish island'	HA	Well-drained saline soils. Never found in wet run-off areas preferring the dunes beside the lake.	80.0%
<i>Halosarcia calypttrata</i>	HC	Slightly higher rises in wet saline areas with good drainage.	65.0%
<i>Halosarcia halocnemoides</i>	HH	Wettest and most saline parts of the wetland.	22.5%
<i>Halosarcia halocnemoides</i> subsp. <i>caudata</i>	HHC	Wettest and most saline parts of the beach profile.	32.5%
<i>Halosarcia indica</i> subsp. <i>bidens</i>	HIS	Well drained saline soils at upper margins of lake.	40.0%
<i>Halosarcia peltata</i>	HPT	Lowest part of wetland in wet areas, run off areas and gutters. Seems to tolerate high salinity.	55.0%
<i>Halosarcia pergranulata</i>	HP	Lower parts of the wetland in wet areas that are not necessarily very saline.	60.0%
<i>Halosarcia pruinosa</i>	HPR	From lake playa through beach profile and into wetter depressions in dunes. Tolerates high salinity, water logging and dry conditions.	65.0%
<i>Halosarcia undulata</i>	HU	Lower part of wetland.	67.5%

Outdoor Trial

To assess the effects of salinity within a more realistic environment representative of natural habitats, seeds were germinated on nursery benches. To prevent rain or watering from diluting the applied salinity concentrations, two nursery benches were covered in plastic with seedling trays placed underneath to represent a hot house. The plastic covering was adjusted to ensure that the maximum temperature did not go beyond 35°C as previous studies have shown this as the optimal temperature for similar samphires and members of the Chenopodiaceae family (Malcolm 1964). This was achieved by adjusting the amount of air flow through the hot house. Seeds were germinated in soil collected from Lake Carey, which is silty clay with gypsum crystals (Coleman 2003). Salt was leached from the soil prior to the addition of seeds by saturating the soil in a wheelbarrow until the salt content was reduced to 0.6 parts per thousand (ppt) which was determined using an Electrical Conductivity (EC) meter (Hanna Combo pH & EC meter). The trays were lined with mesh to avoid loss of soil. The nursery trials were two weeks longer than the laboratory trials, as it took longer for initial germination. Most of the nursery plots formed a salt crust during the trial due to evaporative wicking.

Three replicates of 40 seeds from each species were placed into slide holders on filter paper and secured with a polypropylene fleece ('Plant Cover'—PP Non Woven Fleece (17 g/m²) to prevent seeds from being lost. Ten seeds were placed in each slide holder with two holders buried in the soil and two placed on top of the soil to establish if light also affected germination. Seeds were watered with saline water representing each concentration on a regular basis with watering increasing over time as required. Watering began at weekly intervals and then increased to every second day for two weeks, followed by daily watering for two weeks to maintain soil moisture. Number of seeds germinating were recorded on a weekly basis for six weeks and removed. Mean germination was calculated by dividing the total number of germinants per concentration by the number of clean (non-contaminated) seeds.

After six weeks the seed trays were watered three times a day with fresh water to flush the salt and test the ability of all seeds to recover for a further two weeks. All germinants were counted at the end of this time and the average calculated by dividing the number of NaCl concentrations (4) by total mean germination.

$$\frac{\sum x^1}{\sum c^1} = y$$

where x^1 = total mean germination
 c^1 = number of concentrations

Statistical Analyses

The statistical package Minitab 14 was used to analyze the data. The data was not transformed because not all species had the same response to salinity of the irrigation water and a significant relationship was achieved without transformation for all of the laboratory trials. For the laboratory trials the entire data set was examined for interaction between species and salinity using an ANOVA two-way test. For individual species a regression analysis was completed between proportion of seeds germinated against salinity with only the probability and R^2 being reported. The main issue with the nursery trials was the low germination. An ANOVA analysis of the nursery data was not done because of the large number of zero scores. Instead, these data were analysed only with regression analysis as described above.

RESULTS

Viability Trials

Five of the nine species had a seed viability of 60% or greater. *Halosarcia* 'Angel Fish Island' had the highest germination at 85% and *H. halocnemoides* was the species with the lowest seed viability of 22.5%.

Laboratory Trials

With increasing NaCl concentrations there was a marked decrease in germination for all species except *H. halocnemoides*, which increased by 11% at higher NaCl concentrations (Figure 2). The majority of species had some germination at all concentrations. However, *H. indica* subsp. *bidens* seeds did not germinate at 20 or 30 g/l NaCl, *H. 'Angelfish Island'* seeds did not germinate at 30 g/l NaCl, and *H. halocnemoides* seeds did not germinate at 0 g/l NaCl. Seeds of *H. 'Angelfish Island'* showed the greatest salt sensitivities; seed germination decreased 81% between 0 and 30 g/l NaCl (Figure 2). *H. calypttrata* seeds showed the greatest salt tolerance with 42% seed germination at 30 g/l NaCl (Figure 2). Of the nine species, *H. pergranulata*, *H. indica* subsp. *bidens* and *H. halocnemoides* had the poorest germination in the laboratory trials.

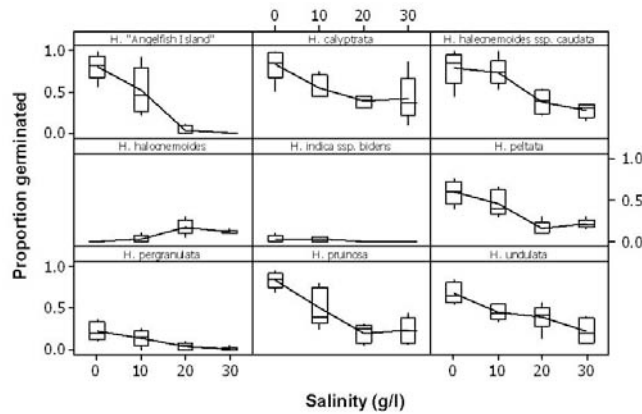


Figure 1—The effects of salinity on seed germination for the 9 study species in the 4 week laboratory trials.

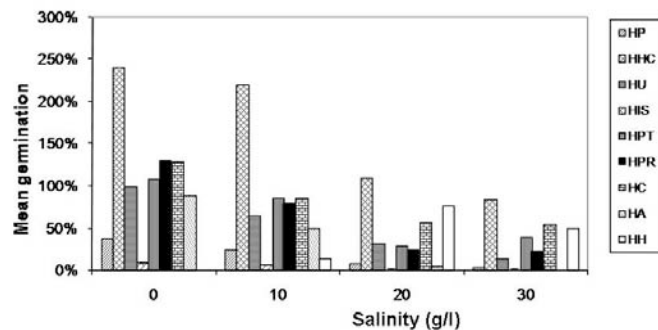


Figure 2—Germination of nine samphire species, expressed as a percentage of seed viability (from Figure 1) for 0, 10, 20 and 30 g/l NaCl concentrations in 4 week long laboratory trials. See text for an explanation of values > 100%. Error bars show standard error.

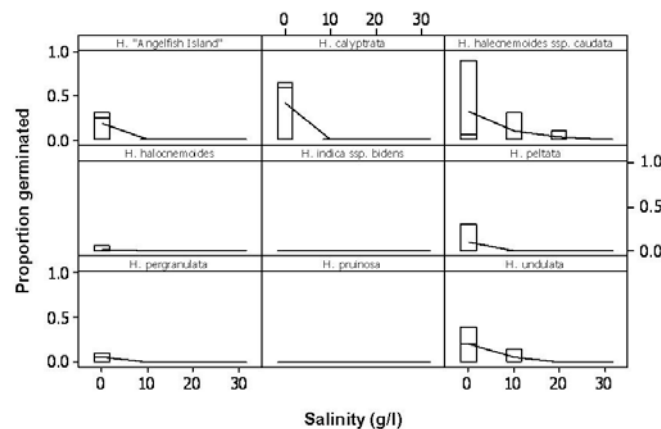


Figure 3—The effects of salinity on seed germination for the 9 study species grown in outdoor trials. The box plot display consists of a rectangular box representing roughly the middle 50% (interquartile range) of the data, and lines ("whiskers") extending to either side indicating the general extent of the data. The median value is shown as a cross bar inside the box and the line connects the mean values.

The Two-way ANOVA analysis of the entire data set showed that there was a significant relationship between salinity and germination, species and germination and an interaction between the factors:

Source	DF	SS	MS	F	P
Species	8	6.6757	0.83447	48.47	0.000
TDS (g/l)	3	3.9852	1.32839	77.17	0.000
Interaction	24	2.6574	0.11072	6.43	0.000
Error	144	2.4789	0.01721		
Total	179	15.7972			

In all cases other than *H. halocnemoides* there was negative linear relationship between germination and salinity of the irrigation water (Table 2). *H. halocnemoides* had the highest proportion of seeds germinate at 20 g/l. *H. indica* subsp. *bidens* had a very poor germination rate at all salinities which may explain the significant probability but a low R^2 of 17%.

Germination was tested against the viability levels of each species (Figure 4). The viability-adjusted germination for *H. halocnemoides* subsp. *caudata* at all concentrations far exceeds that expected with a viability of only 32.5%. Germination of *H. peltata*, *H. pruinosa* and *H. calyptata* also exceed their viability at 0 g/l NaCl. Viability results were therefore defective for some species.

Outdoor Trials

In contrast to the laboratory trials, seed germination in the outdoor trials was very low. Only seeds of two species buried in the soil germinated at 10 and 20 g/l NaCl and no germination occurred at 30 g/l NaCl (Figure 4). There was no germination of the seeds placed on the surface. *H. halocnemoides* subsp. *caudata* and *H. undulata* seeds both germinated at 10 g/l NaCl and *H. halocnemoides* subsp. *caudata* and *H. pruinosa* had 1 to 2 germinants at 20 g/l NaCl. The highest germination across all salinities was for *H. calyptata* followed by *H. halocnemoides* subsp. *caudata*. *H. halocnemoides* subsp. *caudata* is the most salt tolerant and *H. indica* subsp. *bidens* being the most salt sensitive with no germination recorded.

The regression statistics relating germination to salinity are shown in Table 2. The probability and regression did not have the same frequency of significance as for the laboratory trials but the low germination rate would be major factor in this result. The results of the analysis showed that germination of four species was negatively affected by salinity, germination of three species was not and the remaining two did not have any seeds germinate.

All species had the ability to recover from high salt concentrations once flushed with fresh water (Figure 5). The species with the greatest increase in germination, once flushed, was *H. 'Angelfish Island'*. *H. peltata* seeds had a great increase in germination on the surface. The species with the least ability to recover was *H. halocnemoides*. Averaged across all the species, there was an increase of 58% in germination once salt was removed.

Table 2—Regression analysis relating the germination of different samphire species to salinity.

	Lab. trial		Soil trial	
	Probability	R ²	Probability	R ²
<i>H. 'Angelfish Island'</i>	0.000	75%	0.040	29%
<i>H. calypttrata</i>	0.002	38%	0.039	30%
<i>H. halocnemoides</i> subsp. <i>caudata</i>	0.000	63%	0.141	12%
<i>H. halocnemoides</i>	0.002	39%	0.192	8%
<i>H. indicia</i> subsp. <i>bidens</i>	0.042	17%	na	na
<i>H. peltata</i>	0.000	58%	0.192	8%
<i>H. pergranulata</i>	0.000	57%	0.056	25%
<i>H. pruinosa</i>	0.000	64%	na	na
<i>H. undulata</i>	0.000	60%	0.039	30%

DISCUSSION

There is a significant relationship between reduced germination of samphire species present at Lake Carey and salinity. The two trials (laboratory and outdoor) supported similar results in that all seed germination decreased with increased NaCl concentrations. The strong results from the controlled laboratory trials, however, were not replicated by the soil trials. Germination for the nine studied samphire species will occur at 0 g/l and 10 g/l NaCl followed by a rapid decline at 30 g/l NaCl. Seed germination dramatically increased following flushing with fresh water.

The decrease in germination for all species (except *H. halocnemoides* subsp. *caudata*) within the laboratory trials suggests that the concentration of salt does have an effect on seed germination; the higher the NaCl concentration the lower the germination. *H. halocnemoides* subsp. *caudata* was the only species where germination increased, likely because its habitat is the most saline parts of Lake Carey (Table 3). For the three species that had low germination (*H. indica* subsp. *bidens*, *H. pergranulata* and *H. halocnemoides*), the temperatures used in the experiment may not have been optimal (Malcolm 1964). Although

H. pergranulata seeds were scarified, there was little germination for this species. A test between non-scarified and scarified seeds was not undertaken although the experiments completed by Barret (2000) concluded that there was an increase in germination from scarification.

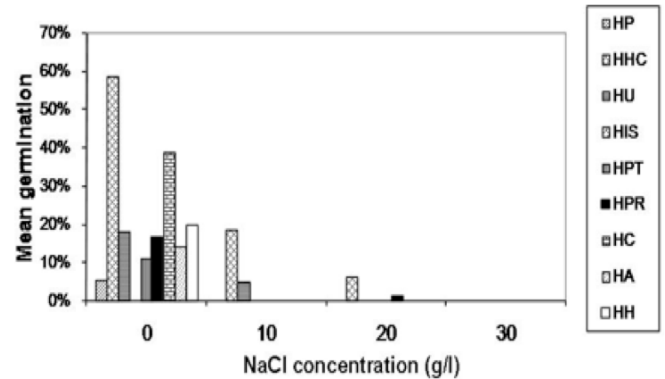


Figure 4—Viability-adjusted germination in outdoor settings across all species at differing NaCl concentrations with standard errors. Error bars show standard error.

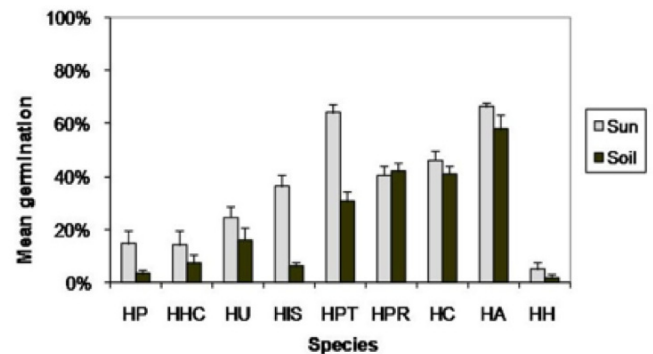


Figure 5—Comparison of ability to recover once flushed with fresh water for seeds in soil and sun in outdoor trials for all 9 study species. Error bars show standard error.

There was very little germination of seed either on the surface or buried in the soil in the outdoors trials, which makes it difficult to determine which species were the most salt sensitive or salt tolerant in the natural soil of the area. *H. halocnemoides* subsp. *caudata* had the most germination over 10 g/l NaCl and naturally occurs in the most saline areas, although there was little germination for *H. halocnemoides*, which inhabits similar areas. The main difficulty with the nursery trials was controlling the salt content of the plots and therefore the salinity of the pore water. The salt crusts that formed likely had a negative impact on germination.

A lack of moisture may also have contributed to the limited germination within the outdoor trials although some seeds receiving 0 g/l NaCl concentration still germinated. The irrigation frequency remained the same for all treatments. When watering frequencies were increased there was no direct increase in germination.

Once the viable seeds were watered three times a day with fresh water to flush the salt from the soil, there was a substantial increase in germination across all species. This suggests that a flush of fresh water is required to increase germination once seeds have been exposed to salt concentrations above 20 g/l NaCl. This supports previous findings at Hannan Lake, Western Australia that halophytes primarily germinate in periods of lowered soil salinity; an adaptive trait that ensures favorable growth conditions for young seedlings (English et al. 2002). We have observed that samphire species at Lake Carey germinate when rain flushes salt from the surface soil. In this case an analysis of the recruitment versus rainfall showed that there was a strong but not significant relationship between total recruitment to rainfall at all sites monitored over seven years.

It is inferred from this experiment that if the addition of hypersaline water to the lake reduces the period when seeds are exposed to freshwater conditions in the top soil at Lake Carey's edge, the germination of these samphires will be reduced. However the evidence is that once a suitable flushing event occurs, significant recruitment will occur.

This experiment has shown that the samphires tested in this trial require freshwater to germinate but that they will accommodate hypersaline conditions for a period and recover to germinate when the salinity drops to a low salinity.

ACKNOWLEDGEMENTS

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Microbial Diversity and Microbial Abundance in Salt-Saturated Brines: Why are the Waters of Hypersaline Lakes Red?

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ABSTRACT

Salt-saturated lakes such as the North Arm of Great Salt Lake and saltern crystallizer ponds contain 10^7 - 10^8 and more red microorganisms ml^{-1} . Even the Dead Sea occasionally turns red due to microbial blooms. Three types of organisms may contribute to the coloration: the alga *Dunaliella salina* rich in β -carotene, halophilic Archaea (family *Halobacteriaceae*) containing 50-carbon bacterioruberin carotenoids and sometimes also retinal proteins (bacteriorhodopsin, halorhodopsin), and the recently discovered *Salinibacter* (*Bacteroidetes*) which contains pigment salinixanthin (an unusual acylated C_{40} -carotenoid glucoside) as well as different retinal pigments. Bacteriorhodopsin and halorhodopsin enable the cells to directly use light energy for respectively the outward pumping of protons driving ATP generation and for the inward transport of chloride ions. The carotenoid pigments (β -carotene, α -bacterioruberin and derivatives, salinixanthin) primarily appear to protect the cells against photooxidative damage. Salinixanthin also acts as a light harvesting antenna for xanthorhodopsin, the proton-pumping retinal pigment of *Salinibacter*. Quantitative assessment of the relative importance of the different pigments in the coloration of red brines of natural salt lakes and solar saltern crystallizer ponds suggests that α -bacterioruberin and other carotenoids contributed by members of the *Halobacteriaceae* are generally responsible for most of the color of the waters. The quantity of β -carotene present in *Dunaliella* cells often greatly exceeds that of the haloarchaeal bacterioruberin pigments. However, the large amounts of β -carotene contribute only little to the optical properties of the brines because of the dense packing of the pigment in little globules within the chloroplast. Presence of salinixanthin and of bacteriorhodopsin and derivatives in the biomass can often be demonstrated as well, but these pigments have never been shown to contribute greatly to the overall optical properties of the waters. Thus, carotenoids of the bacterioruberin group appear to be the main factor causing the characteristic red color of hypersaline brines worldwide.

INTRODUCTION

“An embankment is made and ditches to draw clear sea water. It is left for a long time until the color becomes red. If the south wind blows with force during the summer and autumn the salt may grain over night. If the south wind does not come all the profits are lost.”

The above lines were derived from the ‘Peng-Tzao-Kan-Mu’, a compilation of ancient Chinese pharmacology by Li Shih-Chen (1518-1593), as cited in an essay on the history of salt making by Baas Beeking (1931) and attributed by him to much earlier times. Red colors are associated with many natural and man-made salt lakes whenever the NaCl concentration approaches saturation (Javor 1989). A more recent essay on salt and salt production was entitled “Red, the magic color for solar salt production” (Litchfield 1991). Such statements clearly show how prominent the color of salt-saturated brines often is. Figure 1 depicts a crystallizer pond of the salterns of Eilat, Israel; similar bright colors are characteristically found worldwide in salt production facilities. Such red brines are by no means restricted to man-made ponds for the production of solar salt. The North Arm of Great Salt Lake, Utah, with salt concentrations above 300 g l^{-1} shows similar colors (Post 1977; Baxter et al. 2005), and even the Dead Sea, a lake that is dominated by divalent cations (currently about $2.45 \text{ M } [\text{Mg}^{2+} + \text{Ca}^{2+}]$ vs. about $1.75 \text{ M } [\text{Na}^+ + \text{K}^+]$) has occasionally turned red when rainwater floods reduced the salinity of the upper water layers (Oren 2002a).



Figure 1—A crystallizer pond of the Israel Salt Company at Eilat, photographed on April 27, 2008.

Microscopic examination of red saltern crystallizer brines shows the presence of massive numbers of a variety of microorganisms. Figure 2 shows a typical picture of such a microbial community, consisting of prokaryotic cells, often present in numbers as high as 2×10^7 – 10^8 ml⁻¹ and sometimes even higher, as well as red-orange cells of the flagellate unicellular green alga *Dunaliella salina*, typically present in numbers of a few hundreds to a few thousands ml⁻¹. The prokaryotic community is dominated by red Archaea of the family *Halobacteriaceae* (Oren 2006), and in many cases the most abundant type was reported to consist of flat, square or rectangular cells. Many such cells can be recognized in the left panel of Figure 2.

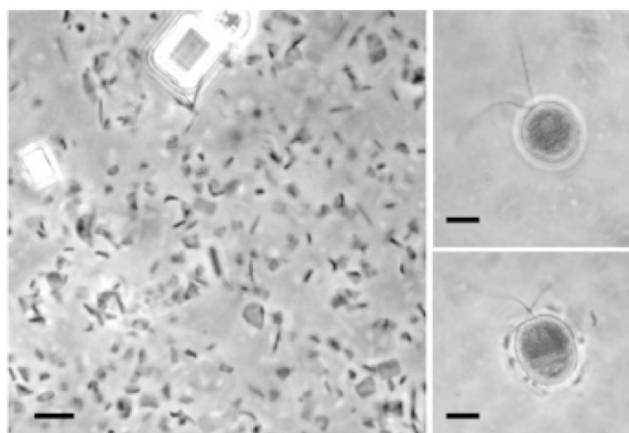


Figure 2—Microorganisms from the crystallizer brine shown in Figure 1: Flat square halophilic Archaea resembling *Haloquadratum walsbyi* and other types of prokaryotes (left panel), and *Dunaliella salina* cells (right panels). Phase contrast. The bars represent 5 μ m. The cells shown in the left panel had been concentrated by centrifugation, and therefore no gas vesicles are visible within haloarchaeal cells. Two small halite crystals are seen in the left panel.

The world of halophilic microorganisms is very diverse (Oren 2002a, 2002b, 2002c, 2007), and different types of red, orange or purple pigments are found in many salt-loving organisms. Many, often contradictory statements can be found in the literature about the reasons why salt-saturated brines are red (see Oren et al. 1992). Some authors have attributed the color to the massive presence of β -carotene inside the *Dunaliella* cells. Others have argued that carotenoid pigments of the halophilic Archaea may be the main cause of the red coloration. Yet another idea, brought forward from time to time, is that retinal-containing proteins such as bacteriorhodopsin produced by some halophilic Archaea may be responsible for the color of saltern crystallizer ponds and other salt lakes. In most cases no experimental evidence has been provided to support the statements made, and the nature of the organisms present and their pigments was not further documented. As a result, considerable confusion still exists about the true nature of

the optical effects caused by the presence of pigmented microorganisms in the brine.

In this paper I will try to present an overview of our current understanding of the pigments that may contribute to the color of hypersaline brines and the microorganisms that harbor those pigments, and then assess to what extent these pigments indeed contribute to the intense color of saltern ponds such as the one shown in Figure 1. The conclusions are widely applicable. However, it should be realized that it is quite probable that the coloration of brines at different geographical locations and with different chemical and physical properties may be due to different types of pigments and different types of microorganisms.

Dunaliella salina and its β -Carotene Content

Dunaliella salina, first described from salterns near Montpellier in the south of France in 1838 and named in 1905 (Oren 2005), is found worldwide in saltern evaporation and crystallizer ponds as well as in many natural salt lakes. *Dunaliella* is a unicellular eukaryotic alga that taxonomically belongs to the Chlorophyceae. Cells grown in the laboratory under low light and high nutrient concentrations are typically green because of their content of chlorophyll *a* and *b*. However, when exposed to stressful conditions of high light intensities and nutrient limitation, cells become colored orange-red due to the accumulation of large amounts of β -carotene. β -carotene has a characteristic absorption spectrum with a broad peak between 400 and 500 nm and an absorbance maximum at 451 nm (Figure 3). The β -carotene is located in small globules within the interthylakoid space of the cell's single chloroplast (Ben-Amotz et al. 1982, 1988). In some *Dunaliella* strains the pigment may be accumulated in quantities up to 8-10% of the cells' dry weight and possibly even more. The oily globules contain a mixture of the all-*trans* and the 9-*cis* isomers of β -carotene, together with minor amounts of other mono-*cis* and di-*cis* stereoisomers (Ben-Amotz et al. 1982). The higher the light intensities to which the cells have been exposed, the higher the ratio between the amount of the 9-*cis* to the all-*trans* form.

The β -carotene content of *Dunaliella* is mainly determined by the total irradiance the alga receives during a cell division cycle. High light intensities, nutrient limitation stress or supraoptimal salt concentrations lead to a decrease in the amount of chlorophyll per cell and an increase in its β -carotene content (Ben-Amotz & Avron 1983; Lers et al. 1990). Ultraviolet radiation also has a strong inducing effect on β -carotene biosynthesis.

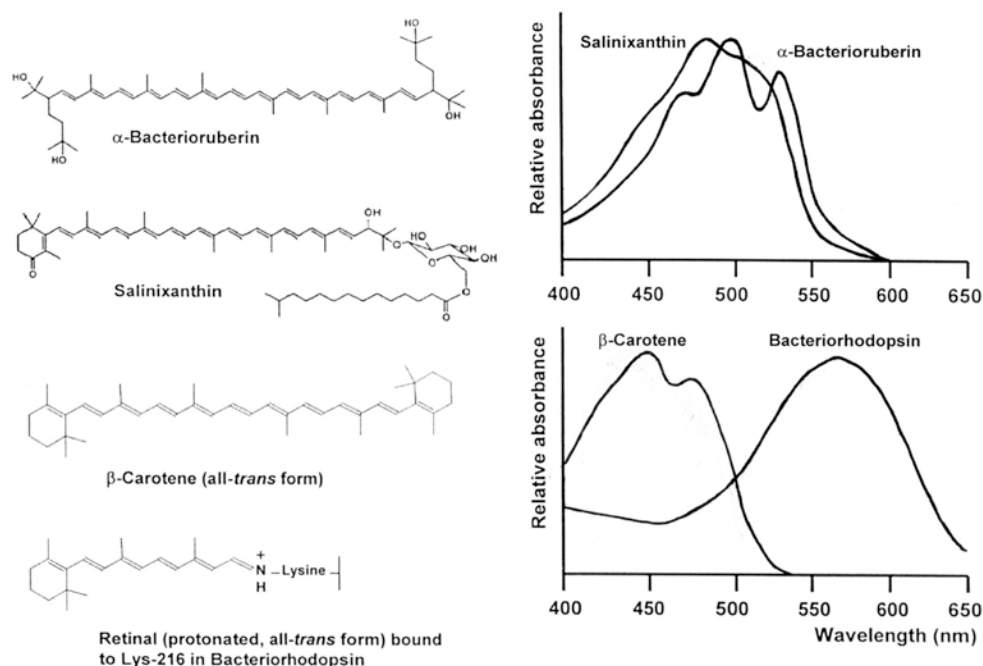


Figure 3—The principal orange, red and purple pigments encountered in the biomass of saltern crystallizer ponds, and their absorption spectra: the C_{50} carotenoid α -bacterioruberin of halophilic Archaea (family *Halobacteriaceae*), salinixanthin, the acylated C_{40} -carotenoid glucoside of *Salinibacter ruber*, β -carotene accumulated by the unicellular green alga *Dunaliella salina*, and bacteriorhodopsin of *Halobacterium* and some other members of the *Halobacteriaceae*.

Dunaliella cells that have accumulated large amounts of β -carotene tolerate much higher light intensities than green cells that had not synthesized major amounts of the pigment. The action spectrum of the photoprotection parallels the absorption spectrum of the carotenoid pigments (Ben-Amotz et al. 1989). The mechanism of the protective effect, however, is still not completely clear. It may be based on the destruction of singlet oxygen radicals produced during photosynthesis under high radiation. However, the β -carotene is located in globules within the interthylakoid space rather than in the thylakoids themselves, and the question should therefore be asked whether the distance between the carotene globules and the thylakoid-bound chlorophyll still allows effective quenching of chlorophyll-generated radicals. It is more probable that the carotene globules may protect the cells against high light damage by acting as a screen that absorbs excess radiation.

The Carotenoid Pigments of Halophilic Archaea

Most species of the halophilic Archaea (family *Halobacteriaceae*) are colored pink-red due to a high content of carotenoid pigments in their cell membrane. Only a few species in the group such as *Natrialba asiatica* do not show such a pink color. In some other species the extent of pigmentation depends on the salinity at which the cells had been grown: *Haloferax mediterranei* is only

weakly pigmented at high salinity, but produces massive amounts of carotenoid pigments when incubated at suboptimal salinities. However, those species that appear to be most abundant in saltern crystallizer ponds and other salt lakes are all brightly colored, including the square archaeon *Haloquadratum walsbyi* that is commonly found in salterns worldwide (Bolhuis et al. 2004; Burns et al. 2004).

Extracts of halophilic archaeal cells in organic solvents are pink and show absorbance maxima at 498 and 530 nm with a shoulder at 470 nm (Figure 3). This characteristic absorption spectrum is mainly due to the presence of C_{50} straight-chain derivatives of α -bacterioruberin (Kelly et al. 1970). Different derivatives of α -bacterioruberin are present as well, such as monoanhydrobacterioruberin and bisanhydrobacterioruberin (Kelly et al. 1970; Kushwaha et al. 1975). Several other derivatives have been found in minor amounts (Rønnekleiv et al. 1995). For a more complete account of the carotenoid pigments encountered in the group and their chemical structures see Oren 2002a and Oren 2006.

Studies in the early 1960s using *Halobacterium salinarum* as a model organism have clearly shown the advantages that pigmentation provides to cells. Non-pigmented mutants can easily be isolated, and such mutant strains grow in the dark as well as the red-pink wild type, but when incubated

at light intensities equivalent to full sunlight, the white mutants are rapidly outcompeted by the pigmented parent strain. The carotenoid pigments thus protect the cells against damage by excessive light (Dundas & Larsen 1962). The carotenoid pigments of *Hbt. salinarum* were also claimed to protect the cells against UV radiation and aid in photoreactivation (Wu et al. 1983). A protective role of bacterioruberin by providing resistance to agents that damage DNA such as ionizing radiation or hydrogen peroxide was also shown in *Hbt. salinarum*, a colorless mutant being more sensitive to such agents than the pigmented strain. The frequency of DNA strand-breaks induced by ionizing radiation (^{60}Co γ -rays) was significantly reduced by the presence of bacterioruberin pigments (Shahmohammadi et al. 1998).

The Retinal Pigments of Halophilic Archaea

The retinal pigments present in members of the *Halobacteriaceae* have received much interest since the structure and function of bacteriorhodopsin were elucidated in the early 1970s (Oesterhelt & Stoeckenius 1971). *Halobacterium salinarum* has four such retinal-containing proteins: the outward proton pump bacteriorhodopsin (maximum absorbance at 568 nm) (Figure 3), the inward chloride pump halorhodopsin (578 nm), and two sensory rhodopsins involved in light sensing for phototaxis: a sensor for green light (sensory rhodopsin I) to which the cells are attracted, and a sensor for blue light (sensory rhodopsin II) that acts as a repellent. Not all members of the *Halobacteriaceae* possess genes encoding all these retinal proteins. Many species lack bacteriorhodopsin; *Haloquadratum walsbyi* has two bacteriorhodopsins and one halorhodopsin, but lacks sensory rhodopsins, which would not be useful in these organisms that lack active motility (Bolhuis et al. 2006). Bacteriorhodopsin and halorhodopsin contain seven transmembrane helices with the retinal being covalently attached through the formation of a Schiff base between the aldehyde function of the chromophore and the ϵ -amino group of a lysine residue in the seventh helix. In *Hbt. salinarum*, bacteriorhodopsin is organized in special patches in the cytoplasmic membrane (the 'purple membrane'), while in certain other species the retinal pigments appear to be more evenly distributed over the cell membrane. More in-depth information on the biology, biochemistry, and biophysics of the haloarchaeal retinal proteins can be found in specialized reviews (Oesterhelt 1988; Lanyi 1990; 1993, Oesterhelt 1995; Lanyi 1998; Lanyi & Luecke 2001; Oren 2002a).

When bacteriorhodopsin is excited by light of a suitable wavelength, a complex photocycle is initiated that lasts about 10 milliseconds. During the process, the retinal group undergoes isomerization from the all-*trans* to the 13-*cis* isomer. In addition, the Schiff base between the retinal and lysine-216 is deprotonated and protonated again. As the

proton is released at the outer side of the membrane but replenished from the internal side, the completion of the photocycle results in transport of a proton from the cell to the surrounding medium and the generation of a proton gradient across the cell membrane. This proton gradient can then drive the synthesis of ATP and other energy-requiring processes. Light can thus relieve energy starvation at low nutrient and low oxygen concentrations (Brock & Petersen 1976), and even anaerobic photoheterotrophic growth is possible with light serving as the energy source (Hartmann et al. 1980).

Under suitable conditions *Halobacterium salinarum* produces large quantities of bacteriorhodopsin. The color of the culture then changes from red-pink or red-orange (due to the presence of bacterioruberin carotenoids) to purple. Genome sequencing (Ng et al. 2000) and molecular biological studies have contributed much to our understanding of the biosynthesis of bacteriorhodopsin and its regulation. *Hbt. salinarum* has a cluster of three genes: *bop*—the gene encoding the bacterio-opsin (the protein backbone of bacteriorhodopsin), *brp*—a bacterio-opsin-related protein, and *bat*—the bacterio-opsin activator. Expression of the *bop* gene cluster is induced by low oxygen tension and by light (Shand & Betlach 1991), and much information has accumulated on the factors that influence the biosynthesis of the protein and of the retinal moiety, all coordinated in a regulon that reacts to changes in light intensity and oxygen concentration (Baliga et al. 2001; DasSarma et al. 2001).

In view of the obvious advantages that the possession of bacteriorhodopsin may bestow on halophilic Archaea, it is surprising that attempts to quantify its presence and importance in natural communities of halophilic Archaea have been so rare. The color of haloarchaeal blooms in salterns and natural salt lake is generally red-pink rather than purple. Occurrence of bacteriorhodopsin was reported in a community of *Halobacteriaceae* that developed in the Dead Sea in 1980–1982, and concentrations of the pigment were then estimated to be in the range of 0.6–0.7 nmol l⁻¹ at the time the prokaryote community contained 4–5 × 10⁶ cells ml⁻¹ (Oren & Shilo 1981). Indications were also obtained that this bacteriorhodopsin may have contributed to light-dependent fixation of CO₂ fixation in a mechanism that still has not been fully elucidated (Oren 1983). Javor (1983) found 2.2 nmol l⁻¹ bacteriorhodopsin in the archaeal community in the crystallizer ponds of the salterns of Exportadora de Sal, Guerrero Negro, Baja California, Mexico. Flash spectroscopy provided evidence for the presence of both bacteriorhodopsin and halorhodopsin activity in a community of halophilic Archaea in a shallow coastal hypersaline salt flat on the Sinai peninsula, Egypt (Stoeckenius et al. 1985).

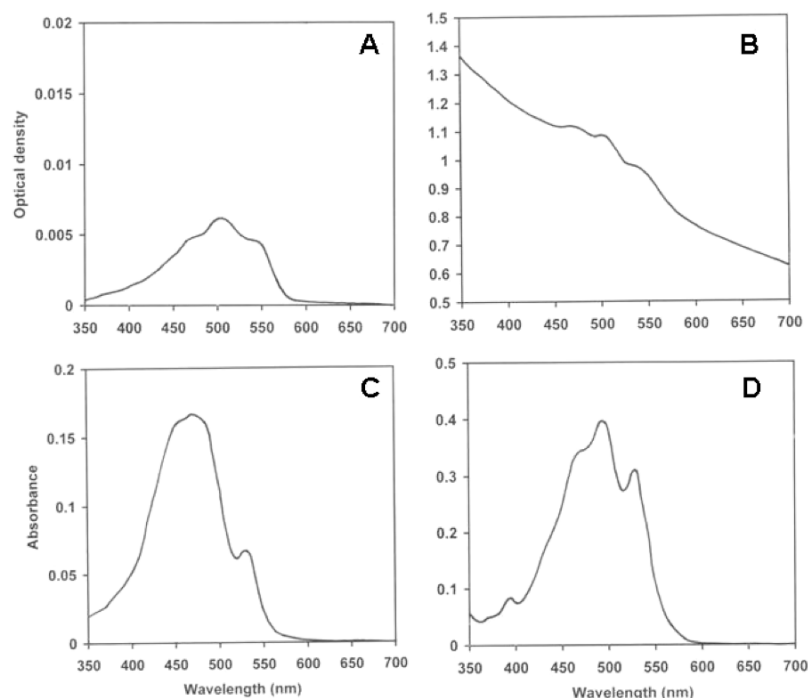


Figure 4—Absorption spectra of brine samples and pigment extracts prepared from them. Brine was sampled from the crystallizer pond of the Israel Salt Company, Eilat, shown in Figure 1. (A) Absorption spectrum of the brine, measured against water to which a small amount of milk was added to adjust for the turbidity of the sample. (B) Absorption spectrum of biomass concentrated from the brine by centrifugation. A portion of 360 ml of brine was centrifuged (30 min, 6000 x g), the pellet resuspended in 4 ml of supernatant brine, and the spectrum was recorded with supernatant brine as the blank. (C) Absorption spectrum of biomass from 50 ml of brine, collected by filtration on GF/C filters and extracted in 3 ml methanol/acetone 1:1 (vol/vol). (D) Absorption spectrum of biomass from 360 ml brine, collected by centrifugation (30 min, 6000 x g), followed by filtration on GF/C filters and extraction in 3 ml methanol/acetone. For explanations see text.

The Carotenoid and Retinal Pigments of *Salinibacter ruber*

Halophilic Archaea of the family *Halobacteriaceae* are not the only pigmented prokaryotes that can contribute to the red coloration of salt lakes and saltern crystallizer ponds. It was recently recognized that a rod-shaped, extremely halophilic representative of the domain Bacteria, phylum *Bacteroidetes*, may also be present in significant numbers in those environments in which red halophilic Archaea thrive. *Salinibacter ruber*, first isolated from Spanish saltern crystallizer ponds (Antón et al. 2002; Oren et al. 2004) now appears to be distributed worldwide in salterns and salt lakes approaching halite saturation. Cultures of *Salinibacter* are orange-red, and contain one major hydrophobic pigment, salinixanthin, identified to be a novel type of acylated C₄₀-carotenoid glucoside (Lutnæs et al. 2002). Its absorption spectrum shows a peak at 482 nm and a shoulder at 506-510 nm (Figure 3). Presence of salinixanthin in the biomass collected from the salterns of Alicante, Spain, could readily be detected by HPLC analysis of the hydrophobic pigments extracted from the cells, and it was estimated that 5-7.5% of the total

prokaryote-derived pigment extracted from those salterns originated from bacterial rather than from archaeal extreme halophiles (Oren & Rodríguez-Valera 2001).

Analysis of the genome sequence of *S. ruber* (Mongodin et al. 2005) showed that, although *Salinibacter* is a representative of the Bacteria, it possesses genes homologous to haloarchaeal bacteriorhodopsin, halorhodopsin, and two sensory rhodopsins. While the functioning of the product of the putative halorhodopsin-like gene as a chloride pump still has to be proven, it was shown that the product of the bacteriorhodopsin-like gene, termed xanthorhodopsin, indeed acts as a light-driven proton pump. Further analysis of photochemical processes in *Salinibacter* demonstrated that the carotenoid salinixanthin can transfer absorbed light energy with a high efficiency to the xanthorhodopsin proton pump (Balashov et al. 2005; Balashov & Lanyi 2007). Such direct energy transfer between membranal carotenoids and retinal proteins has never yet been demonstrated in *Halobacterium* or any of the other members of the *Halobacteriaceae*. The finding shows that, at least in some halophilic prokaryotes, the red-orange carotenoid pigments may have additional

functions beyond protection against high light intensities and scavenging of toxic free radicals and to prevent photooxidative damage.

How Do the Different Microbial Pigments Influence the Color of Hypersaline Brines?

As shown above, many different types of red, orange and purple pigments can be produced by halophilic microorganisms: β -carotene of *Dunaliella*, bacterioruberin carotenoids of the *Halobacteriaceae*, salinixanthin of *Salinibacter*, and the different retinal pigments: bacteriorhodopsin and halorhodopsin of haloarchaea and xanthorhodopsin and other retinal proteins of *Salinibacter*. The question should therefore be asked to what extent each of these pigments contributes to the color that we see when we look at red brines of saltern crystallizer ponds (Figure 1), the North Arm of Great Salt Lake, and other similar environments colored by dense communities of halophilic microorganisms.

The answer to the above question may appear simple and straightforward: a simple way to assess the contribution of each pigment could be the extraction of the pigments followed by measurement of the absorption spectrum of the extract. Yet, such a simple approach can easily lead to erroneous conclusions. This is clearly demonstrated by the following case study, based on the same saltern crystallizer brine shown in Figure 1 and its microbial communities illustrated in Figure 2. Microscopic enumeration of the biota gave numbers of 280 *Dunaliella* cells and about 4×10^7 prokaryotic cells ml^{-1} in this brine.

When brine from the crystallizer pond was collected by filtration on glass fiber filters that retain all or at least most of the biomass, and the filters were then extracted with methanol/acetone (1:1, vol/vol), all hydrophobic pigments (carotenoids, chlorophylls) are released, and the components of the resulting extract can be evaluated by measuring the absorption spectrum, if necessary followed by HPLC separation and characterization of each individual pigment fraction. While satisfactory for many purposes, such a protocol does not provide information on the presence of bacteriorhodopsin, halorhodopsin and other retinal proteins, which are not extracted by organic solvents. The resulting spectrum (Figure 4C) shows a very large peak of β -carotene (the broad absorbance maximum around 450 nm). Some bacterioruberin carotenoids are present as well, as witnessed by the small peak at 530 nm. The major bacterioruberin peak at 496 nm is hidden below the large β -carotene peak. The absence of a peak at 665-670 nm shows that the *Dunaliella* cells contained very little chlorophyll. Based on the spectrum shown in Figure 4C the obvious conclusion would be that *Dunaliella*-

derived β -carotene contributes most to the light absorption by the brine and is mainly responsible for its color, and that the bacterioruberins of the halophilic Archaea add a minor contribution as well.

An altogether different spectrum was obtained when the biomass was collected by centrifugation instead of by filtration. In this case the brine was centrifugated for 30 minutes at 6000 x g, the pellet was resuspended in a small portion of brine, cells were then collected on glass fiber filters as described above, extracted with methanol/acetone, and the absorption spectrum recorded. The resulting spectrum (Figure 4D) looks like a nearly pure bacterioruberin spectrum (compare Figure 3), and no obvious contribution from β -carotene is seen. Microscopic examination of the cell pellet shows the reason: no *Dunaliella* cells are found in the pellet. Due to the high content of carotene globules the cells are lighter than the brine, and they float upwards rather than sink during centrifugation. When the pellet obtained after centrifugation is resuspended in brine rather than extracted in organic solvent, and the in-vivo absorption spectrum measured against brine cleared of particles (Figure 4B), no straight baseline is obtained. The particles in the suspension not only absorb light but also cause a considerable light-scattering effect. As light scattering is wavelength-dependent, much higher optical densities were measured in the blue part of the spectrum than in the red part. The peaks that rise out of the baseline are those of bacterioruberin pigments. No prominent peak in the area of 570-580 nm is seen, and this proves that bacteriorhodopsin and halorhodopsin do not greatly contribute to the color of the cells (note that in the sample of Figure 3B such pigments would have been preserved, while the treatments applied in Figure 3C and 3D did not extract retinal proteins).

When finally a spectrum was made of brine that was neither filtered nor centrifugated, and a correction was made for sample turbidity and light scattering by adjusting the scattering properties of the water blank with a small amount of milk, the true in vivo spectrum of the crystallizer pond biomass was obtained (Figure 4A). Surprisingly, this spectrum resembles that of halophilic Archaea and their bacterioruberin carotenoids, and no contribution of *Dunaliella* β -carotene is noticeable. This is unexpected, as β -carotene was the most abundant pigment in the biomass (compare Figure 4C). The reason why these large amounts of β -carotene contribute so little to the optical properties of the brine is the location of the pigment inside the cells. The haloarchaeal pigments are distributed more or less evenly over the entire cell membrane. This, and the fact that the halophilic Archaea are so abundant in the brine (4×10^7 cells ml^{-1}), enables the bacterioruberin pigments to absorb light, and the resulting spectrum is therefore that of the

haloarchaeal carotenoids. *Dunaliella* cells are rare (only about 280 cells ml⁻¹), and their massive amount of β -carotene is densely concentrated in tiny globules within the chloroplast. In this way the pigment contributes very little to the overall optical properties of the brine. When, however, the algal carotene is extracted with organic solvents, it becomes obvious how much had been accumulated in the *Dunaliella* chloroplasts (Oren et al. 1992; Oren & Dubinsky 1994).

FINAL CONCLUSIONS

1. Different pigments may contribute to the color of red brines in salt lakes: the 50-carbon carotenoid α -bacterioruberin and derivatives synthesized by halophilic Archaea of the family *Halobacteriaceae*, the 40-carbon carotenoid β -carotene that is accumulated within the chloroplast of the alga *Dunaliella salina*, the acylated C₄₀-carotenoid glucoside salinixanthin of the recently discovered *Salinibacter ruber* (*Bacteroidetes*), and the membrane-bound retinal-containing proteins bacteriorhodopsin and halorhodopsin, found in many representatives of the *Halobacteriaceae* as well as in *Salinibacter*.

2. In solar saltern crystallizer ponds and probably in many natural hypersaline salt lakes as well, β -carotene of *Dunaliella* is the most abundant pigment, and its presence dominates the absorption spectrum of biomass extracted with organic solvents that dissolve hydrophobic pigments.

3. However, the color of the brine is, at least in many cases, not primarily due to the algal β -carotene but rather to the presence of archaeal bacterioruberin pigments. This is due to different location of the pigments involved. Halophilic archaea are found in large numbers in the brines, and the bacterioruberin pigments are in most cases evenly distributed over the entire cytoplasmic membrane. On the other hand, the number of *Dunaliella* cells is several orders of magnitude lower, and in these cells the pigment is densely concentrated within globules located between the thylakoids of the chloroplast. Thus the pigment contributes only little to the optical properties of the brines, and its quantity becomes apparent only upon extraction with organic solvents.

4. Presence of retinal pigments (bacteriorhodopsin, halorhodopsin, sensory rhodopsins) within the natural community of halophiles in salt lakes has only rarely been quantified. Such pigments have occasionally been shown to be present, but in vivo absorption spectra of the biomass collected from natural brines never showed a prominent absorption peak that can be attributed to such retinal proteins.

5. Presence of the acylated C₄₀-carotenoid glucoside salinixanthin, a red-orange pigment found in the recently discovered red extremely halophilic bacterial species *Salinibacter ruber*, can be demonstrated at least in some salt-saturated hypersaline brines. Quantitatively its contribution to the pigmentation was always found to be a minor one in comparison with the amount of bacterioruberin pigments from the halophilic Archaea that inhabit the same brines.

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A Population-Specific Marker within the Superspecies *Artemia franciscana*

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Artemia franciscana is one of the two *Artemia* species that are found in the New World with a wide distribution in the American continent. Commercially available cysts mainly originate from Great Salt Lake, Utah and from San Francisco Bay, California. Cysts from the latter have been used in many (deliberate or not) inoculations around the world. These two populations show a number of differences (i.e., in diapause deactivation treatment or in temperature tolerance) supporting the notion that *A. franciscana* is a “superspecies”. In addition, evidence from morphological, karyological, electrophoretic, and DNA sequencing data has shown significant sub-structuring and differentiation within *A. franciscana*. In this work, the second p26 intron was studied in order to trace the “hidden diversity” within the *A. franciscana* complex and to evaluate its utility as a population-specific marker for various applications. Ten *A. franciscana* populations from USA, Chile, Brazil, and Vietnam were screened and the sizes of the amplicons revealed six different genotypes (Table 1). Primer design was based on the conserved regions of the second and third exons of p26 gene sequences of *A. franciscana* (GenBank accession numbers DQ310577, DQ310575 and AF031367). BLAST searches confirmed the identity of the PCR product on the basis of detected similarities with second and third exons of p26 sequences from other *Artemia* representatives. San Francisco Bay yielded a single fragment of ~1500 bp (using 1.8% agarose gels and visualized under UV light) whereas Great Salt Lake showed two patterns, namely

~2000 bp and 2000/1500 bp. Populations that are known to originate from San Francisco Bay inoculated material, such as MAC (Macau, Brazil) and VC (Vinh Chau, Vietnam), showed the same genotype as the source population. Among the six Chilean populations studied, substantial heterogeneity in genotypic profiles was revealed (Table 1). Populations CHA, LLA, LVI, and IQU were monomorphic while observed heterozygosity values for CON and CEJ were 0.45 and 0.49, respectively. The same estimate for the GSL population was 0.55. For the whole data set, 30 of 45 comparisons for population differentiation showed significant ($p < 10^{-5}$) heterogeneity in allele frequencies. Twenty single-pair crosses between Great Salt Lake (~2000 bp) and San Francisco Bay (~1500 bp) were performed and screened with the p26 marker in order to confirm the pattern of inheritance. Although preliminary, our results indicate that both fixed differences and polymorphic patterns exist in p26 genotypes within the *A. franciscana* complex. The developed marker seems to be of diagnostic value in stock identification as shown by the obtained genotypes in feral populations (MAC, VC). The p26 gene could be informative in population genetic surveys investigating levels of variability, population structure and differentiation, and patterns of gene flow. The obtained genetic diversity estimates reflect a common fact in *A. franciscana* populations which is a trend towards the maintenance of high levels of genetic variability in their gene pools and great interpopulation genetic heterogeneity.

Table 1—Numbers of individuals (*N*) with different p26 genotypes in the examined *Artemia franciscana* populations (SFB: San Francisco Bay, California, USA; GSL: Great Salt Lake, Utah, USA; CHA: Chaxas, Chile; CON: El Convento, Chile; LLA: Llamara, Chile; LVI: Los Vilos, Chile; IQU: Iquique, Chile; CEJ: Cejas, Chile; VC: Vinh Chau, Vietnam; MAC: Macau, Brazil).

	SFB	GSL		CHA	CON					LLA	LVI	IQU	CEJ			VC	MAC
N	48	23	28	38	33	5	3	10	18	40	40	30	8	13	20	65	25
2000 bp		-	-			-	-		-				-		-		
1500 bp	-		-	-			-	-			-	-		-	-	-	-
1400 bp					-			-	-	-							

Microbial Communities in Salt Lakes: Phylogenetic Diversity, Metabolic Diversity, and *In Situ* Activities

Summary of a Roundtable Discussion on our Current Understanding, Limitations to our Knowledge, and Future Approaches, Salt Lake City, 12 May 2008

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ABSTRACT

A roundtable discussion was held on Monday, 12 May 2008, following the sessions on 'Microorganisms in Hypersaline Environments' at the 10th Conference on Salt Lake Research & 2008 FRIENDS of Great Salt Lake Issues Forum, University of Utah, Salt Lake City. Among the aspects discussed were: 1. The gap between our knowledge of the microorganisms isolated in culture and the true microbial diversity as apparent when using culture-independent techniques, in most cases based on the characterization of small subunit ribosomal RNA genes; 2. The metabolic diversity of the microorganisms inhabiting saline and hypersaline lakes and the lack of information to what extent the metabolic potential of the microbiota as apparent from culture studies or detection of functional genes is realized in the environment; 3. The limited understanding of the diversity of algae, bacteriophages and protozoa in hypersaline lakes and their relative importance of such microbial predators and grazing animals on the regulation of the microbial community sizes in such lakes; 4. The impact of high throughput "-omics" technologies for assessing the diversity and metabolism of hypersaline environments. In recent years a number of comprehensive studies were performed in selected hypersaline environments by large interdisciplinary teams of scientists. Such studies contribute invaluable information to define the nature and function of the microbial communities in such environments. However, the inability to independently grow specific organisms compared to the genetic diversity revealed by non-cultivation techniques indicates that additional work is needed to develop and define *in vitro* cultivation conditions. More of such studies are needed, with the appropriate funding, to solve the basic questions relating to the importance of microorganisms in saline lakes and other hypersaline ecosystems.

INTRODUCTION

During the 10th Conference on Salt Lake Research & 2008 FRIENDS of Great Salt Lake Issues Forum, held at the University of Utah, Salt Lake City, a session of eight talks, accompanied by a number of posters, addressed the microbial diversity of saline and hypersaline lakes and other hypersaline ecosystems. Among the environments discussed were Great Salt Lake (Utah), the Salton Sea (California), the Dead Sea (on the border between Israel and Jordan), Tuz Lake (Turkey), Lonar Lake (India), solar salterns, and Permian halite deposits. Following the oral presentations on Monday, 12 May, a roundtable session was held, in which different aspects relating to the diversity and activities of microorganisms in hypersaline environments were discussed, attempting to place the information presented in the individual talks in a more general context. Below we present a summary of the main items discussed and the conclusions reached during this roundtable session.

What Microorganisms are the Main Players in Salt Lakes and Other Hypersaline Environments? Culture-Dependent Versus Culture-Independent Approaches

It is surprising at what high rate new species of halophilic and halotolerant bacteria are being described in recent years, not only in absolute numbers (Oren 2008) but also relative to numbers of novel non-halophiles. Out of the 528, 593, and 631 names of species of prokaryotes (Archaea and Bacteria combined) that were validly published in 2005, 2006, and 2007 according to the rules of the International Code of Nomenclature of Prokaryotes (numbers derived from www.bacterio.cict.fr), at least 37, 39 and 74 refer to organisms that have their optimal growth above seawater salinity; thus, over 8.5% of all newly named prokaryotes are halophiles according to this definition. Hence, hypersaline environments and halophilic microorganisms are becoming increasingly popular research targets, especially in Asia.

The number of completed or nearly completed genome sequences of halophiles is also rapidly rising, which provides new information about the genetic diversity and metabolic potential never realized before. The first halophilic archaeal genome to be sequenced was *Halobacterium* strain NRC-1 (Ng et al. 2000), followed by *Haloarcula marismortui* and *Haloferax volcanii* from the Dead Sea, *Halorubrum lacusprofundi* from Deep Lake, Antarctica, the flat square *Haloquadratum walsbyi* from Spanish salterns (Bolhuis et al. 2006), and the alkaliphilic *Natronomonas pharaonis* from the soda lakes of Wadi Natrun, Egypt. To date, genomes of 17 isolates of *Halobacteriales* are completely closed and are ready for use in identifying untold diversity in hypersaline environments (Liolios et al. 2008; www.genomesonline.org). In the domain Bacteria the genome sequence information on the extremely halophilic red *Salinibacter ruber* was published (Mongodin et al. 2005), and the same is true for the moderately halophilic *Chromohalobacter salexigens*, also isolated from solar salterns. Genomic sequences expected to be completed released in the coming year include the anaerobic thermophilic halophile *Halothermothrix orenii* isolated from a salt lake in Tunisia and the eukaryotic brine alga *Dunaliella*.

The rapid increase in our understanding of the microbial diversity in hypersaline systems is due to the intensive use of genetic tools that provide additional dimensions for assessing diversity without the need to culture organisms. In recent years, DNA isolation techniques from environmental samples have greatly improved and are no longer the limiting factor. The discovery of unique genes and organisms has fueled even more interest in hypersaline environments. Application of molecular methods, in most cases based on the characterization of small subunit ribosomal RNA genes (16S rRNA for prokaryotes, 18S rRNA for eukaryotes) amplified directly from DNA isolated from the biomass shows that most sequences recovered have no equivalent in the database of known species. The results are indeed typical of all such whole-environment genetic studies. The application of 16S rRNA-based culture-independent techniques to hypersaline environments was pioneered by Benlloch et al. (1995) in the salterns of Alicante, Spain, and has since been applied to a variety of environments: saltern ponds (Benlloch et al. 2002; Legault et al. 2006), Mono Lake, California (Humayoun et al. 2002), Great Salt Lake, Utah (Baxter et al. 2005), and the Great Salt Plains, Oklahoma (Kirkwood et al. 2008). This was a key discussion point of the symposium that indicated additional work is needed to

bring concordance to the organisms found thus far. The diversity detected using such approaches depends to a large extent on the money available for sequencing; if funding is appropriate, very large numbers of novel 16S rRNA sequences can be retrieved, as is shown by the study by Humayoun et al. (2002) in Mono Lake.

Initial use of the Phylochip to detect microbes in air (Brodie et al. 2007), with the potential to detect over 9000 groups of microbes based on the 16S rRNA gene sequence offers a new option to examine the diversity of hypersaline environments. Use of this technology in Great Salt Lake dramatically expanded the view of the level of diversity that may exist independent of culturing. The true value of this approach remains to be realized, but the preliminary data look very exciting and worth pursuing.

The combination of culture-dependent and culture-independent techniques has provided the basis for our current understanding of microbial diversity in high-salt environments (for an overview see e.g. Oren 2002a, 2002b, 2007). Molecular, culture-independent techniques, when properly applied, can provide information on the nature of the often yet uncultured dominant microorganisms in the environment. The information thus obtained can be used to specifically search for conditions to cultivate and study these organisms if enough sequence is acquired to predict some of the nutritional requirements and genetic regulation mechanisms. This approach has recently been successful in two important cases, with the isolation and characterization of both the square, gas-vacuolate archaeon *Haloquadratum walsbyi* (Burns et al. 2007) and the rod-shaped red *Salinibacter ruber* (Antón et al. 2002), both from solar salterns. A study in Australian saltern crystallizer ponds showed that with appropriate techniques and much patience, many members of the *Halobacteriaceae* present in the brines can be cultured (Burns et al. 2004). Extensive subculturing in liquid media is necessary as isolation on solid agar is not possible for some species.

The authors of this roundtable discussion summary suggest that the best approach is a combination of both cultivation-dependent and cultivation-independent methods—to fully characterize the microbial communities in salt lakes and other hypersaline environments. Environmental sequences alone without culture-based characterization will not adequately advance understanding of the causes of diversity, but can direct culture efforts toward the isolation of yet uncultivated microbes.

Metabolic Potential and *In Situ* Metabolic Activities of Microbial Communities at High Salt Concentrations

More important than the phylogenetic diversity of the species of halophilic microorganisms present in salt lakes is the function these microorganisms perform in the ecosystem. Here our understanding is still rather limited. One popular experimental approach is to look for the metabolic potential of the community, and this can be done in a number of ways. The potential of the community (or of selected isolates obtained from the community) to degrade any of a large number of carbon sources can provide information on processes that the community can theoretically perform. The Biolog[®] system enables high-throughput testing of a large number of carbon sources. Some modification of the standard protocol may have to be applied when high salt concentrations are present, but overall this assay has proved successful in a number of hypersaline systems (Litchfield et al. 2001). Alternatively, one may use “metabolomics”, a set of exploratory LC/MS/MS techniques to examine the small molecules present in an environmental sample. This approach is littered with pitfalls that are largely due to ensuring accurate identification of the molecular mass with the database of identifiers. Nevertheless, metabolomics is an important method to apply in understanding the metabolic potential of hypersaline environments since relatively few of the organisms are available *in vitro* to serve as models for metabolic study.

Molecular techniques have in recent years extended our knowledge about the metabolic potential of microbial communities. Detection of “functional” genes specific for certain metabolic processes can be used to assess the presence or absence of certain groups of microorganisms or of certain biochemical pathways. The water column of Mono Lake, California has been a popular environment for testing this approach. Genes used for this purpose include ribulose biphosphate carboxylase/oxygenase involved in autotrophic fixation of carbon dioxide (Giri et al. 2004), ammonia monooxygenase, the key enzyme of autotrophic oxidation of ammonia to nitrite (Ward et al. 2000), bisulfite reductase as an indicator for the presence of bacteria performing dissimilatory sulfate reduction, and genes involved in the methanogenic pathway (Scholten et al. 2005).

Analysis of genome libraries generated from DNA isolated from the environment can provide much more extensive information, and the recently developed “Geochip” which

contains probes for over 4000 functional genes from (non-halophilic) Bacteria, is currently being tested to study of the potential metabolic diversity in hypersaline environments. The chips were designed for use in soil, specifically for bioremediation applications. Their use in hypersaline ecosystems may necessitate redesign. Future Geochips may need to include probes specific for archaeal and more diverse biochemical pathways that may play roles in hypersaline metabolism. Many halophiles (notably the *Halobacteriaceae*, but also *Salinibacter* and possibly the anaerobic fermentative *Halanaerobiales* as well) contain a large excess of acidic amino acids in their proteins, and this should of course be reflected in the nucleotide sequence and codon usage of the encoding genes, so that gene probes based on relatively well-conserved genes from non-halophiles may not always react with the homologous halophile gene. A conclusion of the session was that it may be time to redesign some of the high-throughput techniques to include more probes for Archaea and for halophilic and halotolerant algae in addition to the eubacterial probes that are well known and used extensively on the Geochip.

In evaluating the function of metabolic activity in a microbial community, one must assess to what extent the potential, as evaluated using DNA-based techniques or from culture-dependent laboratory studies, is indeed realized in the environment. It is sometimes stated that “If you’ve got it, you’re using it”, thus assuming that the possession of one or more of the key genes for a certain metabolic pathway is sufficient to prove that that metabolic pathway indeed is operative in the organisms or in the community *in situ*. There is no *a priori* justification for such a statement in environments that are not extreme. However, in extreme environments, the parameters are narrower, and this statement may be insightful in realizing that specific metabolites are hallmarks of that location and they can only arise via a single or a limited number of mechanisms. Testing for the microbial conversions to occur in salt lakes under field conditions is always much more difficult than demonstrating presence of the genes necessary for such conversions. Studies that go all the way to prove that the genetic potential is realized in the environment are unfortunately very rare.

An interesting case in this respect is the recent finding of *amoA* genes, encoding the key enzyme of autotrophic nitrification, in the nearly salt-saturated North Arm of Great Salt Lake, as reported at the conference. Theoretical considerations based on thermodynamic calculations make it improbable that autotrophic nitrification can function at

such high salt concentrations (Oren 1999, 2001). If indeed it can be proven that ammonia is oxidized to nitrite at measurable rates in Great Salt Lake brines, this would necessitate a change in our concepts on how nitrifying bacteria function or indicate that new biochemical mechanisms are yet to be discovered in these environments. In that case special efforts should be made to isolate the organism(s) responsible for the process (which is not easy, as even non-halophilic nitrifiers are notoriously difficult to grow), but only in that way can we extend our understanding and assess why the earlier theoretical models may not apply for Great Salt Lake nitrifiers.

Molecular biology techniques may help here in the future as well. For example, if we can assess the expression of *amoA* genes within the microbial community by quantifying mRNA levels, then we will have additional evidence that the genes are being transcribed. Still, the final proof will be the direct measurement of the enzyme activity itself. Techniques of metabolomics have not yet been extensively applied to hypersaline environments, but such techniques which assay specific metabolites in the field do have a considerable potential for revealing processes performed by the microbial community present. The potential impact of lateral gene transfer from one organism to another (or from phage) is hard to calculate, but in environmental metabolomics or genomics, where one is examining the broad view of processes occurring within the whole microbial community, this is not a concern.

Bacteria in the Food Chain of Hypersaline Lakes

It is clear that prokaryotes—Bacteria as well as Archaea—form an essential link in the food chain in saline and hypersaline environments. While we have a reasonably good understanding of the factors that promote development of halophilic and halotolerant prokaryotes, we know much less about the factors that may cause their decline: formation of non-culturable states, predation by protozoa, predation by higher animals, and lysis by bacteriophages.

Our knowledge of protozoa preying on prokaryotes and algae at the highest salinity is very limited. Comprehensive studies of the microbial food web along the salinity gradient in a Spanish saltern showed that the higher the salinity, the less important protozoa become in regulating prokaryote community densities (Pedrós-Alió et al. 2000; Joint et al. 2002). In spite of this, there is a long list of heterotrophic ciliate, flagellate and amoeboid protozoa that have been

observed to occur at high salinities (Hauer & Rogerson 2005). The recent finding of flagellate protozoa preying on prokaryotes in solar saltern crystallizer brines in Korea (Park et al. 2003) shows that a reevaluation of the role of protozoa in saline and hypersaline lakes is warranted.

In spite of the abundance of brine shrimp species (*Artemia* spp.) in many salt lakes worldwide, we know little about the importance of these brine shrimps in regulating prokaryote community densities. Recently a number of studies have been initiated to examine the nature of the prokaryotes associated with *Artemia* and its intestinal system.

At the highest salinities bacteriophages appear to be more important in regulating community densities of halophilic Archaea and Bacteria than are protozoa (Pedrós-Alió et al. 2000). Only few studies have thus far been devoted to the diversity of bacteriophages in saline and hypersaline lakes. Following a bloom of red halophilic Archaea in the Dead Sea in 1992–1993, virus-like particles abounded in the water column, outnumbering prokaryote cells by an order of magnitude (Oren et al. 1997). Some characterization of bacteriophage numbers and properties has also been performed in Mono Lake (Jiang et al. 2004), in the Salton Sea (Wood et al. 2002), and in Spanish solar salterns (Diez et al. 2000). However, our understanding of the biology of these phages and their interrelationships with the prokaryotes they infect is in most cases very limited.

The Importance of Interdisciplinary Studies to Advance our Understanding of the Microbial Ecology of Salt Lakes

In recent years there have been a number of comprehensive studies in which single hypersaline environments were studied simultaneously by large interdisciplinary teams of scientists. Such studies, which applied a wide range of complementary approaches on the same environment and the same samples, have contributed invaluable information on the nature and functioning of the microbial communities in such environments much more than a large number of isolated studies could have done.

The MIDAS ('Microbial Diversity in Aquatic Systems') workshop, held at the salterns of Santa Pola, Alicante, Spain in May 1999 and sponsored by the European Union, presents a beautiful example of what can be achieved when a number of international research teams, each with its own expertise, work together to learn about the microbiology of a hypersaline environment. Publications emerging from that

workshop have dealt with the prokaryotic genetic diversity along the salinity gradient using different approaches (Benlloch et al. 2002; Casamajor et al. 2002), viral diversity along the salt gradient (Diez et al. 2000), the diversity of planktonic photoautotrophic microorganisms (Estrada et al. 2004), assessment of primary production, nutrient assimilation and microzooplankton grazing (Joint et al. 2002), and evaluation of the factors that control heterotrophic prokaryotic abundance (Gasol et al. 2004). Moreover, the samples collected from the crystallizer ponds during that workshop have yielded some of the first isolates of *Salinibacter ruber*, the novel type of extremely halophilic bacteria phylogenetically affiliated with the *Bacteroidetes* but physiologically closely resembling the archaeal family *Halobacteriaceae* (Antón et al. 2002).

The 'Microbial Observatories' program, initiated by the U.S. National Science Foundation, has enabled more such studies to be made in hypersaline environments. Studies in the Great Salt Plains, Oklahoma in recent years have shown how much novel information can be obtained when properly funded. The scientific output thus far includes papers on the characterization of halotolerant aerobic heterotrophic bacteria from the Great Salt Plains (Caton et al. 2004), carbon substrate utilization, antibiotic sensitivity, and numerical taxonomy of bacterial isolates (Litzner et al. 2006), aerobic biodegradation of aromatic compounds by the halophilic communities (Nicholson & Fathepure 2005), DNA-repair potential of *Halomonas* spp. isolated from the from the ecosystem (Wilson et al. 2004), a novel bacteriophage (Seaman & Day 2007), and diversity of diatoms (Potter et al. 2006), cyanobacteria (Kirkwood et al. 2008) and other algae (Henley et al. 2006).

More such studies are needed in other salt lakes and other salt-stressed ecosystem, with appropriate funding, to solve the basic questions relating to the importance of prokaryotic and eukaryotic microorganisms in saline lakes and other hypersaline ecosystems. Studies of interdependence between photoautotrophs and heterotrophs could be of particular value.

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Report of the ISSLR Conference Working Session on Lessons Learned for Setting Agendas for Saline Lakes Research

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ABSTRACT

Minutes of a session of the joint meeting of the 10th International Conference on Salt Lake Research & FRIENDS of Great Salt Lake Issues Forum summarize and categorize approximately 150 comments made by 80 participants on lessons learned for setting research agendas for saline lakes. Successful research agendas for saline lakes appear to have three components: strong science, proactive communication, and sufficient support. This paper summarizes diverse perspectives expressed during the working session.

INTRODUCTION

On May 15, 2008, eighty attendees of the 10th ISSLR conference shared perspectives concerning the reasons why some saline lakes research programs succeed and others fail. The session was prompted by a desire to learn from each other and by the immediate need of the State of Utah for guidance to prioritize research needs for Great Salt Lake. The ISSLR working session did not focus on the relative merit of specific research objectives but focused on lessons learned on how to set research agendas. This short report does not attempt to analyze participants' perspectives or to evaluate the efficacy of this working session compared to attempts of similar nature.

After brief introductory remarks exhorting participants to set aside their personal projects and to think as decision makers, work-session attendees divided into groups of 2-8 people, designated a scribe for their group, and brainstormed three questions: (1) what has worked, (2) what has not worked, and (3) what advice they had with respect to setting research priorities for saline lakes. After an hour of lively discussion, attendees reconvened for a 20 minute committee of the whole and scribes reported group insights. The following compendium of comments includes inconsistent, even contradictory, comments. It is intended as shared, wisdom from a diverse group of individual researchers and managers who have observed successes and failures of science and public policy. It does not represent consensus of the working session participants, the conference leaders, the ISSLR, or even the author's views. Comments from scribes were abbreviated and organized in three general categories: comments with respect the scientific substance of research; comments with

respect to communication of research needs and findings; and comments with respect to obtaining sufficient funding for research programs. The scribes attempted to capture the substance of participant comments in the following listings, but both scribes and the author recognize they may not have fully communicated subtleties of participant insights.

DISCUSSIONS

Context: Every saline lake is unique. Different lakes require different research approaches. Research related to different saline lakes is at different stages. Long- and short-term research goals should be framed for the physical conditions, socioeconomic conditions, and management needs of a specific lake. However, saline lakes share sufficient commonalities that colleagues can learn from successes of well-funded, science-driven programs, and perhaps avoid pitfalls of unsuccessful research programs. Participants recognized and did not enumerate the variety of values and variety of threats facing saline lakes.

What has worked to successfully achieve the scientific objectives of research programs: establish long-term research goals; develop a clear picture of the hydrogeomorphic conditions of a system; understand mass balances (quantity and quality); know where water comes from, where and how it is stored, and where it goes; take time to identify key substantive issues and assure that research addresses the highest priority issues; invest in baseline monitoring because monitoring is essential for analyses of change; plan long-term integrated land management of entire catchment areas; attempt to understand the system as a whole; encourage collaborative cross-disciplinary studies; encourage expert-driven full-lake oriented studies that produce general knowledge of a saline lake; and conduct retrospective analyses to evaluate research quality and applicability.

What has worked to facilitate communication: hold coordination meetings of technical researchers; seek public participation; consult with decision makers and diverse stakeholders; assure proper diagnosis of stakeholders; disseminate research and policy results to the public; seek multidisciplinary input through workshops and meetings to apply scientific perspectives to technical, political, and

social challenges; develop bottom-up approaches with input from front-line managers and local researchers; use web-sites to share research and news; apply geospatial tools to store, display, interpret, and communicate results of scientific studies to wide ranges of scientists, stakeholders, and managers; use landscape analysis as a way to transfer knowledge of hydrogeomorphic settings; and apply technically-based and agreed-upon methods and communicate technical results.

What has worked to successfully obtain financial and societal support: take advantage of eco-disasters, make positive use of crises; fund applied studies that produce general knowledge of a saline lake; litigate or threaten litigation such as evocation of the public trust doctrine; have a point-agency whose mission is the lake and all its stakeholders; find, educate and nurture powerful “champions” who support the research; and address “low hanging fruit” meaning make early easily-attained progress on issues of immediate interest to stakeholders.

What has undermined achieving the scientific objectives of saline lake research: not coordinating efforts; setting too many top priorities (it’s the same as having no priorities); not sufficiently planning or allocating sufficient resources or time to do the work; failing to manage expectations of scientists, managers and stakeholders; not acting because of overwhelming needs; reacting to, rather than in anticipation of crises; following research agendas torqued by industry or environmentalist pressures; failing to work across political jurisdictions for multi-jurisdictional closed-basin systems; competing with research for fresh water systems; not appreciating the local and global role of saline systems such as saline wetlands; trying to do too much with too little; cutting corners; and skipping peer review.

What has undermined successful communication of research on saline lakes: use of jargon that obfuscates science and derails decision makers; use of different units in diverse languages to describe saline lake characteristics, such as salinity (i.e., use of TDS, ppt, percent, and specific gravity); not having standard methods for working in saline lakes; and working in an environment where trust is lacking.

What has undermined financial and societal support for research on saline lakes: using top-down approaches to set priorities; depending on local community support when the understanding of a lake is limited; relying on support from an unsupportive department of government with potentially different mandates such as an agency dedicated solely to extractive resources, or an agency with higher priorities, or an agency too small to have political

clout; receiving unbalanced lobbying efforts; and making decisions about a lake on information so grossly inadequate that major consequences are not anticipated.

Specific advice for successfully achieving scientific objectives of saline lake research: establish / continue baseline monitoring; analyze data sets and drivers to anticipate future impacts and research needs; prioritize topical research based on human health, ecosystem health, and economic impacts; recognize how scale affects research definition, plans, and approaches; target potential for early successes to show payback for investment of public funds; fund ecosystem initiatives appreciating in advance their magnitude and that they require long-term commitment; encourage collaborative, cross-disciplinary research; take advantage of the relative simplicity of saline lake ecosystems; understand closed-basin dynamics; and focus locally and think globally, meaning recognize how each system is unique but research is instructive beyond its borders.

Specific advice for successful communication of scientific research on saline lake and for establishing a strong funding base with political support: define stakeholders for specific projects; understand and address local stakeholders’ issues and questions; involve stakeholders meaningfully in two-way, not-one way, communication; meet early and meet often to share information among scientific researchers; require publication and shared research results; require efficient allocation of research funds tied to ecosystem and other indicators; understand and address local stakeholder issues and questions; quantify and widely tout the economic, social, and environmental values of the lake; recognize the vulnerabilities of a lake and protect it; quantify the global as well as local value of the saline systems; counter perceptions of worthless, dead systems with scientific and economic examples; be alert for opportunities for eco-disaster tourism; harness the energy of crises to gain advocacy; build constituencies; identify common enemies; and forge ahead.

SUMMARY

From the organizers’ perspective, the session was successful. It encouraged shared knowledge such as P. Coleman’s web link *Methods Manual for Salt Lake Studies* (http://en.wikibooks.org/wiki/Methods_Manual_for_Salt_Lake_Studies). It directly impacted the allocation and focus of State of Utah 2008–2009 discretionary research funds. As expected, Great Salt Lake Tech team chose to invest most of the recently appropriated funding for expansion and upgrades of baseline monitoring. However, largely in response to working session coaching

to invest some funds in high-profile research with promise for rapid results, it designated \$100000 for short-term, “hot topic” research to be identified by stakeholders.

The session highlighted the opportunity of international conferences for sharing ideas on mechanisms for furthering research in addition to their usual role of sharing research accomplishments. The session was boisterous and provocative. Scribes recorded approximately 150 comments. The comments divided about equally in four categories: (1) specific needs of specific lake systems (not itemized), (2) proactive communication of research needs and results, (3) ways to secure funding, and (4) approaches to strong science and quality control. There was no consensus. However, participants suggested that a panel of experts, such as a committee of the National Research Council, might develop a global agenda, meaning a blue

print or working plan including schedules that could serve as a generic model for approaching saline lake research. Although discussion was wide-ranging and opinions were diverse, all agreed that lack of monitoring and research has been detrimental to effective management of saline lakes and that investment in understanding saline systems is especially needed with increased societal demand for water and expected changes in climate.

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Spiral Jetty from atop Rozel Point, in mid-April 2005 by Soren.harward. Wikimedia Commons at en.wikipedia.org. Accessed February 2009.

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