Extremophiles (2009) 13:355–361
DOI 10.1007/s00792-008-0223-1

ORIGINAL PAPER

Competitive exclusion of Cyanobacterial species in the Great Salt Lake

Hillary C. Roney · Gary M. Booth · Paul Alan Cox

Received: 25 July 2008 / Accepted: 16 December 2008 / Published online: 8 January 2009

Abstract The Great Salt Lake is separated into different salinity regimes by rail and vehicular causeways. Cyanobacterial distributions map salinity, with *Aphanothece halophytica* proliferating in the highly saline northern arm (27% saline), while *Nodularia spumigena* occurs in the less saline south (6–10%). We sought to test if cyanobacterial species abundant in the north are competitively excluded from the south, and if southern species are excluded by the high salinity of the north. Autoclaved samples from the north and south sides of each causeway were inoculated with water from each area. *Aphanothece, Oscillatoria, Phormidium*, and *Nodularia* were identified in the culture flasks using comparative differential interference contrast, fluorescence, and scanning electron microscopy. *Aphanothece halophytica* occurred in all inocula, but is suppressed in the presence of *Nodularia spumigena*. *N. spumigena* was found only in inocula from the less saline waters in the south, and apparently cannot survive the extremely hypersaline waters of the northern arm. These data suggest that both biotic and abiotic factors influence cyanobacterial distributions in the Great Salt Lake.

Communicated by L. Huang.

H. C. Roney · P. A. Cox (✉)
Institute for Ethnomedicine, Box 3464,
Jackson Hole, WY 83001, USA
e-mail: paul@ethnomedicine.org

G. M. Booth
Department of Plant and Wildlife Science,
Brigham Young University, Provo, UT 84602, USA

Keywords Competitive exclusion · Halophilic bacteria · *Aphanothece* · *Nodularia* · *Oscillatoria* · *Phormidium* · Gause's principle

Introduction

Cyanobacteria are well-adapted for living in harsh conditions, including photosynthetic areas beneath Antarctic ice, hot springs and geysers in Yellowstone, and hypersaline lakes, including the Great Salt Lake (Dyer 2003). Two common cyanobacteria species that have been identified from the Great Salt Lake are *Aphanothece halophytica* and *Nodularia spumigena* (Brock 1976; Felix 1978; Felix and Rushforth 1980), with *N. spumigena* episodically blooming in Farmington Bay (Marcarelli et al. 2006). Since nitrogen is the limiting nutrient in the Great Salt Lake (Oren 2002) it is interesting to note that both *A. halophytica* and *N. spumigena* can fix nitrogen.

The Great Salt Lake is a hypersaline remnant of the Pleistocene Lake Bonneville (Oren 2002) which was 557 km long and 233 km wide with an area of 51,800 km^2 in what is now Utah, Idaho, and Nevada (Utah Geological Survey 1990). As Lake Bonneville retreated, the lake lost all outlets, so salinity increased.

After completion in the mid 19th century of the transcontinental railway near Promontory Point, Utah, trains had to traverse many additional rail kilometers around the northern end of the Great Salt Lake. To reduce this distance, the Union Pacific Railroad constructed a 19 km rail causeway across the Great Salt Lake in 1959 (Fig. 1), replacing an earlier trestle built in 1902 by the Southern Pacific Railroad. Unlike the former wooden trestle, which did not impact water flow, the 1959 causeway built with rock fill, hydrologically divided the

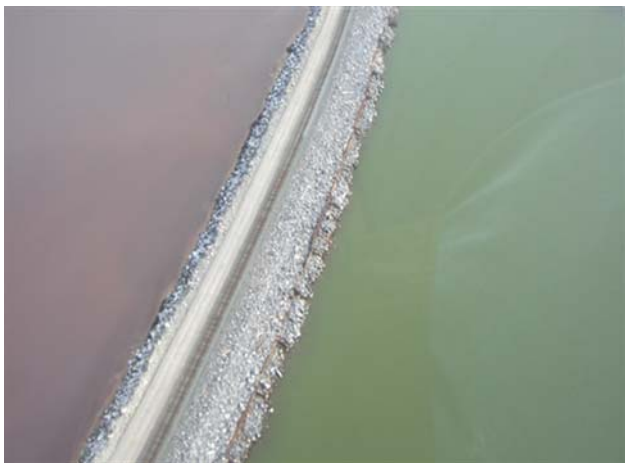

Fig. 1 Great Salt Lake Rail Causeway with hypersaline water in the north (*left*) and less saline water on the south (*right*)

waters of the Great Salt Lake into two portions, a northern arm with negligible freshwater inputs, and a southern arm with more than 90% of the freshwater flow (Butts 1980; Oren 2000; Stephens and Gillespie 1976; Sturm 1980). Three major rivers–the Bear, Weber, and Jordan–all flow into the Great Salt Lake south of the railway causeway (Gwynn 2002). The overall salinity of the Great Salt Lake, which to that point had been linked solely to changing water levels, quickly adjusted with the northern arm becoming even more hypersaline, moving from an average of 15% in the 1870s to as high as 28% salinity in the 1960s (Sturm 1980). In 1970, the northern arm held approximately 330–350 g salts per liter, while the south arm held 120–130 g salts per liter (Oren 2002). While the major cation in the water is Na, Mg, K, Ca, in decreasing order of abundance are important as is the anion SO_4 (Sturm 1980).

These habitat changes were later partially replicated with construction of a second barrier to lake water flow. A causeway for vehicular use has been periodically constructed from Syracuse, Utah to Antelope Island, and was most recently rebuilt in 1992 (Gwynn 2002). These vehicular and railway causeways resulted in the partitioning of the Great Salt Lake into three different salinity regimes: the northern arm, with an average of 27% salinity, the middle arm with average salinity of 10–16%, and the southern arm, with average salinity of 6% or less (Utah Geological Survey 1990). These three different salinity regimes, any one of which would be considered hypersaline, allowed species to sort according to ecological tolerances. The results are striking: each large area of the lake has different colored water, resulting in part from different concentrations of *Artemia fransiscana* brine shrimp cysts and microscopic green algae such as *Dunaliella salina* and *D. viridis* as well as species of the Archean genus *Halobacterium* (Post 1981), but also perhaps due to different concentrations and species compositions of cyanobacteria.

A study was designed to determine whether cyanobacterial distributions in the Great Salt are influenced by abiotic factors, biotic factors, or both. To explore this question, experiments were designed to examine two hypotheses; Hypothesis 1: Cyanobacterial species abundant north of the railway causeway are competitively excluded from the south by other species, and Hypothesis 2: Cyanobacterial species that thrive and bloom south of the Antelope causeway cannot grow in high salinity waters from the north.

Materials and methods

Experimental cultures

A total of 28 water samples from both the north and south sides of Antelope Island causeway and the north and south sides of the railway causeway (seven from each site) were collected in December 2007. Water temperatures and GPS coordinates were recorded at each site. To avoid pseudoreplication, six water samples from both sides of the vehicular and railway causeways, approximately four liters in volume, were used as inocula. The seventh jar from each side was approximately eight liters in volume and used for media after filtering and autoclaving. Approximately 30 ml of the filtered media water was placed in autoclavable, sterile 50 ml nalgene plastic flasks. In total there were 96 flasks inoculated with 10 ml of unsterilized water from either the north or the south of the railway and the vehicular causeways totaling six replicates of inoculum water for each medium type. No nutrients or other growth media were added to the water (Dyer 2003). In addition, 21 control flasks were prepared using autoclaved media and autoclaved inocula. A random number table was used to decide from which sample jars to draw the inoculum. In addition, one control flask was prepared which consisted of autoclaved distilled water inoculated into autoclaved distilled water medium. After all 129 flasks were inoculated; they were placed in a heated green house with constant 8.5 h/day illumination. Each flask was gently shaken by hand periodically for aeration. These liquid cultures were incubated for 7 weeks.

Cyanobacterial identification

For aquatic cyanobacteria, identification by light microscopy (phase and/or interference contrast), and scanning electron microscopy (SEM) are preferred (Cronberg and

Annadotter 2006). Identification and abundance counts of cyanobacteria from the culture flasks were performed using differential interference contrast (DIC) and epi-fluorescence imaging, with SEM for verification.

Data analysis

To ensure arbitrariness of cyanobacterial counts, microtransects of water cultured from each flask, based on two microscope slides were conducted. Each microtransect was replicated twice for each slide, with data entered on a six-cell mechanical lab counter. When mass colonies of cyanobacteria where encountered precluding individual counts, the colony were assessed as "large" or "very large", with medians and nonparametric analyses used to analyze qualitative data. In each of the 16 possible combinations of 4 types of media (railway north, railway south, Antelope north, and Antelope south) and 4 types of inocula (railway north, railway south, Antelope north, and Antelope south), the median counts of the 4 major cyanobacterial species (*A. halophytica, Oscillatoria* sp., *Phormidium tenue*, and *N. spumigena*) were ranked.

A two-way Analysis of Variance (ANOVA) was calculated for abundance of *A. halophytica* in the culture flasks with "large" colonies scored as 500 and "very large" colonies scored as 1,000 for this purpose. To reduce impact about outliers and ensure consistency of distribution across the observed range, all data were transformed with a square root transformation prior to analysis as is standard for count data. F statistics for the transformed data were calculated to test three different pairs of hypotheses, with the null hypothesis to be rejected at the $P < 0.05$ level:

Hypothesis pair #1

H_0: no variation in cyanobacterial counts exists due to differences in media.
H_1: variation in cyanobacterial counts exists due to differences in media.

Hypothesis pair #2

H_0: no variation in cyanobacterial counts exists due to differences in inocula.
H_1: variation in cyanobacterial counts exists due to differences in inocula.

Hypothesis pair #3

H_0: no variation in cyanobacterial counts exists due to interactions.
H_1: variation in cyanobacterial counts exists due to media and inocula interactions.

For cyanobacterial taxa which proved to be of rare occurrence in the culture flasks, exact logistic tests, rather than an ANOVA, were calculated.

Results

Experimental cultures

When sampling for the experimental cultures of the Great Salt Lake, profoundly different colors on either side of the railway causeway were observed from the air (Fig. 1). These differences were also apparent in water samples taken from deep water on either side of the causeway rather than evaporative ponds (Fig. 2). Salinity from the sample sites was previously measured by hydrometers—south side of Antelope causeway 20.6 ppt, north side of Antelope causeway 75 ppt, south side of railway causeway 155 ppt, and north side of railway causeway 195 ppt (Roney 2007). Salinity values of the flasks were altered slightly by addition of inocula, except when the same inoculum was added to the same medium.

At the time of sampling in December 2007, ambient air temperature was −2.2°C on the railway causeway (41°13′16″N112°32′3′34″W) and −2.8°C on the vehicular causeway (41°4′44″N112°12′57″W), with water temperatures north of the railway causeway at 2.0°C, south of the railway causeway at 4.3°C. Water temperature north of the vehicular causeway was 3.3°C, while the water temperature south of the causeway was 2.5°C. Analysis by light microscopy showed no growth in any of the 21 control flasks, which were found to be sterile.

Cyanobacterial identification

Four genera of cyanobacteria, *Aphanothece, Oscillatoria, Phormidium* and *Nodularia*, were identified (Figs. 3, 4, 5, 6), with identifications confirmed by Dr. James Metcalf

Fig. 2 Water samples taken on north (*left*) and south (*right*) side of railway causeway. Color differences primarily due to *Dunaliella* distributions although the cyanobacterium *Aphanothece* flourishes in the hypersaline waters in the north

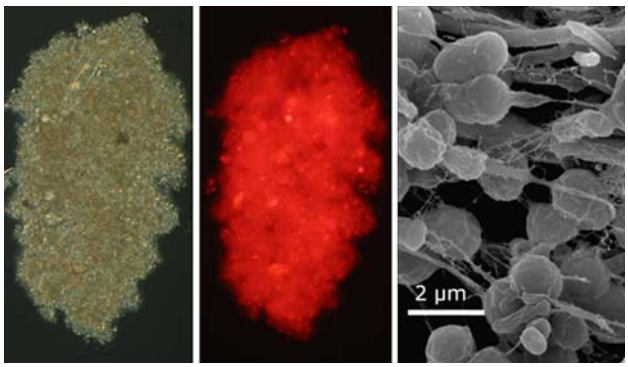

Fig. 3 *Aphanothece halophytica*: differential interference contrast (*left*); fluorescence (*middle*); scanning electron microscopy (*right*)

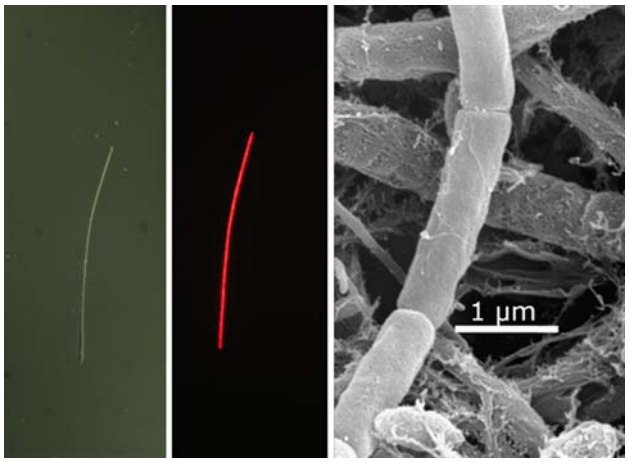

Fig. 4 *Oscillatoria* sp.: differential interference contrast (*left*); fluorescence (*middle*); scanning electron microscopy (*right*)

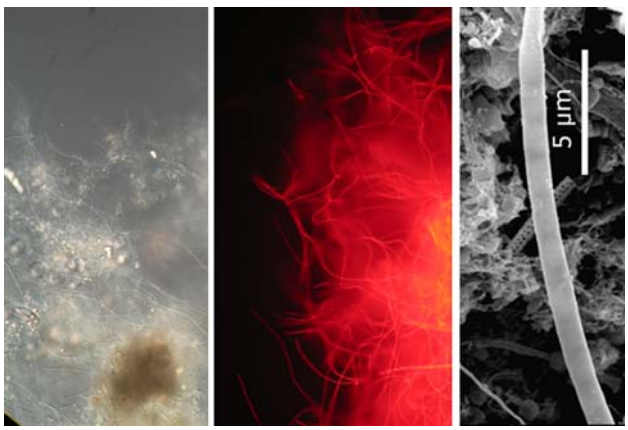

Fig. 5 *Phormidium tenue*: differential interference contrast (*left*); fluorescence (*middle*); scanning electron microscopy (*right*)

(University of Dundee, Scotland). In addition, a fifth cyanobacterial genus, *Spirulina*, was observed, but was not found in any transect through any of the microscope slides.

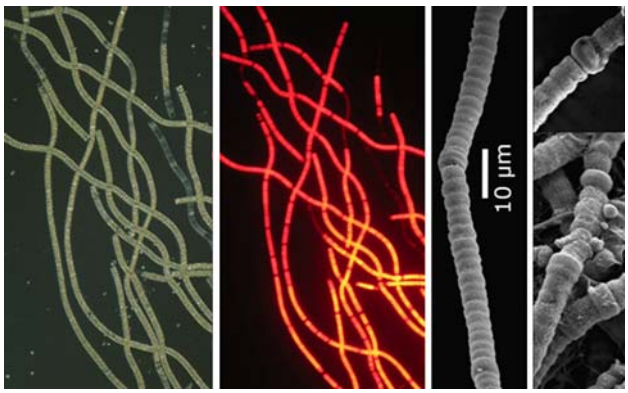

Fig. 6 *Nodularia spumigena*: differential interference contrast (*left*); fluorescence (*middle*); scanning electron microscopy (*right*)

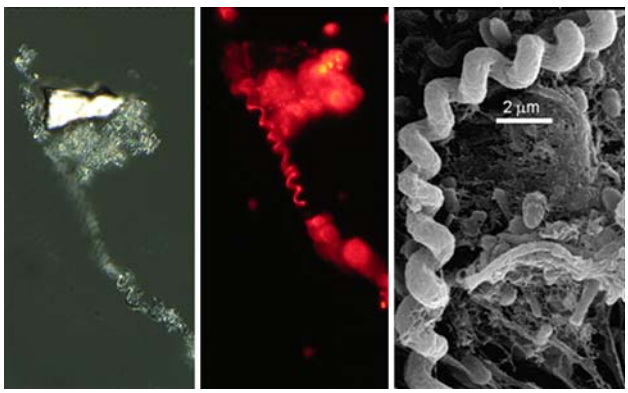

Fig. 7 *Spirulina* cf. *labyrinthiformis*: differential interference contrast (*left*); fluorescence (*middle*); scanning electron microscopy (*right*)

Because of its trichomes and its affinity for saline waters, this species is referable to *Spirulina labyrinthiformis* (Fig. 7), although Nübel et al. (2000) have placed a similar salt-tolerant species into the new genus, *Halospirulina*. Comparisons between differential interference contrast microscopy, fluorescence microscopy, and scanning electron microscopy allowed different observations of cyanobacterial morphology and size to be compared for taxonomic identification.

Data analysis

Comparative medians of the four cyanobacterial genera for each inocula type in the four media are shown in Fig. 8, which demonstrates that *A. halophytica* appears throughout all the four types of media and inocula, but that *Nodularia spumigena* is abundant only in inocula from Antelope south waters. Since there are 24 different permutations of the ordered ranks of *Aphanothece* (A), *Oscillatoria* (O), *Nodularia* (N), and *Phormidium* (P) plus an additional 16 permutations of three and two-way ties, as well as one

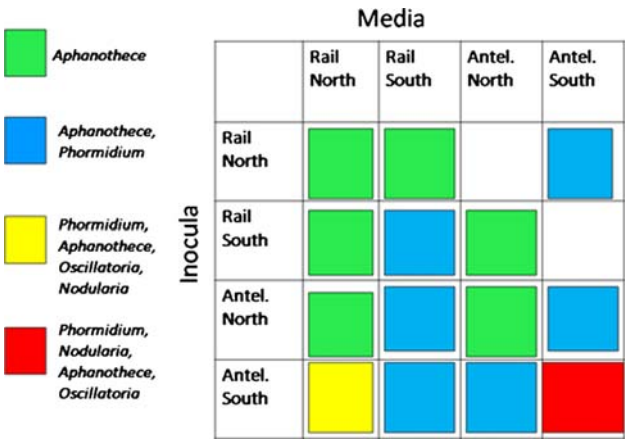

Fig. 8 Rankings of cyanobacterial dominance by medians. Media/Inocula in the *upper left corner* of the chart are extremely hypersaline, while those in the *lower right corner* are far less saline

Table 1 ANOVA of *Aphanothece halophytica* distributions in autoclaved media from the Great Salt Lake

Source	Variation	Degrees free	Mean square	*F* Statistic	Significance
Media	64.6	3.0	21.5	11.4	$P < 0.01$
Inocula	26.1	3.0	8.7	4.6	$P < 0.01$
Interaction	105.6	9.0	11.7	6.2	$P < 0.01$
Subtotal	196.3	15.0			
Error	151.1	80.0	1.9		
Total	347.4	95.0			

possible case of a four-way tie in rank, there are 41 different possible rankings of the four cyanobacterial species. These different rankings can perhaps most easily be portrayed as different colors (Fig. 8). *A. halophytica* was dominant in all cultures flasks, except those in which *N. spumigena* and *Oscillatoria* sp. occurred.

A two-way ANOVA for the distributions of *A. halophytica* was performed as indicated in Table 1. The F statistics for the ANOVA allows each of the null hypotheses in the three pairs of hypotheses to be rejected at the $P < 0.05$ level. Therefore, it can be concluded that media and inocula, as well as the interaction between media and inocula significantly affected the growth of *A. halophytica* in the culture flasks. Exact tests were calculated for *N. spumigena* and *Oscillatoria* spp. using the exact option of pro logistic in SAS. In these analyses, counts were ignored, and instead, presence/absence data were used. For *Oscillatoria*, the *P* value for the exact test of the medium was 0.0993; thus the effect due to media differences was not significant. However, the *P* value for the exact test of inoculum was 0.0016; hence there was a significant inoculum effect in distribution of *Oscillatoria*. The exact test for the inocula/media interactions was not significant with a *P* value of 0.1963. Leaving interaction out of the model, the additive model (with additive effects of inoculum and medium) showed the odds of a positive response for inocula from rail south, rail north, or Antelope north was just 6.3% relative to inoculum from Antelope south (95% confidence interval: 0.6–64.2%). Thus, Antelope south inocula had a significant positive effect on the presence of *Oscillatoria* in the culture flasks.

A similar analysis was conducted for the presence or absence of *N. spumigena* in the culture flasks. The exact test for the inocula/media interaction had a *P* value of 0.0032; hence the interaction was significant. The odds of a positive effect were extremely high for the combination of Antelope south inoculum with Antelope south medium. For all other combinations, the odds of a positive response were extremely low. For future studies of algal-cyanobacterial interactions, counts were also made of the green alga *Dunaliella salina* and *D. virids* in the flasks; an ANOVA of square root transformed data count for *Dunaliella* showed significant differences in distributions similar to *Aphanothece* distributions; these data will be reported elsewhere.

Discussion

Both the ranking of median abundances in the culture flasks and the results of the two-way ANOVA support the overall hypothesis: cyanobacterial species abundant north of the railway causeway (e.g. *A. halophytica*) are competitively excluded from the south by other species, in this case *N. spumigena* and *Oscillatoria* spp. It appears that the cyanobacterium *A. halophytica* can grow in less saline waters as well as the extreme saline waters north of the railway causeway—since it is found in all inocula—but its growth appears to be suppressed in the south by the presence of *N. spumigena*, which periodically blooms in the Great Salt Lake.

In previous years, we have noted large *N. spumigena* blooms in the low salinity regime of Farmington Bay, as well as in water samples collected south of the railway causeway (Roney 2007), particularly when winds have concentrated blooms near the causeway. Rushforth and Felix (1982) recorded *N. spumigena* as rare in the south arm; perhaps they took their samples at a dormant season, as *N. spumigena* blooms episodically. The absence of *N. spumigena* in the southern arm of the Great Sale Lake may have influenced the ability of *A. halophytica* to migrate and prosper in the fresher water environment of the south arm instead of thriving in the hyper-saline north arm. In all of our samples south of the Antelope causeway (Farmington) since 2004, *N. spumigena* was present in the water column.

The second overall hypothesis—that cyanobacterial species that thrive and bloom south of the Antelope

causeway cannot grow in the high salinity of the north—is also supported by these experimental data. *N. spumigena* was found only in inocula from the less saline waters south of the Antelope Island causeway, and apparently cannot survive the high saline waters north of the railway causeway.

Experimental support for these two general hypotheses helps shed light on our original question: are cyanobacterial distributions in the Great Salt Lake influenced by abiotic factors, biotic factors, or both? From these experiments, it appears that both abiotic (salinity) and biotic (interspecies competition) factors seem to affect distributions of cyanobacterial species. *N. spumigena* distributions seem to be primarily influenced by salinity, since it can only grow in fresher waters. By contrast, *A. halophytica* distributions seem to be primarily influenced by competition from *N. spumigena* and *Oscillatoria* sp. There are, of course, other geochemical processes which we did not measure but which may affect distributions. We are also interested in the relationship between the green alga *Dunaliella* and cyanobacteria. Our initial analysis suggests a commensalism with *Dunaliella* benefitting from the presence of nitrogen fixing *Apanothece*: in our microscopic analysis we often observed *Dunaliella* cells clustered around mass colonies of *Apanothece*. It would be interesting if nitrogen fixed by *Apanothece* in hypersaline environments contributed to exceptional salt tolerance of *Dunaliella* (Zamir et al. 2004).

These experimental results are consistent with Gause's principle, which predicts that no two species can indefinitely occupy the same niche (Gause 1969; Hardin 1960), since there is a clear niche partitioning between *A. halophytica* and *N. spumigena* in the Great Salt Lake. These two species cannot occupy the same hypersaline habitat north of the railway causeway, since *N. spumigena* cannot tolerate hypersaline conditions, and *A. halophytica* is suppressed in the presence of *N. spumigena* in the less saline southern waters.

However, this leaves unanswered the question of why *A. halophytica* is not totally excluded from the south, since it occurs in all samples of inocula, regardless of salinity. Perhaps *A. halophytica* is periodically excluded from southern waters by *N. spumigena* blooms, but during intervals between blooms, the extremely small *A. halophytica* persists, albeit at lower levels. Thus, Gause's Principle should perhaps include a clarification: two species cannot indefinitely occupy the same niche, except when that niche is temporally partitioned, as occurs with episodic blooms of *Nodularia*. This structuring of the cyanobacterial regimes by salinity (Williams 1998) is consistent with the intermediate salinity hypothesis of David Herbst (1999): "Abundance of salt-tolerant organisms is limited by physiological stress at high salinities, and by ecological factors, such as predation and competition, in more diverse communities at low salinities". Since *N. spumigena* distributions cannot survive the high salinity stress of waters from the north arm of the Great Salt Lake, and *Aphanothece halophytica* is competitively excluded by *N. spumigena* at lower salinities, the intermediate salinity hypothesis may apply.

The precise set of conditions that trigger episodic *N. spumigena* blooms is unknown. Our data suggest that these episodic blooms play a major role in excluding *A. halophytica* from vast areas of the Great Salt Lake. Being able to predict the occurrence of *Nodularia* blooms would not only be of theoretical importance; it might also lead to a better understanding of cyanobacterial blooms and cyanotoxin impacts on wildlife and human health (Cox et al. 2005; Metcalf et al. 2008).

Acknowledgments We thank J. Metcalf for assistance in cyanobacterial identification, J. Gardner for assistance in scanning electron microscopy, and B. Schaalje for assistance with biostatistics. We are grateful to the Wood Family Foundation for the Mus Views DIC/Fluorescent Microscopy Facility at the Institute for Ethnomedicine, and A. Fransiscana and R. Smithson for inspiration in our studies of the Great Salt Lake.

References

Brock TD (1976) Halophilic blue-green algae. Arch Microbiol 107:109–111

Butts DS (1980) Factors affecting the concentration of Great Salt Lake brines. In: Gwynn JW (ed) Great Salt Lake: a scientific, historical, and economic overview. Utah Geological and Mineral Survey, Salt Lake, pp 163–167

Cox PA, Banack SA, Murch SJ, Rasmussen U, Tien G, Bidigare RR, Metcalf JS, Morrison LF, Codd GA (2005) Diverse taxa of cyanobacteria produce β-N-methylamino-L-alanine, a neurotoxic amino acid. Proc Natl Acad Sci USA 102(14):5074–5078

Cronberg G, Annadotter H (2006) Manual of aquatic cyanobacteria: a photo guide and a synopsis of their toxicology. International Society for the Study of Harmful Algae, Copenhagen, pp 1–106

Dyer BD (2003) A field guide to bacteria. Cornell University Press, New York, pp 1–355

Felix EA (1978) MS Thesis: the algal flora of the Great Salt Lake. Department of Botany and Range Science Brigham Young University, Provo, pp 1–37

Felix EA, Rushforth SR (1980) Biology of the South arm of the Great Salt Lake, Utah. In: Gwynn JW (ed) Great Salt Lake: a scientific, historical, and economic overview. Utah Geological and Mineral Survey, Salt Lake, pp 305–312

Gause GF (1969) The struggle for existence. Hafner, New York, pp 1–163

Gwynn JW (2002) Great Salt Lake: an overview of change. Utah Dept. Natural Resources, Salt Lake, pp 1–584

Hardin G (1960) The competitive exclusion principle. Science 131:1292–1297

Herbst DB (1999) Biogeography and physiological adaptations of the brine fly genus *Ephydra* (Diptera: Ephydridae) in saline waters of the Great Basin. Great Basin Natur 59:127–135

Marcarelli AM, Wurtsbaugh WA, Griset O (2006) Salinity controls phytoplankton to nutrient enrichment in the Great Salt Lake, Utah, USA. Canad J Fisheries Aquat Sci 63:2236–2248

Metcalf JS, Banack SA, Lindsay J, Morrison LF, Cox PA, Codd GA (2008) Co-occurrence of β-N-methylamino-L-alanine, a

neurotoxic amino acid with other cyanobacterial toxins in British waterbodies, 1990–2004. Environ Microbiol 10(3):702–708

Nübel U, Garcia-Pichel F, Muyzer G (2000) The halotolerance and phylogeny of cyanobacteria with tightly coiled trichomes (*Spirulina turpin*) and the description of *Halospirulina tapeticola* gen. nov., sp. nov. Intl J Syst Evol Microbiol 50:1265–1277

Oren A (2000) Salts and Brines. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Kluwer, Dordrecht, pp 281–306

Oren A (2002) Halophilic microrganisms and their environments. Kluwer, Dordrecht, pp 1–575

Post FJ (1981) Microbiology of the Great Salt Lake north arm. Hydrobiol 81–82:59–69

Roney H (2007) Competitive exclusion of cyanobacteria in the Great Salt Lake. Honors Thesis Brigham Young University, Provo, pp 1–36

Rushforth SR, Felix EA (1982) Biotic adjustments to changing salinities in the Great Salt Lake, Utah. USA Microb Ecol 8:157–161

Stephens DW, Gillespie DM (1976) Phytoplankton production in the Great Salt Lake, Utah, and a laboratory study of algal response to enrichment. Limnol Oceanog 21:74–87

Sturm PA (1980) The Great Salt Lake brine system. In: Gwynn JW (ed) Great Salt Lake: a scientific, historical, and economic overview. Utah Geological and Mineral Survey, Salt Lake, pp 147–162

Utah Geological and Mineral Survey (1990) The Great Salt Lake information sheet. Utah Geol Surv Pub Inf Ser 8:1–2

Williams WD (1998) Salinity as a determinant of the structure of biological communities in Salt Lakes. Hydrobiol 381:191–201

Zamir A, Azachi M, Bageshwar U, Fisher M, Gokhman I, Premkumar L, Sadka A, Savchenko T (2004) Molecular and functional adaptations underlying the exceptional salt tolerance of the alga *Dunaliella salina*. In: Ventosa A (ed) Halophilic microorganisms. Springer, Heidelberg, pp 165–176