Journal of
Plankton Research

plankt.oxfordjournals.org

J. Plankton Res. (2013) 35(5): 1154–1166. First published online June 4, 2013 doi:10.1093/plankt/fbt053

Salinity and nutrients influence species richness and evenness of phytoplankton communities in microcosm experiments from Great Salt Lake, Utah, USA

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Received January 25, 2013; accepted May 6, 2013

Corresponding editor: Beatrix E. Beisner

Harsh environments are typically characterized by some dominant variable limiting diversity, making them interesting systems for studying how species diversity patterns change with abiotic conditions. Several environmental factors with the potential to influence phytoplankton diversity in hypersaline lakes were examined with microcosm experiments using inoculum from the Great Salt Lake, Utah. Experimental combinations of salinity and nutrient supply were run at three different temperatures. Results confirm salinity as a strong determinant of phytoplankton diversity, while also demonstrating the importance of nutrient supply, where species richness decreased with increasing salinity and increased with nutrient enrichment. Community evenness decreased with nutrient enrichment, indicating few species were favored by nutrient enrichment, becoming very abundant. Community biomass was positively correlated with richness and negatively correlated with evenness. Additionally, the abundance of particular species, most notably *Dunaliella* sp., was strongly affected by salinity, temperature and nutrient enrichment following patterns observed in the Great Salt Lake, where its abundance increased with salinity, cooler temperatures and increased nutrient enrichment. These results add to growing evidence that while salinity is a dominant factor influencing diversity in

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hypersaline lakes, other abiotic factors are also important and can interact with salinity to influence phytoplankton communities.

KEYWORDS: Great Salt Lake; *Dunaliella* sp.; species richness; evenness; phytoplankton biomass; salinity

INTRODUCTION

Biodiversity has been and continues to be the focus of extensive research (Lubchenco, 1991; Storch *et al*., 2005), and understanding factors influencing biodiversity, species abundance patterns and species coexistence are therefore of great interest to ecologists (Huston, 1979). Humans increasingly alter and modify landscapes in ways that impact biodiversity; thus, it becomes ever more important to understand the consequences that our actions have on biodiversity (Ehrlich and Wilson, 1991; Chapin *et al*., 2000).

Extreme environments are typically characterized by a dominant environmental variable limiting species richness, and in hypersaline lakes, diversity is largely limited by the ability of each species to tolerate salinity stress, known as halotolerance. In many saline lakes around the world, a negative correlation between species richness and salinity has been observed (Hammer, 1986; Williams *et al*., 1990), yet experimental evidence demonstrating this relationship is limited. Even though they are found worldwide and comprise nearly as much water as freshwater ecosystems (Horne and Goldman, 1994), hypersaline lakes are comparatively understudied systems compared with their freshwater counterparts (Collins, 1977; Por, 1980; Williams *et al*., 1990; Williams, 1998; Williams, 2002) despite comprising commercially (i.e. mining of minerals and harvesting brine shrimp eggs) and ecologically (i.e. resting and feeding areas for migrating birds) important ecosystems (Hammer, 1986; Williams, 2002; Belovsky *et al*., 2011).

In the hypersaline Great Salt Lake (GLS) (Utah, USA), the largest hypersaline lake in North America and the fourth largest in the world, variability in salinity has largely been influenced by seasonally fluctuating freshwater inputs and by a rockfill railroad causeway constructed in 1957, which essentially divided the lake into two (Madison, 1970). Between 1900 and 1959, salinity in the GSL fluctuated from 20 to 27% due to variation in annual precipitation (Stephens, 1990). Since construction of the causeway, salinity in the north arm has ranged from 16 to 29%, while in the south arm of the lake, salinity has ranged from 6 to 28%, because a majority of freshwater enters this arm (Stephens, 1990; Stephens, 1998). Consequently, a connection between decreasing salinity in the south arm of the lake and an increase in numbers and relative abundance of phytoplankton species has been observed (Felix and Rushforth, 1977; Felix and Rushforth, 1979; Felix and Rushforth, 1980; Rushforth and Felix, 1982; Stephens, 1990; Wurtsbaugh and Berry, 1990; Wurtsbaugh, 1992; Stephens, 1998). While it is likely that the overall increase in number of phytoplankton species observed in the south arm is due to the changes in salinity, experimental studies have not documented this.

The past observational studies have also noted that when salinities in the south arm of GSL are high (>15%), phytoplankton diversity is low and composition is dominated by two species of green algae from the halotolerant genera *Dunaliella*, *D. viridis* and *D. salina* (Stephens and Gillespie, 1976; Rushforth and Felix, 1982; Stephens, 1990; Wurtsbaugh, 1995; Stephens, 1998). In studying the halotolerance of *Dunaliella* sp. from GSL, Brock (Brock, 1975) found that a variety of algae grew at the lower salinities tested, while at higher salinities only *Dunaliella* sp. grew. However, these bioassays examined growth of isolated phytoplankton species, which may not reflect responses in mixed species assemblages.

Phytoplankton communities are influenced by a variety of factors impacting species composition and diversity, ranging from bottom-up (e.g. nutrients) to top-down factors (e.g. grazing). Across many aquatic communities, species richness has been observed to increase with fertilization, while community evenness decreases with fertilization (Hillebrand *et al*., 2007). Primary productivity, which is influenced by nutrient supply, has also been shown to influence species richness in lake ecosystems (Dodson *et al*., 2000). More recently, however, species richness has also been viewed as a determinant of primary productivity (Gross and Cardinale, 2007; Gamfeldt and Hillebrand, 2008; Korhonen *et al*., 2011). In the GSL, bottom-up factors in addition to salinity (e.g. nutrient concentrations and temperature) have also been observed to influence phytoplankton dynamics (Stephens and Gillespie, 1976; Wurtsbaugh, 1988; Marcarelli *et al*., 2006; Belovsky *et al*., 2011).

Because varying salinity in the GSL has been linked to shifts in phytoplankton diversity within the lake, we were interested in examining the role of salinity in determining

phytoplankton species richness and diversity, while also examining other factors (i.e. nutrient supply and temperature) shown to influence phytoplankton dynamics in the GSL (Marcarelli *et al.*, 2006; Belovsky *et al.*, 2011). We conducted a series of bioassay experiments that varied salinity, nutrient concentration and water temperature using inoculum from the GSL. We focused on three primary research questions: (i) how do changes in treatment combinations of salinity, nutrient addition and temperature affect phytoplankton species richness and evenness? (ii) How do species from the historically dominant genus *Dunaliella* respond to abiotic variables in multispecies assemblages? (iii) How are species richness and evenness correlated with total community biomass?

METHOD

Experimental design

In a series of seven bioassay experiments, we manipulated salinity (30, 60, 90, 120 and 150 g L^{-1}), nutrient addition (control: added nutrients only from inoculum; low: 50 μm N + 3.2 μm P added; and high: 250 μm N + 16 μm P added) and temperature (10, 20 and 30°C) (Table I). The molar ratio of the nutrient solution was at Redfield ratio, where nitrogen was supplied as NH_4NO_3 (49.6%), $CaNO_3$ (46.8%) and N-NO_3 (3.5%) and phosphorus as P_2O_5 (69.6%) and KH_2PO_4 (30.4%). Brine for experiments was a mixture of NaCl rock salt and Instant Ocean® (Spectrum Brands, Inc., Madison, Wisconsin, USA) at a ratio of 1.5:1. Salinity of brine was measured using a 1.000/1.220 specific gravity 300-mm hydrometer at 20°C, and values (in ‰) converted to g L^{-1}. From surface water collected in the south arm of the lake along the causeway to Antelope Island, 10 mL of inoculum, which had been filtered through a 100-μm netting to remove macrozooplaknton (i.e. *Artemia* sp.), was placed in Nalgene bottles containing 400 mL of brine solution and nutrients. Each treatment combination of nutrient/salinity had a minimum of three replicate bottles for each of the temperature runs (Table I). In several instances, there was a considerable difference between the temperature of the lake and the temperature used in the experimental run (Table I), which was not equivalent across all assays, yet we do not believe that this represented extreme stress on seed communities, as water from the lake was allowed to stabilize to room temperature overnight before each experimental run, and ultimately, the blocked design we employed (*see below*) accounted for variability between runs (Table I). Admittedly, the variability in salinities of the various inocula used was also narrow (60–90 g L^{-1}, Table I), yet the salinity treatments were well within the

Table I: Initial conditions of source algae and experimental treatments for the seven bioassay experiments run in this study

Experiment no.	Source date	Source salinity (g L^{-1})	H_2O Temperature (°C)	Source richness	Source evenness	Experiment temperature (°C)	Salinity treatments (g L^{-1})	Nutrient treatments	Number of replicates	*n*	Average days to equilibrium (by salinity)
1	12 March 1999	60	6.5	12	0.1911	10	30, 60, 90, 120, 150	C, L, H	5	75	31, 31, 31, 33, 35
2	3 September 1999	70	23	15	0.1468	30	30, 60, 90, 120, 150	C, L, H	3	45	14, 17, 17, 21, 45
3	3 December 1999	70	3	16	0.0253	10	30, 60, 90, 120, 150	C, L, H	3	45	25, 25, 44, 55, 72
4	1 March 2000	90	11.7	19	0.0113	20	30, 60, 90, 120, 150	C, L, H	3	45	24, 35, 35, 47, 68
5	28 July 2000	90	24	15	0.1554	10	30, 60, 90, 120, 150	C, L, H	3	27	35, 59, 61, lost, lost
6	13 November 2000	90	5	16	0.3183	20	30, 60, 90, 120, 150	C, L, H	3	45	21, 21.3, 31.7, 34, 40.2
7	23 May 2001	90	17	8	0.4404	30	30, 60, 90, 120, 150	C, L, H	3	45	19, 16.3, 15.3, 20, 43.7

Note: nutrient treatments: C, control; L, low nutrients; H, high nutrients.

range of salinities that phytoplankton species in the GSL encounter. Not unexpectedly, taxonomy varied between the various inocula used; however, during the analyses, measures were taken to account for this variability (i.e. blocked design, *see below*). Additionally, composition of communities observed in the bottles typically bore little resemblance to inoculum communities, with species that were either observed at very low numbers or not encountered in the inocula becoming abundant.

At the start of each bioassay experiment, the brine solution, inoculum and nutrient mixture in each Nalgene bottle were thoroughly mixed and 35 mL of the mixture was extracted and placed in a 50-mL test tube with a test tube cap. Test tubes were used throughout an experimental run to determine treatment effects on total community biomass (measured as change in *in vivo* fluorescence over time) of phytoplankton. Using a Turner Designs TD 700 fluorometer, we measured *in vivo* fluorescence in each test tube initially and then every 2 days throughout the experiment. Nalgene bottles and test tubes were positioned randomly in rectangular trays and kept in a temperature-controlled incubation chamber, with light intensities of approximately 150 μmol photons s^{-1} m^{-2} and a light–dark cycle of 14 h/10 h. Trays with bottles and test tube racks were systematically rotated every other day to reduce differences in irradiation between bottles and test tubes. Additionally, bottles and test tubes were lightly agitated every other day to ensure mixture of the brine solution. Once fluorescence began to reach an asymptote in each test tube (three consecutive measurements with no increase in fluorescence), nutrients were assumed to be depleted and a 25-mL sample of brine was extracted from the corresponding Nalgene bottle and preserved with Lugol's solution for analysis of community composition. We continued to monitor fluorescence in each test tube an additional three sample periods to verify that growth did not again increase. Conditions in test tubes may not have been identical to conditions in corresponding Nalgene bottles, but these were used only to estimate equilibrium in total biomass in Nalgene bottles (Table I).

The number and density of each phytoplankton species was estimated by counting three viewing strips (one diameter length) of a Palmer–Maloney counting cell at a magnification of ×250 and obtaining an average value. For species identification, a magnification of ×400 was used when identification at ×250 was not possible. Additionally, the entire chamber was scanned at lower magnification (×100) for rare taxa. Counting at these magnifications did not allow us to detect picoplankton (<2 μm), so they are not part of our analysis. To ensure accuracy of results, counting procedures were standardized across all experimental runs and all samples counted by one person (C.A.L.). Phytoplankton were identified to the lowest taxonomic category possible (usually genus or species) using Felix and Rushforth (Felix and Rushforth, 1979) and (Prescott, 1962; Patrick and Reimer, 1975). Biovolume for each species was estimated by measuring length and width measurements for 20 individuals and using equations for geometric shapes that most closely resembled cell morphologies (Hillebrand *et al.*, 1999). Cellular biomass of each sample was then determined by multiplying the number of cells of each species by its mean biovolume. Total community biomass for each sample was calculated as the sum total of all species-specific biomass estimates.

Data analysis

Univariate and multivariate analyses

With species richness (total species: S), Pielou's evenness ($J' = H'/\mathrm{Log}(S)$, where H' = Shannon Weiner Diversity Index and S = total species) and phytoplankton biomass as dependent variables, individual unbalanced blocked ANOVAs were conducted for each of the three temperatures tested, with inocula as a block to account for variability between inocula, and salinity and nutrient addition as treatments. Unbalanced ANOVAs were employed because several replicates were lost due to problems with the environmental chamber during one experimental run (Table I). Appropriate transformations of data were used to increase normality (i.e. *ln* for abundance data and arcsine square root for proportional data). For post-hoc pair-wise comparisons, Fisher's LSD tests were performed (equivalent to multiple *t*-tests between all pairs of groups) because we were only interested in planned comparisons between certain treatment combinations. Due to the high number of pair-wise comparisons being performed, using other commonly used pair-wise tests (i.e. Tukey and Bonferroni) would be overly conservative and inflate the probability of type II errors. To accomplish this, we divided the experimental alpha value by the number of individual comparisons we were interested in testing (i.e. $n = 3$ for comparisons between nutrient treatments at each of the salinities tested for an $\alpha = 0.017$, and $n = 10$ for comparisons between salinity treatments for each of the nutrients tested, for an $\alpha = 0.005$). All of the above statistical analyses were conducted using SYSTAT 12.0 (SPSS Inc., Chicago, IL, USA).

Differences in phytoplankton community structure between treatments were analyzed with PRIMER software application (version 6.1; Plymouth Marine Laboratory, Plymouth, UK). The SIMPER function was used to calculate average densities for each species from the salinity × nutrient treatments at each temperature.

Compositional similarities between samples were computed with the Bray–Curtis coefficient. Average compositional similarities between treatments were plotted for each of the temperatures tested using non-metric multidimensional scaling (NMDS). Permutation multivariate analysis of variance (PERMANOVA) was performed for each temperature using all data, to test for differences in composition between treatments. The test statistic for PERMANOVA is the pseudo-F ratio, which is tested for significance by using a permutation test, which randomly shuffles sample labels within and among treatment groups using 999 permutations and the pseudo F-ratios of the randomly assigned communities compared with the pseudo F-ratio of the observed communities (Anderson, 2001).

RESULTS

The number of days needed for community biomass to reach equilibrium did not differ between nutrient treatments, but generally increased with salinity and decreased with temperature (Supplementary data, Fig. S1).

Species richness and Pielou's evenness

For each of the three temperatures tested, significant salinity and nutrient effects on species richness were detected with a blocked ANOVA, with significant interaction effects at 10°C (Table II, Fig. 1a–c). For each of the temperatures tested, species richness was generally higher in the two nutrient treatments than in the control treatment (pair-wise comparisons, Supplementary data, Table SI). Species richness also generally decreased with increasing salinity in the 10 and 20°C treatments, while decreasing only at the higher salinities tested at 30°C (pair-wise comparisons, Supplementary data, Table SII).

For each of the three temperatures tested, significant treatment effects on evenness were detected with a blocked ANOVA (Table II, Fig. 1d–f). Evenness was generally higher in the control treatment than that in the two nutrient treatments for each of the temperatures tested (pair-wise comparisons, Supplementary data, Table SI). Evenness was also generally insensitive to increasing salinity in the 20 and 30°C treatments, while at 10°C, evenness decreased slightly with increasing salinity (pair-wise comparisons, Supplementary data, Table SII).

Total community biomass and diversity

Two measures of productivity (total community biomass and maximum fluorescence of each sample) were highly correlated ($r = 0.854$, $P \leq 0.0001$, $n = 253$), and results obtained using either measure were qualitatively the same, but we used total community biomass since this is a commonly used proxy for productivity.

Significant treatment effects on total community biomass across the three temperatures tested were detected with a blocked ANOVA, with significant interaction effects at 20 and 30°C (Table II, Fig. 2a–c). In general, for each of the temperatures tested, biomass was significantly higher in the low and high treatments compared with the control treatment, while differences between high and low nutrient treatments were not significant in most cases (pair-wise comparisons, Supplementary data, Table SIII). Generally, for each of the nutrient treatments, biomass was insensitive to

Table II: Results from blocked ANOVAs examining the effect of nutrient level (high, medium and low) and salinity (30, 60, 90, 120 and 150 g L^{-1}) on species richness, Pielou's evenness and ln *phytoplankton biomass at the three temperatures (10, 20 and 30°C); F statistic and* P *value (in parentheses) reported*

Source	*df*	Species richness	Pielou's evenness	*ln* biomass
10°C				
Inocula	1, 131	2.61 (0.108)	5.61 (0.019)	0.64 (0.425)
Nutrient	2, 131	31.10 (<0.0001)	20.30 (<0.0001)	221.44 (<0.0001)
Salinity	4, 131	62.51 (<0.0001)	4.77 (<0.0013)	5.54 (0.0004)
Salinity × nutrient	8, 131	3.75 (0.0006)	1.28 (0.257)	1.23 (0.286)
20°C				
Inocula	1, 74	22.65 (<0.0001)	0.22 (0.639)	20.79 (<0.0001)
Nutrient	2, 74	7.11 (0.0015)	16.87 (0.0001)	123.10 (<0.0001)
Salinity	4, 74	35.59 (<0.0001)	2.16 (0.082)	7.72 (<0.0001)
Salinity × nutrient	8, 74	1.93 (0.068)	1.05 (0.405)	6.68 (<0.0001)
30°C				
Inocula	1, 74	36.32 (<0.0001)	0.00 (0.999)	2.04 (0.157)
Nutrient	2, 74	10.87 (0.0001)	14.18 (<0.0001)	104.89 (<0.0001)
Salinity	4, 74	42.31 (<0.0001)	0.43 (0.789)	4.25 (0.004)
Salinity × nutrient	8, 74	0.91 (0.517)	0.61 (0.766)	3.24 (0.003)

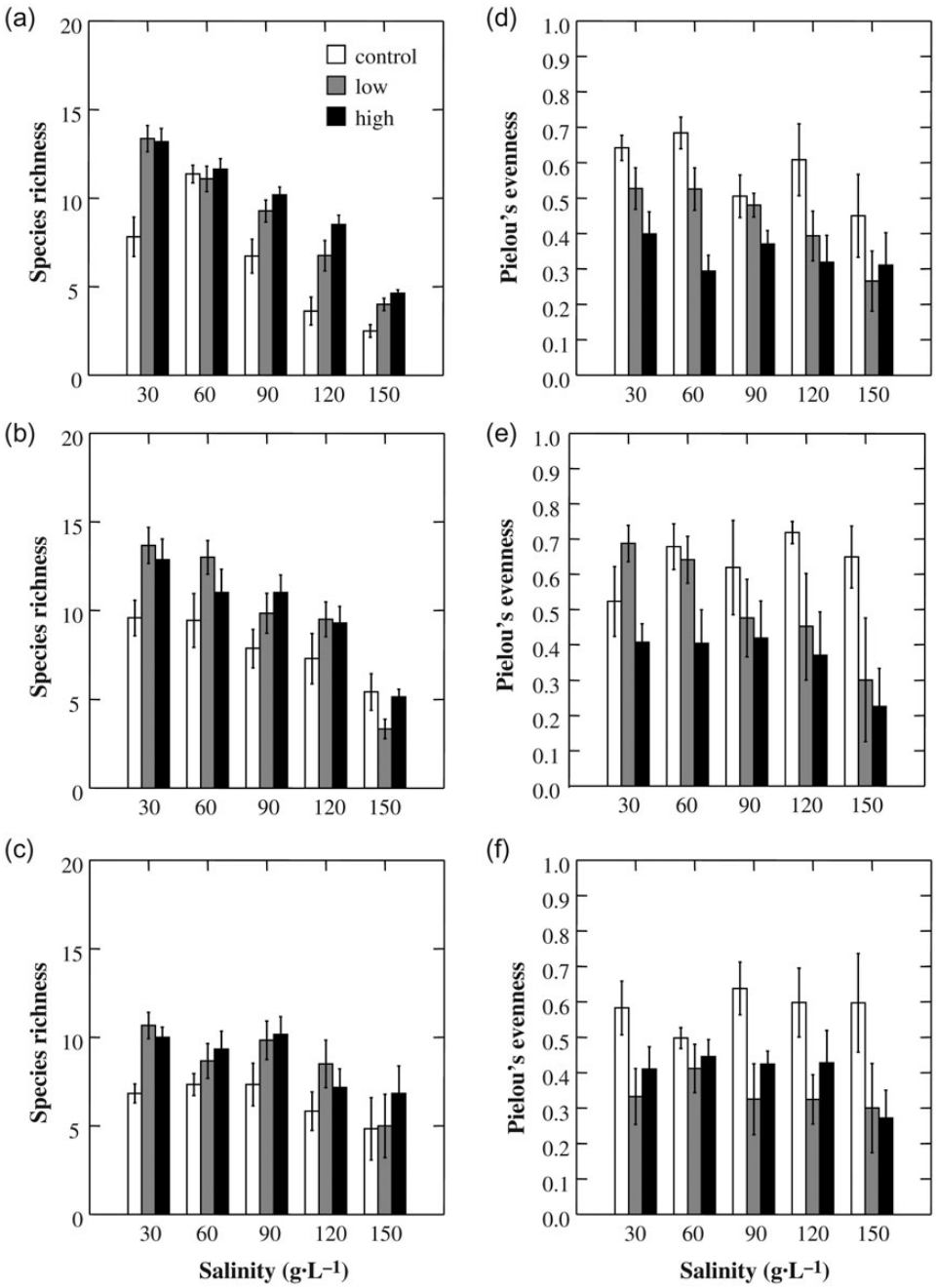

Fig. 1. Effects of nutrient level and salinity on mean species richness (**a**–**c**) and Pielou's evenness (**d**–**f**) for phytoplankton communities at temperatures tested (10, 20 and 30°C). Error bars represent ±1 standard error.

change in salinity, except at 20°C, where biomass increased slightly at salinities greater than 90 g L^{-1} (pairwise comparisons, Supplementary data, Table SIV). Additionally, species richness and total community biomass were positively correlated ($r = 0.338$, $P \leq 0.0001$, $n = 338$, Fig. 2a), while evenness and total community biomass were negatively correlated ($r = -0.594$, $P < 0.0001$, $n = 338$, Fig. 2b).

Species composition and NMDS

For each of the experimental temperatures tested, significant ($P \leq 0.05$) blocking effects (i.e. inocula), treatment effects of nutrient addition and salinity, as well as their interaction on species composition was revealed by PERMANOVA (Table III). NMDS of species composition using averaged species biomass values revealed

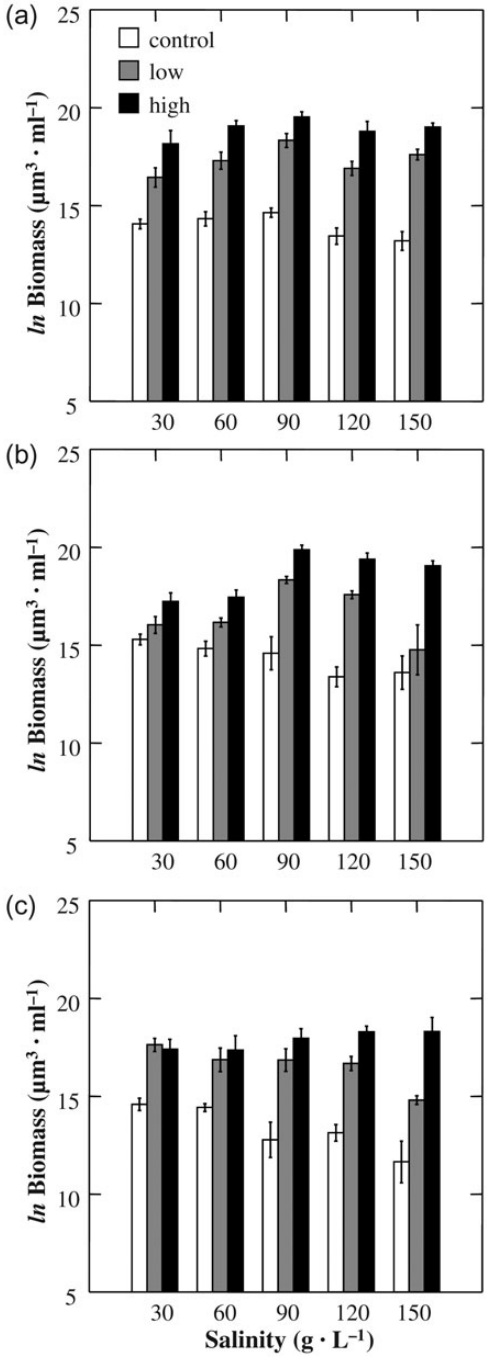

Fig. 2. Effects of nutrient level and salinity on mean total community biomass for phytoplankton communities at temperatures tested (10, 20 and 30°C). Error bars represent ± 1 standard error.

treatment effects for each of the temperatures tested (Fig. 3). At 10°C, greater similarity in species composition was observed between the high and low nutrient treatments compared with the controls (Fig. 3a, Supplementary data, Table SV). In the high and low nutrient treatments, species composition in the two highest salinity treatments tested (i.e. 120 and 150 g L^{-1}) was clearly different from all other salinities. Species composition at 20°C was more variable between salinities, but NMDS revealed the high and low nutrient treatments were separated from the control treatment (Fig. 3b and Supplementary data, Table SVI). At 30°C, species composition in the high and low nutrient treatments was generally more similar to each other than to the control treatment, except in the low nutrient treatment at 150 g L^{-1} salinity, which was very similar to the control treatment at 150 g L^{-1} (Fig. 3c and Supplementary data, Table SVII).

Table III: Results from blocked PERMANOVA for species composition based on square-root-transformed biomass values using Bray–Curtis similarities, examining the effect of nutrient level (high, medium and low) and salinity (30, 60, 90, 120 and 150 g L^{-1}) for experiments at different temperatures (10, 20 and 30°C)

Source	*df*	Pseudo F	*P*
Multivariate test (10° C)			
Inocula	2, 146	24.60	0.001
Nutrient	2, 146	44.06	0.001
Salinity	4, 146	13.71	0.001
Salinity × nutrient	8, 146	5.23	0.001
Residual	130, 146		
Multivariate test (20° C)			
Inocula	2, 99	12.64	0.001
Nutrient	2, 99	18.13	0.001
Salinity	4, 99	7.97	0.001
Salinity × nutrient	8, 99	2.70	0.001
Residual	83, 99		
Multivariate test (30° C)			
Inocula	1, 89	16.01	0.001
Nutrient	2, 89	10.20	0.001
Salinity	4, 89	3.84	0.001
Salinity × nutrient	8, 89	1.85	0.001
Residual	74, 89		

Dunaliella viridis

In these experiments, *Dunaliella viridis* was observed to be much more frequent and in much higher abundance than *Dunaliella salina*; therefore, we focused our analyses on *D. viridis*. The relative abundance of *D. viridis* generally increased with increasing salinity at the three temperatures tested, with higher relative abundance also observed at 10 and 20°C compared with 30°C (Fig. 4a–c, Table IV). Also, the relative abundance of *D. viridis* was generally higher in the nutrient addition treatments. Furthermore, across all temperatures tested, the relative abundance of *D. viridis* decreased nonlinearly as species richness increased (Figs. 5 and 6).

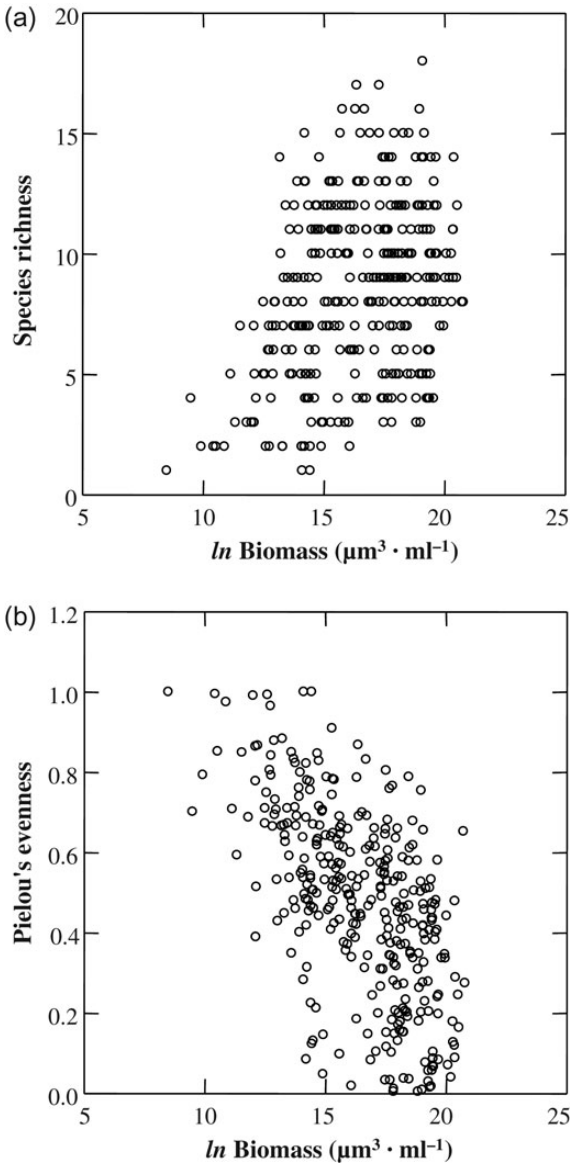

Fig. 3. Scatterplots showing the relationship between productivity, measured as total average community phytoplankton biomass and (**a**) species richness ($r = 0.338$, $P \leq 0.0001$, $n = 338$) and (**b**) Pielou's evenness ($r = -0.594$, $P < 0.0001$, $n = 338$).

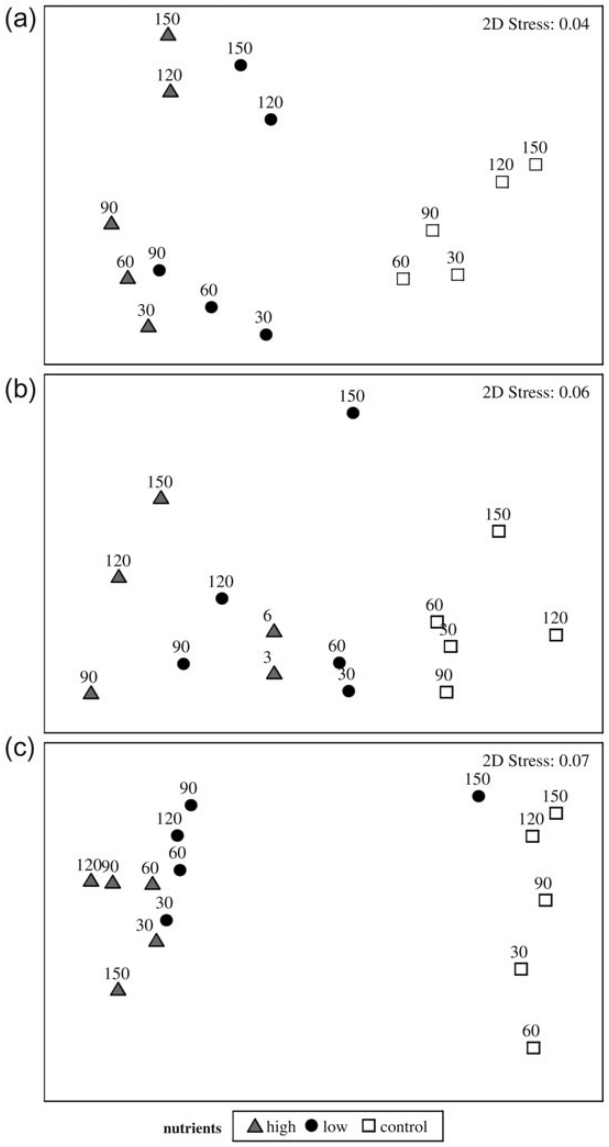

Fig. 4. Multivariate analysis NMDS plot of average species composition based on square-root-transformed Bray–Curtis similarities for each using salinity and nutrient treatment combinations at 10°C (**a**), 20°C (**b**) and 30°C (**c**). Numbers next to symbols represent salinity (g L^{-1}).

DISCUSSION

Species richness, evenness and biomass

Previous examinations of phytoplankton communities in the south arm of the GSL have attributed observed increases in species richness to a long-term trend of decreasing salinity resulting from the effects of a rockfill railroad causeway (Felix and Rushforth, 1979; Rushforth and Felix, 1982). Experimental evidence from this study supports the hypothesis that phytoplankton species richness in the GSL generally increases with declining salinity and is consistent with observations from other hypersaline lakes (Hammer, 1986; Williams *et al.*, 1990). However, this trend was not consistent across all the temperatures we tested, as the trend was not as strong in the 30°C experiments, suggesting temperature also influences the strength of this relationship. We observed decreased species richness at the lower salinities tested (i.e. 3, 6 and 9 g L^{-1}) across nutrient treatments in the 30°C experiments compared with the experiments conducted at other temperatures. These results suggest that 30°C temperatures may have been unfavorable for some species (i.e. several species of green algae such as

Table IV: Results from blocked ANOVA examining the effect of nutrient level (high, medium and low) and salinity (30, 60, 90, 120 and 150 g L^{-1}) on relative abundance of D. viridis *at the three temperatures (10, 20 and 30°C); F statistic and* P *value (in parentheses) reported*

Source	*df*	F_{stat}	*P*
10° C			
Inocula	1, 131	95.51	<0.0001
Nutrient	2, 131	0.229	0.796
Salinity	4, 131	73.94	<0.0001
Salinity × nutrient	8, 131	3.72	0.0006
20° C			
Inocula	1, 74	34.30	<0.0001
Nutrient	2, 74	12.17	0.0001
Salinity	4, 74	16.44	<0.0001
Salinity × nutrient	8, 74	3.46	0.0019
30° C			
Inocula	1, 74	7.48	0.0078
Nutrient	2, 74	1.74	0.1821
Salinity	4, 74	6.62	0.0001
Salinity × nutrient	8, 74	0.43	0.8999

D. salina, *see below*). Water temperatures of 10°C are typical of early spring and late fall, while water temperatures around 26°C are commonly observed in the GSL during the summer (Belovsky *et al.*, 2011). While 30°C is warmer than the high summer temperatures typically observed in the GSL, it is not inconceivable that surface temperatures near 30°C could be reached in the more shallow portions of the lake during a very warm summer given that high summer temperatures are quite variable and that measurements in the lake were taken from surface water in some of the deeper portions of the lake (Belovsky *et al.*, 2011).

In addition to salinity interacting with water temperature to influence species richness, our experiments also show that nutrient supply interacted with salinity to influence species richness and evenness. Species richness increased with nutrient addition, suggesting that increased nutrient concentrations favored species coexistence. However, the relative influence of just a few species dominated community composition as demonstrated by reduced evenness, indicating that while more species were found in enriched communities, only a few species were favored and most were found at low to very low abundance. These results indicate that nutrient enrichment may favor competitive dominance by relatively few species, results which are consistent with those observed in other aquatic communities, where nutrient enrichment increases species richness but leads to a decrease in community evenness (Hillebrand *et al.*, 2007). The mechanism behind these trends in other plankton

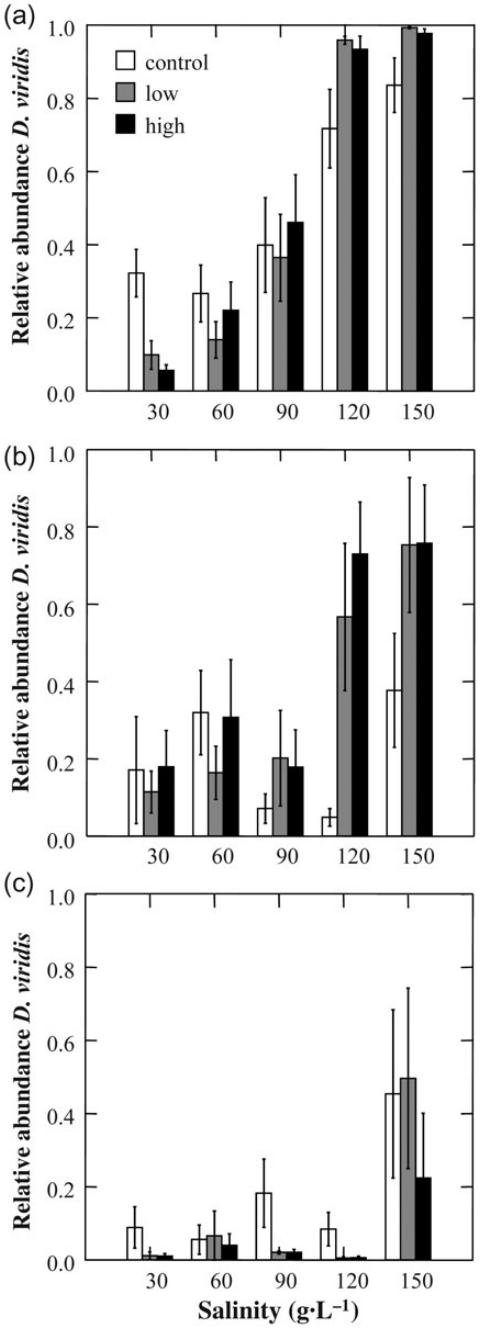

Fig. 5. Effects of nutrient level and salinity on mean relative abundance of *Dunaliella viridis* at 10°C (**a**), 20°C (**b**) and 30°C (**c**). Error bars represent ± 1 standard error.

communities has been suggested to be the inability of producer species to monopolize light use in well-mixed communities leading to competitive dominance rather than exclusion (Hillebrand *et al.*, 2007).

We also observed that species richness and evenness were correlated with total community biomass. Biomass is expected to increase with nutrient supply, and in

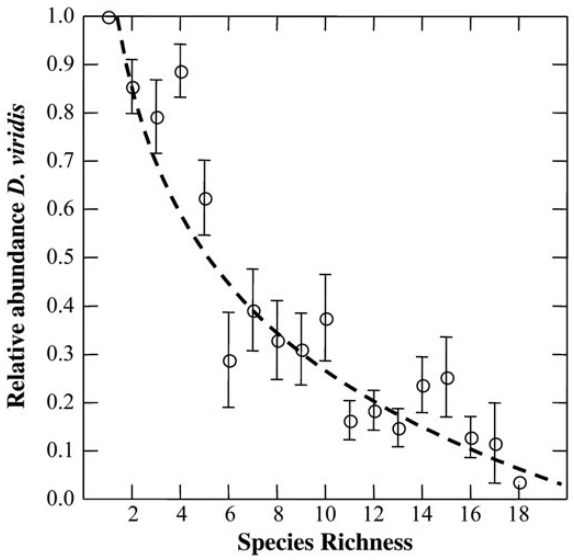

Fig. 6. The relationship between mean relative abundance of *Dunaliella viridis* and species richness. Line represents the mean change in relative abundance of *D. viridis* with increasing species richness described by the equation: $\hat{y} = 1.14 - 0.27 \times^{0.5}$ (df = 1, 17; $r^2 = 0.832$; F = 79.24; $P \leq 0.0001$). Error bars represent $\pm$ 1 standard error.

our experiments, we observed higher total community biomass with nutrient enrichment, while species richness and evenness were positively and negatively correlated with biomass, respectively. Species richness of phytoplankton communities in lakes has been observed to exhibit a unimodal response to an increase in productivity (Dodson *et al.*, 2000), while in our experiments, the response was linear. We propose several explanations for why we did not observe this relationship in our experiments: first, the gradient in species richness values we observed was quite narrow, with values ranging from 1 to 19 species, considerably lower than richness values typically observed in freshwater lakes (Dodson *et al.*, 2000), and second, the size of our microcosms was relatively small compared with the range in sizes of lakes observed in the study by Dodson *et al.* (Dodson *et al.*, 2000); therefore, the richness and productivity gradients observed in our microcosms may have been too narrow for us to observe a declining phase of the relationship. However, more recently productivity has also been shown to respond positively to species richness (Cardinale *et al.*, 2009); therefore, productivity can be both a cause and consequence of species richness in communities (Worm and Duffy, 2003; Gamfeldt and Hillebrand, 2008). While our experiments were not specifically designed to test the hypothesis of increasing biomass with greater species richness, we can make some inferences about the correlation between richness and biomass observed in our experiments. We observed greater total community biomass in communities with more species than in communities with fewer species. Higher productivity or total community biomass with increased species richness can result from either a "sampling effect" in which the probability of a community including a highly productive species increases as species richness increases, or by complementarity, which is more efficient utilization of the available resources by ecologically different species as species richness increases (Loreau *et al.*, 2001). However, the response of total community biomass and Pielou's evenness in our experiments suggests that highest biomass accumulation was achieved when community composition was dominated by just a few species. These results suggest that in our experiments, fertilization favored the growth of a few productive species, and therefore, the correlation between total community biomass and species richness was more likely driven by a few productive species than by more efficient utilization of available resources. In fact, we observed that many samples with high total community biomass were dominated by either one of two species of green algae, *D. viridis* or *Tetraselmis contracta*, or a combination of the two. Observational data seem to support these results as within the GSL, highest annual primary productivity has typically been observed in the late winter to early spring when grazing is absent (Stephens and Gillespie, 1976; Belovsky *et al.*, 2011) and composition of phytoplankton is dominated by *Dunaliella* sp. (Stephens and Gillespie, 1976).

The response of *Dunaliella viridis*

Historically, phytoplankton diversity within the GSL has been observed to be low when salinities have been extremely high (>15%), and communities have generally been dominated by *Dunaliella* sp. (Stephens and Gillespie, 1976; Rushforth and Felix, 1982; Stephens, 1990; Wurtsbaugh, 1995; Stephens, 1998). Consistent with these observations, we observed the relative abundance of *Dunaliella* sp., most notably *D. viridis* increased with salinity in our experiments. Brock (Brock, 1975) suggested that *D. viridis* may be a poor competitor with other phytoplankton species (Brock, 1975) and one reason why it is generally abundant at high salinities is that it experiences competitive release. Our results appear to support the idea that *Dunaliella* may be a poor competitor with other phytoplankton species as we observed a decrease in relative abundance of *D. viridis* with increasing species richness. Species such as this, capable of coping with physiologically stressful environments, which likely would be poor competitors against similar species in less stressful habitats have been termed "stress specialists" (Dunson and Travis, 1991). Species from the genus *Dunaliella* are

perhaps the most halotolerant eukaryotic organisms known and are present in significant numbers in many oceans, brine lakes, salt ponds and marshes (Avron, 1992). However, we also observed that other species were capable of withstanding the highest salinities we tested and in some instances were abundant (e.g. the green alga *T. contracta* and the cyanobacteria *Coccochloris elebans*).

It is clear that salinity is a central abiotic factor influencing species richness in hypersaline lakes, and it seems that at higher salinities, many of the rarer species disappear either as a result of the inability to physiologically overcome osmotic stress or by being outcompeted by species less affected by osmotic stress. *Dunaliella* sp. appears to be a better competitor at high salinities, most likely a result of being less affected by osmotic stress than other species are. Additionally, *Dunaliella* sp. was also more abundant in the lower temperature experiments, with highest and lowest relative abundance observed in the 10 and 30°C experiments, respectively. These results are consistent with observations from the GSL, with *Dunaliella* sp. abundance highest in the late winter to early spring when water temperatures are at or around 10°C (Stephens and Gillespie, 1976; Belovsky *et al.*, 2011).

During the winter months with cold water temperatures, zooplankton are largely absent from the GSL and thus grazing pressure on the phytoplankton community is low (Belovsky *et al.*, 2011). At this time, bottom-up effects have been observed to exert strong influences upon phytoplankton communities (Belovsky *et al.*, 2011). Therefore, studying phytoplankton responses in the absence of grazing is imperative. Conversely, factors that shape phytoplankton communities in the GSL also have the potential to influence higher trophic levels (Paton *et al.*, 1992; Belovsky *et al.*, 2011). The brine shrimp *Artemia franciscana* is typically the only abundant species of zooplankton inhabiting the GSL and the availability of phytoplankton has been shown to limit *A. franciscana* (Wurtsbaugh and Gliwicz, 2001; Belovsky *et al.*, 2011). *Dunaliella* sp. is normally very abundant in spring when brine shrimp cysts hatch and the availability of *Dunaliella* sp. is likely to impact the quality of food available to recently hatched brine shrimp, and as results from our experiments suggest the abundance of *Dunaliella* sp. is influenced by the interaction of salinity with other environment variables, as well as by competition from other phytoplankton species. These observations combined with the results from our study highlight the need for greater understanding of the factors influencing phytoplankton composition in the GSL, and the potential for bottom-up effects to impact higher trophic levels in this system (Belovsky *et al.*, 2011). This is especially important given that *A. franciscana* constitutes a major food source for many migrating shorebirds in the Pacific Flyway, which periodically stop at the GSL (Paton *et al.*, 1992; Belovsky *et al.*, 2011).

CONCLUSIONS

As the area surrounding the GSL becomes increasingly urbanized, human impacts upon the GSL ecosystem are likely to increase. Reduced freshwater inputs to the GSL due to inflow diversions for domestic and agricultural needs are a distinct possibility, which further influence the salinity regime of the lake and is a major issue facing other saline and hypersaline lakes (Williams, 2002). Additionally, increased pollution due to the inputs of agricultural waste-water, pesticides in run-off, a variety of organic and inorganic wastes from domestic and industrial sources, increased nutrient inputs entering the lake and biological invasions are also important issues facing GSL and other saline ecosystems (Williams, 2002; Marcarelli *et al.*, 2006). Therefore, as interest in and appreciation for these ecologically important systems increases, it is important to gain greater understanding of how relevant factors such as changing salinity and nutrient inputs influence biological communities in saline and hypersaline lakes, which will aid future management decisions.

Results from our experiments add to increasing evidence that hypersaline lakes are not as simple systems as once thought (Belovsky *et al.*, 2011) and highlight the need for greater understanding of the factors that influence diversity and coexistence patterns in these unique and ecologically important systems. Our results also highlight growing evidence for the utility of studying biodiversity patterns in saline ecosystems, as several of the patterns we observed in these experiments were consistent with trends observed in other aquatic systems.

SUPPLEMENTARY DATA

Supplementary data can be found online at http://plankt.oxfordjournals.org.

ACKNOWLEDGEMENTS

We are grateful to Susan S. Kilham for advice on experimental design and Todd Crowl and Ted Evans for helpful comments on earlier drafts. We also thank Clay Perschon and the Utah Division of Wildlife Resources Great Salt Lake Ecosystem Project for logistical support when obtaining seed algae. We also thank the anonymous reviewers for their constructive comments.

FUNDING

We wish to thank the Great Salt Lake Ecosystem Program, Utah Division of Wildlife Resources (to G.E.B) for funding.

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