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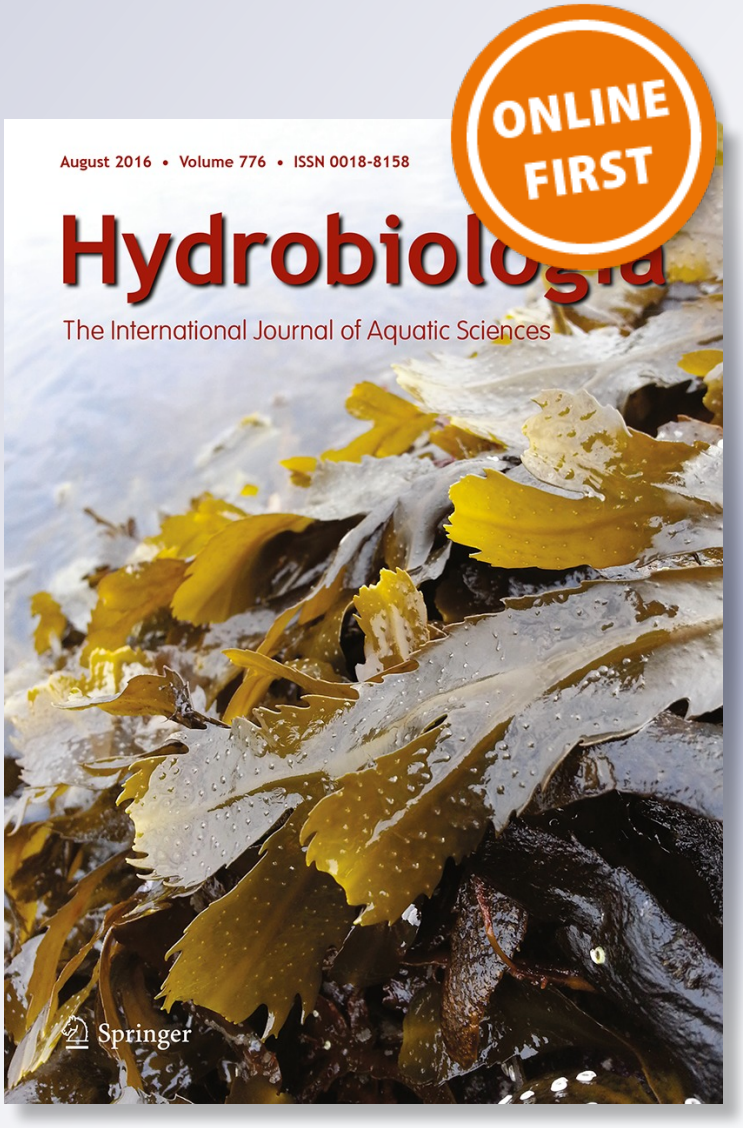

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PRIMARY RESEARCH PAPER

Bioassay analysis of nutrient and *Artemia franciscana* effects on trophic interactions in the Great Salt Lake, USA

Elizabeth M. Ogata · Wayne A. Wurtsbaugh · Trinity N. Smith · Susan L. Durham

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Abstract 14-day microcosm experiments demonstrated the strong interactions between bottom–up and top–down effects of nutrient addition (control, nitrogen, phosphorus, nitrogen + phosphorus) and *Artemia franciscana* grazing on algae in Great Salt Lake water from Gilbert Bay. Nitrogen addition increased phytoplankton chlorophyll concentrations, while phosphorus addition had no stimulatory effect. A combined N + P treatment was synergistic, increasing both phytoplankton and periphyton >10-fold above controls. Our results suggest that phytoplankton were primarily limited by nitrogen and secondarily limited by phosphorus and that periphyton was colimited by nitrogen and phosphorus. The grazing effect increased as *A. franciscana* grew from nauplii to adults and by the final day, *A. franciscana* had markedly reduced both phytoplankton and periphyton abundance in the Control, +N, and +P treatments. Grazing also significantly reduced periphyton in the N + P treatments. Due to high phytoplankton growth rates in the N + P treatment, *A. franciscana* grazing did not significantly reduce chlorophyll concentrations during the bioassay. However, *A. franciscana* in the N + P treatment was significantly larger and had greater reproductive output than in the controls, suggesting that the following generation might have exerted greater grazing pressure.

Keywords Great Salt Lake · Trophic · Nitrogen · Phosphorus · *Artemia franciscana* · Saline

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Introduction

Although ecologists have demonstrated that ecosystems may be controlled both from the "bottom–up" by resource availability and from the "top–down" by predation (Lindeman, 1942; Hairston et al., 1960; McQueen et al., 1986; Carpenter & Kitchell, 1988; Cullen, 1991; Power, 1992), it remains unclear why the relative strength of top–down and bottom–up controls varies between systems (Shurin

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et al., 2006; Gruner et al., 2008) and whether these controls independently or interactively control producer biomass (Gruner et al., 2008). Nearly all aquatic studies on these trophic interactions have been done in fresh or marine waters. Studies conducted in hypersaline environments, as described here, add diversity to the types of communities and environmental conditions represented in cross-ecosystem comparisons of top–down and bottom–up controls.

Trophic interaction studies conducted in hypersaline environments can enable us to better understand why the strength of top–down controls varies across ecosystems. Although food web complexity is thought to reduce the strength of top–down effects, few studies have tested this hypothesis (Shurin et al., 2006). Hypersaline lakes can provide empirical data of simple food webs, as these systems contain relatively simple communities of halotolerant organisms. The dominant macrozooplankton in hypersaline systems, *Artemia*, has been shown to regulate phytoplankton abundance from the top–down in lakes such as the Great Salt Lake (Wurtsbaugh & Gliwicz, 2001; Belovsky et al., 2011), Mono Lake (Jellison & Melack, 1993), and in salterns (Javor, 1989).

Hypersaline environments will also advance our understanding of how nutrients influence food webs from the bottom–up. Historically, phosphorus was assumed to be the primary limiting nutrient in freshwaters. In part, the phosphorus limitation paradigm stemmed from the notion that nitrogen fixation could alleviate limitation by this nutrient (Lewis & Wurtsbaugh, 2008). Recently, however, both nitrogen and phosphorus have been shown to limit primary production in freshwater, marine, and terrestrial environments (Elser et al., 2007). Saline systems offer additional insight on the phosphorus limitation paradigm. High salinity levels have been shown to reduce rates of nitrogen fixation (Herbst, 1998) and saline lakes such as those in the Great Basin (Galat et al., 1981; Jellison & Melack, 2001) and central and northern Great Plains of North America (Salm et al., 2009) have been shown to be primarily limited by nitrogen (Javor, 1989). Saline systems thus offer an opportunity to examine how nitrogen limitation, a previously underappreciated type of nutrient limitation, influences food webs from the bottom–up.

The Great Salt Lake is an ideal system in which to examine the relative strength of top–down effects of grazing and bottom–up effects of nutrients in saline systems. Although high salinity levels are often assumed to limit primary production in hypersaline systems, a long-term study in the Great Salt Lake demonstrated that nutrients and *Artemia franciscana* Kellogg more strongly control phytoplankton abundance than salinity (Belovsky et al., 2011). Indeed, monitoring studies consistently note the strong top–down effect of *A. franciscana* on phytoplankton abundance in the spring, when populations of this macroinvertebrate reach maximal levels (Wurtsbaugh & Gliwicz, 2001; Belovsky et al., 2011). In addition, Great Salt Lake nutrient limitation assays have consistently found nitrogen to be the primary nutrient-limiting phytoplankton growth (Stephens & Gillespie, 1976; Wurtsbaugh, 1988; Marcarelli et al., 2006).

The relative importance of top–down and bottom–up controls on productivity in the Great Salt Lake and hypersaline systems remains unclear. Previous studies either separately tested the effects of top–down or bottom–up controls (e.g., Stephens & Gillespie, 1976; Wurtsbaugh, 1988; Wurtsbaugh & Gliwicz, 2001) or established relationships between nutrient levels, phytoplankton abundance, and *Artemia franciscana* populations with correlations drawn from monitoring data (Belovsky et al., 2011 but see Wurtsbaugh, 2014). Additionally, very little work has been done to understand factors controlling periphyton in the lake. Consequently, we conducted an experiment to directly test the independent and combined effects of nutrient addition and *A. franciscana* grazing on the Great Salt Lake food web. Our research addressed the following questions:

1. What are the effects of nutrient addition (nitrogen, phosphorus, nitrogen + phosphorus) and *A. franciscana* grazing on phytoplankton and periphyton abundance?
2. How do the effects of nutrient addition and *A. franciscana* grazing on phytoplankton change through time as cohorts of *A. franciscana* grow and mature?
3. How do nutrient additions influence *A. franciscana* growth and egg production?

The Great Salt Lake, USA is increasingly impacted by human activities as the watersheds surrounding the

lake undergo rapid urbanization and population growth. An understanding of trophic interactions in the lake will enable managers to understand existing impacts of these disturbances and predict how the lake will respond to future changes.

Materials and methods

Bioassay design

Water for the experiment was collected from Gilbert Bay (41°13.27′N, −112°33.66′W) in the Great Salt Lake on 8 February 2014. At collection, the water temperature was 2.4°C, the salinity was 181 g l^{-1}, and brine shrimps were absent. Prior to the start of the experiment, the water was incubated in an environmental chamber at Utah State University for 11 days at a light intensity of 200 μE m^{-2} s^{-1} with aeration. To allow for sufficient metabolic activity within our 14-day experiment, the water temperature was increased over 11 days until it reached 20°C at the start of the experiment. When temperatures reached 10°C in the stock water, adult brine shrimps from a laboratory stock aquarium were added at a density of approximately 3 shrimps l^{-1} to graze the phytoplankton and thus simulate late spring conditions in the Great Salt Lake (Wurtsbaugh & Gliwicz, 2001; Belovsky et al., 2011).

The bioassay was conducted from 19 February to 5 March 2014. On the initial day of the experiment, the Great Salt Lake stock water was filtered through a 153 μm mesh to remove *Artemia franciscana* and coarse particulate matter and then homogenized in buckets. To measure the initial concentration of phytoplankton chlorophyll *a*, three replicate 10 ml water samples were filtered through Gelman A/E filters with nominal pore size of 1.0 μm and frozen until subsequent analysis. To determine the nutrient concentrations in the water at the start of the experiment, 30 ml samples of water were collected for total nitrogen (TP) and total phosphorus (TP) analyses (2 replicates) and 30 ml samples of water were filtered at vacuum pressures <10 mm Hg through Whatman GF/F filters (0.8 μm) for ammonia (NH_4–N), nitrate plus nitrite (NO_2 + NO_3–N), and soluble reactive phosphorus (SRP) analyses (2 replicates).

Water was added in 900 ml aliquots to 1000 ml acid-washed clear polyethylene terephthalate plastic bottles. Nutrient addition and *Artemia franciscana* grazing treatments were manipulated in a full factorial design. Bottles were randomly assigned to nutrient treatments of either Control (no nutrient addition), nitrogen (+N; 3.5 mg N l^{-1} added as NH_4NO_3), phosphorus (+P; 0.5 mg P l^{-1} added as $NaHPO_4$), or nitrogen and phosphorus (N + P; 3.5 mg N l^{-1} and 0.5 mg P l^{-1}), and either with or without *A. franciscana*. There were 3 replicates of each of the 8 nutrient × grazing treatment combinations. Nutrient concentrations in all bottles prior to nutrient addition on the initial day of the experiment were assumed to be the same as nutrient concentrations of Great Salt Lake water used to fill bottles. Following nutrient additions, initial nutrient concentrations in the +N, +P, and N + P treatments were calculated based on experimental nutrient treatment addition amounts (3.5 mg l^{-1} N and 0.5 mg l^{-1} P). Great Salt Lake *A. franciscana* cysts for the bioassay were hatched in a 30°C, 28 g l^{-1} NaCl solution. Nauplii were separated from unhatched cysts approximately 18 h later and then grown in an algal culture for 3 days prior to the start of the experiment. Although nominally five *A. franciscana* nauplii were added to bottles receiving the +*A. franciscana* treatment, final counts of the shrimp at the end of the experiment revealed that the number of *A. franciscana* in grazing treatment bottles ranged between 3 and 7 *A. franciscana* $bottle^{-1}$ (mean = 5.2), except in one replicate of the +P treatment where there were 12 shrimps. This replicate was subsequently excluded from all analyses. Chlorophyll concentrations in the Great Salt Lake water used to conduct the experiment reflected spring conditions in the Great Salt Lake, where concentrations range from 15 to 30 μg chl l^{-1} (Wurtsbaugh & Gliwicz, 2001; Belovsky et al., 2011). *A. franciscana* densities in the experiment also reflected maximum *A. franciscana* densities in the lake, which usually peak in May or June and have been reported to be 2.1–5.8 adults l^{-1} (Belovsky et al., 2011) and 8.5 *A. franciscana* l^{-1} (Wurtsbaugh & Gliwicz, 2001).

The bottles were incubated on a table with eastern window light (~50% cloudy during experiment). Light intensity readings taken on a cloudy afternoon and with hazy morning light ranged from 150 to 1700 μE m^{-2} s^{-1}. The afternoon temperatures of water in the bottles throughout the experiment ranged from 17.7 and 24.5°C and averaged 21.0°C. The temperature and light fluctuations during the

experiment were relatively low compared to what plankton populations experience in the bays of the Great Salt Lake, where temperatures can vary from 25 to 35°C over a daily cycle. Changes in cloud cover influence light conditions in the lake and daytime light levels can vary from 200 to over 2000 μE m^{-2} s^{-1}. We randomized bottle locations on the incubation table daily to ensure that treatments were exposed to similar light and temperature conditions over the course of the experiment.

Each day the bottles were gently agitated by inverting them three times. The bottles were squeezed with the caps removed to expel gases and allow input of fresh air with CO_2. A 10 ml water sample for phytoplankton chlorophyll measurements was drawn from each bottle at 2-day intervals, filtered, and preserved as described above.

Artemia franciscana lengths were estimated on day 7 of the experiment by photographing the shrimp through the plastic bottles. *A. franciscana* from two replicates of each nutrient treatment were photographed with a single-lens reflex camera with a macro lens. A metal ruler was photographed at the same magnification for scale. Although care was taken to photograph each shrimp only once, it is possible that a shrimp may have been photographed more than once. This likely would have had a minimal effect on the length estimates, as the variance in each bottle ranged between <0.01 to 0.23 mm.

On the final day of the experiment, samples for chlorophyll *a* in phytoplankton were collected from all bottles using the previously described methods. Nutrient samples were collected from two replicates of each treatment type using methods previously described. Water for NH_4–N, NO_3–N + NO_2–N, and SRP analyses were collected directly from each bottle. TN and TP samples were collected after the remaining contents of each bottle were filtered through 153 μm mesh to separate *Artemia franciscana*. This procedure also removed some large detrital particles that had accumulated at the bottom of the bottles. Nutrient samples were refrigerated and analyzed within a day of collection. *A. franciscana* was preserved in 5% formalin and later sexed, counted, and measured with ocular micrometers at 10× magnification. The ovisacs of females in each treatment were dissected and the number of ova counted.

To measure the amount of chlorophyll *a* of periphyton on the bottle walls, the emptied incubation bottles were placed in a freezer to help lyse the cells. 600 or 800 ml of 95% ethanol were then added to the bottles and the air was evacuated from the bottles to ensure that periphyton surfaces were covered with the solvent. The periphyton was extracted for 24 h and analyzed with the Turner 10 AU fluorometer. Chlorophyll *a* from the phytoplankton was extracted in 10 ml of 95% ethanol for 24 h and then analyzed on a Turner 10AU fluorometer utilizing a nonacidification method (Welschmeyer, 1994).

To further assess factors that may influence the nutrient limitation status of algae in the Great Salt Lake, we collected samples from two sites on the railroad causeway in Gilbert Bay (41°13.25′N, −112°40.11′W and 41°13.21′N, −112°36.37′W) on 15 July 2014 for dissolved organic nitrogen analysis. These sites are distant from river inflows where the lake is deep and thus representative of the open, pelagic waters of Gilbert Bay. The water sample from each site was split into three 30 ml samples. One sample from each site was not filtered and was analyzed for TN and TP. The remaining two samples were filtered through Whatman GF/F filters at vacuum pressure <10 mm Hg. To preserve the samples until analysis, 40 μL of sulfuric acid was added to filtered samples to reduce the pH to ~2. One filtered sample was analyzed for total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP), and one filtered sample was analyzed for NH_4–N, NO_3–N + NO_2–N, and SRP. We refer to the combined concentration of NH_4–N (hereafter NH_4) and NO_3–N + NO_2–N (hereafter NO_3) as dissolved inorganic nitrogen (DIN). A blank of 30 ml deionized water and 40 μL of sulfuric acid was included in the dissolved inorganic nutrient analyses to assess potential contamination. Nutrient samples were analyzed within 2 days of collection.

Dissolved organic nitrogen (DON) was calculated as:

$$\text{Dissolved organic nitrogen} = [\text{TDN} - \text{DIN}] \quad (1)$$

The Aquatic Biogeochemistry Laboratory at Utah State University conducted all nutrient analyses. Samples were first diluted to salinities of near 35 g l^{-1} to approximate seawater, for which the analytical methods are appropriate. TN was quantified using a potassium persulfate digestion (Nydahl, 1978) followed by a cadmium reduction for NO_3 (APHA, 1998; EPA method 353.2). TP was quantified using a potassium persulfate digestion followed by an

ascorbic acid molybdenum reaction for SRP (Murphy & Riley, 1962; EPA method 365.1). NH_4 was quantified with an automated alkaline phenol-hypochlorite reaction followed by spectrophotometric analysis (EPA method 350.1; Solorzano, 1969; APHA, 1998).

Artemia franciscana size calculations

Artemia franciscana dry weights were calculated as:

(2) Dry weight individual^{-1} (μg ind^{-1}) = $0.90 \times L^{3.02}$, where L = length of each *A. franciscana* (mm). (Regression derived from Reeve, 1963; see Wurtsbaugh, 1992)

A. franciscana filtration rates were calculated as:

(3) Water volume filtered individual^{-1} day^{-1} (ml ind^{-1} day^{-1}) = $5.45 \times L^{1.82}$, where L = length of *A. franciscana* (mm). (Regression derived from Reeve, 1963; see Wurtsbaugh, 1992)

2011, 2012, 2013 preliminary bioassays

Bioassays were conducted in previous years (2011, 2012, and 2013) with the same experimental design and similar methods. However, in previous years the experiment was conducted with Great Salt Lake water that had been held in a stock aquarium (salinities of 140–160 g l^{-1}) and was not as representative of actual Great Salt Lake nutrient conditions as the 2014 experiment. In addition, mature adult *Artemia franciscana* was added at the start of the experiments and likely had a consistent grazing effect throughout the experiment. Nutrient concentrations were not measured in these experiments.

Statistical analysis

We constructed general linear mixed models to assess the effects of nitrogen addition, phosphorus addition, and number of *Artemia franciscana* on phytoplankton and periphyton log chlorophyll concentrations (phytoplankton and periphyton models, respectively). Nitrogen addition and phosphorus addition were included as categorical fixed effects; the number of *A. franciscana* was included as a continuous fixed effect, assumed to have a linear relationship with log chlorophyll; and a bottle was the experimental unit for the nitrogen, phosphorus, and number of *A. franciscana* factors. To assess changes in phytoplankton chlorophyll concentrations through time, the phytoplankton model included day as a categorical fixed effect and a heterogeneous first-order autoregressive covariance structure was used to model repeated measures on a bottle through time.

Mean phytoplankton and periphyton chlorophyll concentrations on day 14 in nutrient treatments with and without *Artemia franciscana* were estimated from the statistical model at a number of *A. franciscana* equal to five and zero, respectively. Pairwise comparisons among the means of nutrient treatments with and without *A. franciscana* were adjusted for Type I error using the Tukey–Kramer method. Contrast statements were used to obtain a test of interaction of nitrogen addition and phosphorus addition in the absence of *A. franciscana*. To test whether the number of *A. franciscana* influenced chlorophyll concentrations, we used contrast statements to test equality of slopes of the regression of log chlorophyll on the number of *A. franciscana* among nutrient treatments. These tests were performed for each day in the phytoplankton model.

The effects of nutrient treatments on *Artemia franciscana* weights on day 7 and 14 and final egg numbers were assessed using general linear mixed models (day 7 weight, day 14 weight, and egg number models, respectively). In the day 7 weight and final egg number models, nitrogen addition and phosphorus addition were included as categorical fixed effects; a bottle was the experimental unit for the nitrogen and phosphorus factors; and individual *A. franciscana* within a bottle was treated as subsample. In the day 14 weight model, nitrogen addition, phosphorus addition, and *A. franciscana* sex (female, male) were included as categorical fixed effects; a bottle was the experimental unit for the nitrogen and phosphorus factors; groups of *A. franciscana* within a bottle were experimental units associated with the sex factor; and individual *A. franciscana* within each group were incorporated as subsamples. In all three models, pairwise comparisons among nutrient treatment means were adjusted for Type I error using the Tukey–Kramer method. In the day 14 weight model, contrast statements were used to conduct post hoc pairwise comparisons among nutrient treatment means for female and male weights.

We constructed general linear mixed models to assess the effect of nutrient and *Artemia franciscana* treatments on final TN, TP, NH_4, NO_3, and SRP concentrations. Nitrogen addition, phosphorus addition, and *A. franciscana* presence or absence were included as categorical fixed effects. Higher-order interactions that were not significant were dropped, as nutrient analyses were conducted on only two replicates from each treatment. All data analyses were obtained using the GLIMMIX procedure in SAS/STAT 13.2 in the SAS® System for Windows 9.4 TS1M2 (SAS Institute Inc. 2013).

Results

Initial conditions

On the initial day of the experiment, Great Salt Lake water used in the bioassay had a mean chlorophyll *a* concentration of 36.0 μg l^{-1} and respective TN and TP concentrations of 5.52 and 0.44 mg l^{-1}. Molar ratios of the two nutrients were TN:TP = 28:1, DIN:SRP = 5.9:1, and DIN:TP = 1.8:1. Initial total and dissolved nutrient concentrations expected after addition of nutrient treatments are shown in Table 1.

Effects of nitrogen and phosphorus additions on phytoplankton

Statistical results for the phytoplankton general linear mixed model are reported in Online Resource 1 Table S1. In the +N treatment, phytoplankton chlorophyll concentrations increased at the start of the experiment and peaked on day 4 (Fig. 1; nutrient treatment means are reported in Online Resource 1 Table S2). Chlorophyll concentrations in the +N treatment were two-fold greater than those in the Control treatment throughout the experiment and the difference was significant on days 2–12 (Fig. 1). Mean chlorophyll concentrations in the phosphorus-alone treatment were usually below those in the Control treatment, but they were never significantly different from the Controls (Fig. 1).

Nitrogen and phosphorus added in combination had a significant synergistic stimulatory effect on phytoplankton growth (Fig. 1; Online Resource 1 Table S1). By day 2, chlorophyll concentrations in the N + P treatment were three-fold greater than the Control treatment. When chlorophyll concentrations in the N + P treatment peaked at >300 μg l^{-1} on day 10, concentrations were 14-fold greater than those at the start of the experiment. Chlorophyll levels in the

Table 1 Total nitrogen (TN), total phosphorus (TP), ammonia (NH_4), nitrate + nitrite (NO_3), and soluble reactive phosphorus (SRP) concentrations in *Artemia franciscana* (no *A. franciscana*, +*A. franciscana*) and nutrient (Control, +N, +P, N + P) treatments at the start (day 0) and final day (14) of the experiment

Day	*A. franciscana* treatment	Nutrient treatment	TN (mg l^{-1})	TP (mg l^{-1})	NH_4 (mg l^{-1})	NO_3 (mg l^{-1})	SRP (mg l^{-1})
0		Control	5.52 (0.173)	0.44 (0.009)	0.32 (0.032)	0.03 (0.001)	0.13 (0.001)
		+N	9.01	0.44	2.07	1.78	0.13
		+P	5.52	0.94	0.32	0.03	0.63
		N + P	9.01	0.94	2.07	1.78	0.63
14	No *A. franciscana*	Control	4.20 (0.110)	0.36 (0.006)	0.68 (0.092)	0.02 (0.003)	0.14 (0.011)
		+N	8.79 (0.077)	0.35 (0.003)	1.62 (0.069)	1.57 (0.003)	0.14 (0.001)
		+P	4.05 (0.300)	0.96 (0.011)	0.67 (0.031)	0.03 (0.002)	0.81 (0.027)
		N + P	6.24 (0.012)	0.84 (0.004)	0.37 (0.020)	0.03 (0.001)	0.33 (0.001)
14	+*A. franciscana*	Control	4.31 (0.066)	0.36 (0.008)	0.68 (0.177)	0.03 (0.003)	0.14 (0.002)
		+N	10.58 (0.898)	0.35 (0.002)	2.04 (0.071)	1.66 (0.094)	0.14 (0.003)
		+P	3.75	0.87	0.43	0.03	0.87
		N + P	6.00 (0.133)	0.81 (0.029)	0.35 (0.002)	0.03 (0.001)	0.33 (0.044)

Data are means and SE (in parentheses; $N = 2$). Standard Error reported for Day 0 in Control treatment represents SE of Great Salt Lake stock water analyses. One replicate in the +P −*A. franciscana* treatment was excluded as it held 12 *A. franciscana*

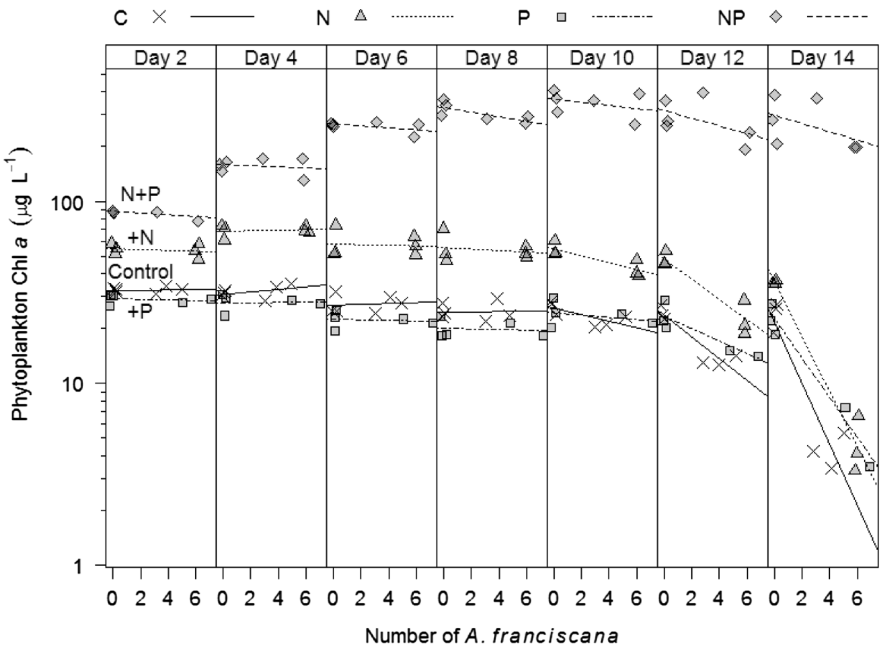

Fig. 1 Observed (*points*) and predicted (*lines*) phytoplankton chlorophyll concentrations in nutrient treatments (Control: Control treatment with no added nutrients; +N: NH_4NO_3 addition; +P: PO_4 addition; N + P: NH_4NO_3 + PO_4 addition) by sampling dates throughout the experiment. Points were jittered along the *x-axis* to reduce overlap. The predicted lines were made by plotting a regression line through back-transformed predicted chlorophyll concentrations for each treatment on each day estimated at the numbers of *Artemia franciscana* between 0 and 7. Table below figure: the $N \times P$ row reports the significance level of tests of interactions between nitrogen and phosphorus additions on log phytoplankton chlorophyll levels on each sampling date estimated at a number of *A. franciscana* = 0. *Control, +N, +P*, and $N + P$ rows report pairwise comparisons among the means of nutrient treatments without *A. franciscana* on each sampling date; means were estimated at a number of *A. franciscana* = 0. Treatments that do not share the *same letters* are significantly different. The *Equal slopes* row reports the significance level of tests of equality among slopes, i.e., whether effect of *A. franciscana* grazing varied among the nutrient treatments

N + P treatment were significantly greater than those in all other nutrient treatments on all days of the experiment (Fig. 1). On day 14, chlorophyll levels in the N + P treatment were 14-, 8-, and 13-fold greater than the Control, +N, and +P treatments, respectively.

Development of *Artemia franciscana* grazing effect

The effect of *Artemia franciscana* on phytoplankton chlorophyll concentrations became more pronounced as the juveniles matured during the experiment (Fig. 1). In all nutrient treatments, *A. franciscana* had a minimal effect on phytoplankton chlorophyll concentrations for 8–10 days (Fig. 1; slope estimates are reported in Online Resource 1 Table S3). On days 10–14, increasing numbers of *A. franciscana* had significant negative effects on chlorophyll concentrations in the Control, +N, and +P treatments (Online Resource 1 Table S3), but did not have a significant effect in the N + P treatment. Phosphorus addition may have reduced the effect of increasing densities of *A. franciscana* on chlorophyll concentrations (*A. franciscana* × phosphorus addition interaction $F_{1,19.87} = 5.81$, $P = 0.026$; Fig. 1); however, there was no evidence that the effect of increasing numbers of *A. franciscana* differed between treatments except on day 14 (Fig. 1), when only the N + P treatment was significantly different from the Control ($P < 0.001$), +N ($P < 0.001$), and +P ($P < 0.010$) treatments.

Periphyton

The effects of nutrients on periphyton chlorophyll concentrations were similar to those on phytoplankton (cf Fig. 2a, b). Accumulations of periphyton during

the experiment were low (range: <0.001–0.097 μg Chl cm^{-2}; means and mean comparisons are reported in Online Resource 1 Table S4). Nitrogen and phosphorus had a significant interactive effect ($F_{1,15} = 8.31$ $P = 0.011$) on periphyton chlorophyll concentrations and the simultaneous addition of nitrogen and phosphorus increased periphyton chlorophyll concentrations 57-fold above the Control (Fig. 2b; general linear mixed model results are reported in Online Resource 1 Table S5; nutrient treatment means are reported in Online Resource 1 Table S4). Periphyton chlorophyll concentrations in the +N and +P treatments were not significantly greater than the Control treatment (Online Resource 1 Table S4).

Artemia franciscana grazing on periphyton had an even larger effect than on the phytoplankton (Fig. 2b; slope estimates are reported in Online Resource 1 Table S6). There was no evidence that the grazing effects varied with N or P additions (Fig. 2b). *A. franciscana* was frequently in contact with the bottle surfaces and appeared to be using their filtering appendages to feed on the walls.

Artemia franciscana size and reproductive output

At the start of the experiment, the mean length of the nauplii was 0.9 mm and the mean estimated weight was 0.46 μg. N and P additions increased the mean weight of juvenile *Artemia franciscana* on day 7 by more than 50% (mean dry weight: control = 8.9 μg, +N = 14.2 μg, +P = 15.9 μg, N + P = 15.0 μg; data not shown). However, the effect was not statistically significant, likely due to our experimental design and variability. Nitrogen addition, the interaction of nitrogen addition × phosphorus addition, and sex had significant effects on *A. franciscana* weights on day 14 (Online Resource 1 Table S7). In each nutrient treatment, female *A. franciscana* was significantly heavier than male *A. franciscana*

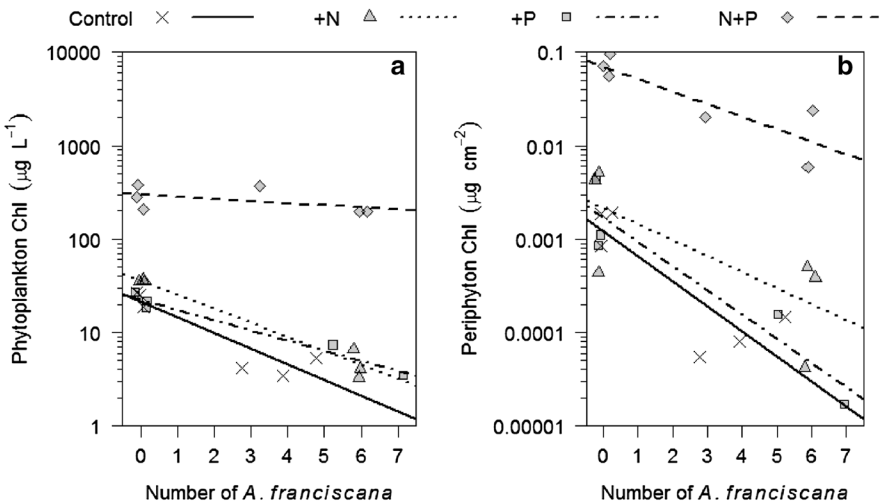

Fig. 2 Observed (*points*) and predicted (*lines*) phytoplankton (μg l^{-1}) (**a**) and periphyton (**b**) chlorophyll *a* concentrations (μg cm^{-2}) on the final day (14) of the bioassay in nutrient treatments with and without *Artemia franciscana* grazers (note log scale and different scales in frames **a** and **b**). Points were jittered along the *x-axis* to reduce overplotting. The *predicted lines* were made by plotting a regression line through back-transformed predicted chlorophyll concentrations for each treatment on each day estimated at numbers of *A. franciscana* between 0 and 7. **a** Phytoplankton chlorophyll concentrations estimated at a number of *A. franciscana* equal to zero were significantly greater in the N + P treatment than the Control treatment (Adjusted $P < 0.001$). The +N and +P treatments were not significantly different from the Control (+N Adjusted $P = 0.228$; +P Adjusted $P = 0.998$). *A. franciscana* had a significant effect on phytoplankton levels in the Control, +N, and +P treatments (Control, +N, and +P: all $P < 0.001$) but not in the N + P treatment ($P = 0.291$). **b** Periphyton chlorophyll concentrations estimated at a number of *A. franciscana* equal to zero were significantly greater than controls in the N + P treatment (Adjusted $P < 0.001$), but not in either the +N and +P treatments (+N Adjusted $P = 0.885$, +P Adjusted $P = 0.972$). *A. franciscana* reduced periphyton levels in all four treatments (Online Resource 1 Table S6) and there was no evidence that the slopes differed significantly among nutrient treatments (Online Resource 1 Table S5)

(Control: Adjusted $P = 0.013$; +N: Adjusted $P = 0.020$; +P: Adjusted $P = 0.010$; N + P: Adjusted $P < 0.001$). On day 14, female *A. franciscana* in the N + P treatment was two-fold heavier than female *A. franciscana* in the +N and +P treatments and 1.6-fold heavier than females in the Control treatment. However, females in the N + P treatment were only significantly larger than those in the +N and +P treatments (Fig. 3a; means and mean comparisons are reported in Online Resource 1 Table S8). Surprisingly, nutrient treatments did not have significant effects on male weights (Online Resource 1 Table S8).

Female *Artemia franciscana* in the N + P treatment had more than twice as many eggs (mean = 186 female^{-1}) as those in other nutrient treatments (Fig. 3b, means and mean comparisons are reported in Online Resource 1 Table S9). Female *A. franciscana* in the +N and +P treatments did not have significantly more eggs than those in the Control treatment (Online Resource 1 Table S9). The length of female *A. franciscana* and the number of eggs were strongly correlated ($r^2 = 0.62$; $P < 0.001$), indicating that the increased brood size of female *A. franciscana* in the N + P treatment may have been partially due to an increase in female size. In addition, one female in the N + P treatment was observed to have a second brood developing in the ovisac, possibly indicating a decreased time interval between broods.

Nutrient concentrations

As expected, nutrient treatments had significant effects on the final concentrations of all nutrients in our experimental bottles (Table 1, Online Resource 1 Table S11). TN, NO_3, and SRP concentrations were significantly impacted by only nutrient treatments; the effect of *Artemia franciscana* was not significant (Online Resource 1 Table S11). Although the +N and N + P treatments began with similar concentrations of N, final concentrations of TN (Adjusted $P < 0.001$) and NO_3 (Adjusted $P < 0.001$) were significantly lower in the N + P treatment than in the +N treatment. Similarly, although the +P and N + P treatments began with similar concentrations of P, the final concentration of SRP (Adjusted $P < 0.001$) was significantly lower in the N + P treatment than in the +P treatment.

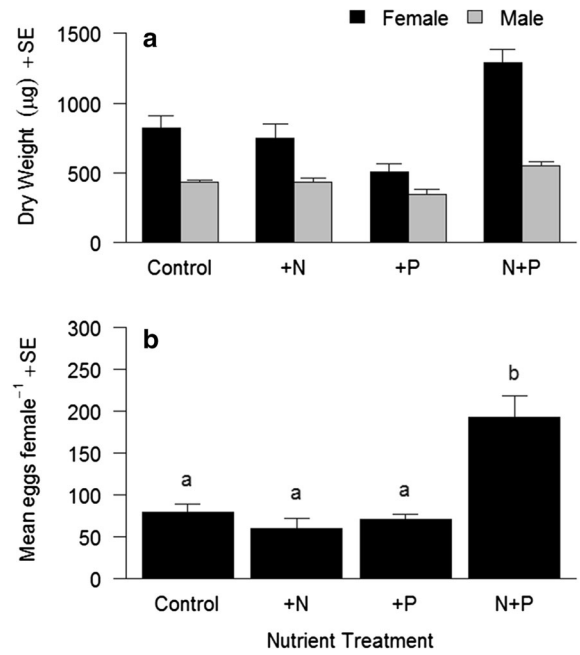

Fig. 3 **a** Mean dry weight of female and male *Artemia franciscana* in the four nutrient treatments on the final day of the 14-day experiment. Female *A. franciscana* in the N + P treatment was significantly heavier than those in the +N (Adjusted $P = 0.010$) and +P (Adjusted $P < 0.001$) treatments, but was not significantly different than those in the Control treatment (Adjusted $P = 0.073$). The weights of female *A. franciscana* in the Control, +N, and +P treatments were not significantly different (Adjusted $P > 0.446$). Nutrient treatments did not significantly affect the weights of male *A. franciscana* (Adjusted $P > 0.128$). Female *A. franciscana* was significantly heavier than male *A. franciscana* in all nutrient treatments (Control Adjusted $P = 0.013$; +N Adjusted $P = 0.020$; +P Adjusted $P = 0.010$; N + P Adjusted $P < 0.001$). **b** Number of eggs female^{-1} in ovisacs of *A. franciscana* in four nutrient treatments on the final day of the experiment. *Letters* above *bars* report pairwise comparisons among nutrient treatment means. Treatments that do not share the *same letters* are significantly different from the others. Female *A. franciscana* in the N + P treatment had significantly more eggs than those in the Control (Adjusted $P = 0.022$), +N (Adjusted $P = 0.003$), and +P (Adjusted $P = 0.047$) treatments. There were no significant differences in the number of eggs produced by female *A. franciscana* in the Control, +N, +P treatments (Adjusted $P > 0.487$)

Artemia franciscana treatments only had significant effects on NH_4 and TP concentrations (Online Resource 1 Table S11). The presence of *A. franciscana* enhanced the positive effect of N addition (Online Resource 1 Table S13) and the negative effect of P addition on NH_4 concentrations (Online Resource 1 Table S14). *A. franciscana* presence reduced the

positive effect of P addition on TP concentrations (Online Resource 1 Table S15).

2011, 2012, 2013 Bioassays

The phytoplankton results of the preliminary bioassays were similar to those found in 2014. In treatments without *Artemia franciscana*, the +N treatment significantly stimulated final phytoplankton chlorophyll concentrations in all years (2011 = 966%, 2012 = 369%, 2013 = 348% relative to the Control treatments; Online Resource 1 Tables S16 and S17). The +P treatment may have had a small stimulatory effect (2011 = 130%, 2012 = 140%, 2013 = 31% of control in treatments without *A. franciscana*), but the difference was never significant ($P > 0.06$). There were significant synergistic stimulatory effects when N and P were added together ($P < 0.01$) and phytoplankton chlorophyll levels in the N + P treatment were over an order of magnitude above controls in all years (2011 = 1202%, 2012 = 1643%, 2013 = 1739% of control in treatments without *A. franciscana*).

Presence of *Artemia franciscana* had a significant negative impact on chlorophyll levels in all years ($P < 0.009$), but the magnitude of the effect varied among years (Online Resource 1 Table S16). In control treatments with *A. franciscana,* phytoplankton chlorophyll levels were 12, 22 and 36% of the control treatments without *A. franciscana* in 2011–2013, respectively (Online Resource 1 Tables S16 and S17). Grazing by adult *A. franciscana* significantly reduced phytoplankton chlorophyll levels in the N + P treatments, but the effects varied among years: phytoplankton chlorophyll levels in the N + P treatment with *A. franciscana* were 22, 56, and 3% of those in the N + P treatment without *A. franciscana* in 2011–2013, respectively (Online Resource 1 Table S17).

Discussion

Bottom–up effects of nutrient additions

The bioassay results indicate that phytoplankton in Gilbert Bay in the Great Salt Lake are primarily limited by nitrogen and secondarily limited by phosphorus. Phytoplankton chlorophyll levels throughout the experiment illustrate the dynamics of primary and secondary limitation. Chlorophyll levels in the +N and N + P treatments increased at the start of the experiment, as nitrogen addition alleviated growth limitation. Chlorophyll levels in the +N treatment declined after day 4, while concentrations in the N + P treatment continued to increase until day 10. Throughout the experiment, the N + P treatment had significantly greater chlorophyll levels than the +N treatment, and on the final day, chlorophyll levels were eight-fold greater in the N + P treatment than in the +N treatment and demonstrate the importance of phosphorus as a secondary limiting nutrient (sensu Tank & Dodds, 2003). Final nutrient concentrations in the microcosms demonstrate the importance of nitrogen and phosphorus, as concentrations of both DIN and SRP were significantly reduced in the N + P treatment by the end of the experiment, probably as a consequence of the increased demand resulting from the higher algal densities.

The results of the 2014 bioassay are consistent with the 2011–2013 bioassays conducted with Great Salt Lake aquaria water. Nitrogen was the primary limiting nutrient all years, as the +N and N + P treatments both increased phytoplankton abundance. N + P treatments were elevated relative to the Control and +N treatments, indicating the role of phosphorus as a secondary limiting nutrient. Although factors such as differences in ambient nutrient concentrations and salinity may have caused the magnitude of nutrient effects to differ between years, the 2011–2014 experiments all demonstrate the importance of nitrogen as a limiting nutrient. In addition, previous Great Salt Lake nutrient bioassays conducted with different methods have also found nitrogen to be the primary limiting nutrient (Stephens & Gillespie, 1976; Wurtsbaugh, 1988; Marcarelli et al., 2006).

In Gilbert Bay, high salinity levels likely reduce the amount of cyanobacterial nitrogen fixation (Herbst, 1998; Marcarelli et al., 2006) and bioavailable nitrogen levels may thus remain low. Dissolved phosphorus may have relatively high concentrations in the water column, as high sulfate concentrations in the lake likely sequester iron, and reduce phosphate precipitation by iron oxyhydroxide (Blomqvist et al., 2004). Phosphorus levels may also be elevated due to limestone phosphoric rock lithology of the lake's watershed (Wurtsbaugh, 1988). Our results are consistent with the review of Javor (1989) who found that most saline lakes are nitrogen limited. Thus, although

the conditions in our experiment differed from actual conditions in the Great Salt Lake, our findings of primary nitrogen limitation and secondary phosphorus limitation are reasonable and likely reflect the lake's nutrient limitation status.

Despite our finding of primary nitrogen limitation, nitrogen was relatively abundant in our total nutrient analyses, and the Great Salt Lake water used to conduct the bioassay had a TN:TP molar ratio of 28:1, suggestive of P-limitation (Smith, 1982). Although TN:TP ratios are a commonly used metric to predict nutrient limitation, the ratio is ineffective when a significant portion of the nitrogen pool is composed of refractory dissolved organic nitrogen (DON) (Lewis & Wurtsbaugh, 2008). DIN:SRP and DIN:TP ratios may more appropriately reflect the relative amount of bioavailable nutrients and have previously been shown to better predict phytoplankton nutrient limitation than TN:TP (Morris & Lewis, 1988). Indeed, the Great Salt Lake water used in our bioassay had molar ratio DIN:SRP = 5.9:1 and DIN:TP = 1.8:1, both indicating N-limitation and consistent with the results of the bioassay.

The accumulation of refractory dissolved organic nitrogen in the Great Salt Lake and other terminal lakes may produce nitrogen-limiting conditions despite high total nitrogen concentrations. Our analysis of dissolved organic nitrogen in Gilbert Bay water collected subsequent to the experiment indicated that dissolved organic nitrogen (DON) was 73% of TN at one of two replicate stations. However, the analysis was problematic at the other station, as DON was greater than TN. Although this is theoretically impossible, researchers have acknowledged that analyses to quantify DON lack sensitivity and precision and the persulfate oxidation method used in our analysis may not efficiently oxidize all refractory compounds (Bronk et al., 2000). The incomplete oxidation of refractory organic nitrogen in our samples may have caused nitrogen levels to appear low in the total nitrogen analysis. The oxidation may have been more complete in TDN analysis, as filtration may have removed clay and sediment or lysed bacterial cells, leading to a more complete digestion and causing nitrogen levels to appear higher in the TDN analysis. Although our analysis did not discriminate between labile and refractory DON pools, we hypothesize that low levels of DIN in the TDN analysis and the N-limitation observed in our assay may indicate that a significant portion of the DON was refractory. An analysis of mixed layer water from the lake indicated that it contained very high dissolved organic carbon (DOC) concentrations (42 mg C l^{-1}; Jones & Wurtsbaugh, 2014). In another terminal lake, Waiser & Robarts (2000) found that DOC averaged 700 years old, indicating that highly refractile dissolved organic compounds can accumulate in terminal, saline systems. Although our work did not directly address the refractile nature of the DON pool in the lake, future work to characterize different DON constituents may better clarify the nuances linking water nutrient concentrations and biological nutrient limitation.

Although several factors are known to influence the availability of nitrogen and phosphorus in the Great Salt Lake, the mechanisms underlying the observed pattern of primary nitrogen limitation and secondary phosphorus limitation in our experiment are less clear. In the +N treatment, nitrogen additions may have caused phosphorus to become limiting, while in the N + P treatment, the simultaneous addition of nitrogen and phosphorus may have allowed phytoplankton abundance to increase without inducing such a limitation. However, at the end of the experiment, the +N treatment still contained a high amount of phosphorus (TP = 0.35 mg l^{-1}; SRP = 0.14 mg l^{-1}). In addition, biochemical-level processes and shifts in algal community composition may have contributed to the synergistic effect of N and P in the N + P treatment (Harpole et al., 2011).

N and P also had a significant interactive effect on the periphyton community in the bioassay, as chlorophyll concentrations only increased markedly with the addition of both nutrients. This indicates that periphyton were colimited by N and P and that multiple nutrient loading may have a similar effect on pelagic and benthic production. Benthic nutrient dynamics are particularly important as periphyton have been shown to be a significant component of whole-lake primary production and respiration processes, particularly in shallow systems (Vadeboncoeur et al., 2002) such as the Great Salt Lake. In the Great Salt Lake, recent analysis of littoral zone carbonaceous biostromatolites indicated that periphyton production may be approximately 30% of phytoplankton production (Wurtsbaugh et al., 2011). In our experiment, periphyton represented maximally 13% (in the N + P treatment) of the total bottle chlorophyll, but this was likely the

result of a slow colonization process on the bottle walls, and also because the algae that were added were likely dominated by planktonic species and not benthic taxa.

The synergistic effect of N and P in both the phytoplankton and periphyton highlights the close biological interplay of the two nutrients and indicates the importance of considering the potential synergistic or supraadditive effect of multiple nutrients on primary producers (Harpole et al., 2011). A metaanalysis of nutrient enrichment experiments in freshwater and marine environments found interactions between N and P to be common (Elser et al., 2007). Although ecological debates have largely focused on whether nitrogen or phosphorus are more likely to be limiting in certain systems (e.g., Lewis & Wurtsbaugh, 2008), our findings of primary nitrogen limitation and secondary phosphorus limitation in the phytoplankton and colimitation in the periphyton supports the importance of considering both nutrients when evaluating controls on primary production.

It is increasingly important to understand the mechanisms driving nutrient dynamics in the Great Salt Lake, as anthropogenic activity may have modified nutrient dynamics from historical conditions and, in the future, may produce biogeochemical conditions not previously observed. Metropolitan growth in Salt Lake City is a considerable source of nutrient loading through wastewater effluent and storm water inputs that are primarily transported into the lake via a large hyposaline nontidal estuary on the southeast corner of the lake (Farmington Bay) (Marcarelli et al., 2006). Although nitrogen fixation in that bay can be extensive, nitrogen-removing mechanisms such as denitrification likely remove large quantities of nitrogen, and both N and P sedimentation may be high. The reduction in TN and TP concentrations in our N + P treatment may have been caused by greater losses of N and P to detrital material or periphyton. Nevertheless, Farmington Bay outflows provide approximately 45% of the nitrogen loading to Gilbert Bay with a TN:TP molar ratio of 30:1 (Wurtsbaugh et al., 2006), although, as demonstrated by our study, the bioavailability of nitrogen and phosphorus will influence their effects on production dynamics. Further analysis of temporal nutrient dynamics and anthropogenic impacts on Great Salt Lake will improve efforts to manage and preserve the lake ecosystem.

Top–down effects of *Artemia franciscana* grazing

Artemia franciscana grazing in the 2014 bioassay regulated phytoplankton via top–down in Control, +N, and +P treatments. The top–down effect of consumers on primary production has been demonstrated in freshwater (Carpenter et al., 1985; Vanni, 1987), marine (Hessen & Kaartvedt, 2014), and hypersaline (Jellison & Melack, 1988, 1993; Wurtsbaugh, 1992) systems. Lampert et al. (1986) empirically demonstrated that high rates of zooplankton grazing, rather than nutrient depletion, cause the clear water phase of low phytoplankton abundance that occurs after phytoplankton blooms.

In the Great Salt Lake, monitoring and laboratory studies have noted a strong negative correlation between *Artemia franciscana* abundance and chlorophyll concentration (Wurtsbaugh & Berry, 1990; Wurtsbaugh, 1992; Belovsky et al., 2011). Seasonal phytoplankton and *A. franciscana* dynamics are strong, as *A. franciscana* cysts hatch in the spring when phytoplankton abundance is high, but by midsummer when the growing *A. franciscana* populations have grazed phytoplankton to levels that become food limiting (chlorophyll $\ll$1.0 μg l^{-1}), the shrimp population declines. Because of the reduced grazing pressure, phytoplankton abundance slowly increases until late fall and remains high during winter conditions when *A. franciscana* populations overwinter as resting cysts (Wurtsbaugh & Gliwicz, 2001).

The grazing effect was less robust in the N + P treatment and increasing densities of *Artemia franciscana* did not significantly reduce phytoplankton chlorophyll levels on any day in the experiment. *A. franciscana* in the N + P treatment were likely unable to increase ingestion rates to compensate for the elevated phytoplankton chlorophyll concentrations, as they were likely feeding to repletion. To evaluate this hypothesis, we estimated filtration rates and ingestion rates of *A. franciscana* in our experiment using the results of a feeding rate experiment conducted by Reeve (1963). *Dunaliella viridis* Teodoresco cell counts were converted to chlorophyll concentration with Great Salt Lake data when concurrent cells counts and chlorophyll measurements were available (W. Wurtsbaugh, unpublished data). This analysis indicated that approximately 6.7 *D. viridis* mm^{-3} is equal to 1 μg l^{-1} of chlorophyll. The conversion of Reeve's results suggests that above 2 μg l^{-1} Chl, 10

mm *A. franciscana* is able to feed to repletion and ingestion rates of *D. viridis* level off near 0.4 µg Chl day^{-1}. Reeve's data suggest that when chlorophyll levels reach 10 µg l^{-1}, *A. franciscana* can obtain its maximum ration by filtering at only 10% of its maximum rate. These results suggest that *A. franciscana* was likely feeding to repletion in all nutrient treatments throughout the experiment and *A. franciscana* in the N + P treatment was thus unable to ingest greater amounts of chlorophyll to compensate for the bottom–up effect of N + P addition.

As in the Great Salt Lake where temporal grazing dynamics are striking, our bioassay also demonstrated how *Artemia franciscana* grazing pressure increased as *A. franciscana* grew and was able to filter and ingest greater amounts of phytoplankton. *A. franciscana* was added as 3-day-old nauplii that have very low filtration rates (ca. 1.5 ml $individual^{-1}$ day^{-1}), allowing the stimulatory bottom–up effect of nutrient addition on phytoplankton to get a head start. A similar pattern occurs during spring conditions in lakes where spring mixing and river inflow increase nutrients which, in combination with higher temperatures and light conditions, stimulate phytoplankton blooms that subsequently support emerging grazer populations (Sommer et al., 2012). By the end of our experiment, grazing rates were sufficiently high and *A. franciscana* reduced final phytoplankton abundance in the Control, +N, and +P treatments to concentrations below the control treatment without *A. franciscana*.

The results of the 2011–2013 bioassays further demonstrate the importance of *Artemia franciscana* filtration rates on phytoplankton abundance. In these bioassays, adult *A. franciscana,* rather than nauplii, were added at the start, creating high grazing rates throughout the experiment. As a result, *A. franciscana* grazing significantly reduced phytoplankton in all nutrient treatments, including those in the N + P treatments. *A. franciscana* grazing had a smaller impact in 2012 likely due to the use of adult *A. franciscana* densities <50% of those in the other years.

The top–down effect of *Artemia franciscana* on periphyton abundance was unexpected, as this zooplankton is thought to primarily graze in the water column on phytoplankton, and their association with the benthic zone has not been extensively studied in the Great Salt Lake or elsewhere. *A. franciscana* had a larger grazing effect on periphyton in Control, +N, and +P treatments than in the N + P treatment. The low phytoplankton abundances (chl <8 µg l^{-1}) in the Control, +N, and +P treatments reflected concentrations observed to limit *A. franciscana* reproductive rates (Gliwicz et al., 1995), and this may have prompted the *A. franciscana* to increase feeding on the periphyton.

The effect of grazers on periphyton in the Great Salt Lake and other saline lakes may be potentially important. In previous Great Salt Lake studies, *Artemia franciscana* grazing has only been hypothesized to influence biostromatolite periphyton production by increasing light availability for the periphyton (Wurtsbaugh et al., 2011). However, Gliwicz et al. (1995) and Barnes & Wurtsbaugh (2015) both observed *A. franciscana* apparently grazing on periphyton in mesocosms mimicking the Great Salt Lake. Additional studies are required to definitely test whether *A. franciscana* is actually ingesting the periphyton, as the swimming activity of the shrimp may have simply dislodged periphyton and caused the observed reduction in periphyton abundance.

Artemia franciscana presence increased NH_4 concentrations in nitrogen addition treatments and decreased concentrations in phosphorus addition treatments. Shifts in the N:P ratio of algae that result from the addition of N and P may alter nutrient recycling by *A. franciscana* (Elser et al., 1996; Elser & Urabe, 1999). For example, an increase in the N:P content of phytoplankton caused by N addition may have increased the amount of excess N excreted by *A. franciscana* as NH_4. Similarly, a decrease in the N:P ratio of phytoplankton caused by P addition may have reduced the amount of excess N excreted by *A. franciscana* as NH_4. Additionally, the higher NH_4 concentrations in the +N treatments, and lower concentrations in the +P treatment may have been driven by phytoplankton uptake. In the +N treatment the algae likely became P-limited by the end of the experiment, so NH_4 excreted by the *A. franciscana* may have accumulated. In the +P treatment nitrogen likely was limiting by the end of the experiment, so algal demand for NH_4 would have been high. Additional research that considers the relative amounts of N and P in the water column and the biomasses of primary producers and *A. franciscana* can better clarify the influence of consumer-driven nutrient recycling on interactions between top–down and bottom–up food web controls.

Comparison of top–down and bottom–up controls and feedback mechanisms

The relative strength of nutrients and grazing treatments on phytoplankton chlorophyll levels varied with the combination of nutrients added, chlorophyll concentration, and the temporal changes in the grazing ability of *Artemia franciscana* populations. On the final day of the experiment, single nutrient treatments were primarily controlled by the top–down effect of *A. franciscana*. In +N and +P treatments, grazing reduced phytoplankton abundance to levels below the Control treatment without *A. franciscana* and neither nutrient added alone counteracted the consumptive effect of *A. franciscana* on phytoplankton. *A. franciscana* was less effective at counteracting the stimulatory effect of N + P addition and *A. franciscana* grazing never had a significant effect on phytoplankton concentrations in the N + P treatment. High rates of primary production stimulated by N and P additions and low grazing rates of nauplii early in the experiment likely allowed phytoplankton in the N + P treatment to increase to levels that the adult *A. franciscana* later in the experiment was unable to reduce. In the Great Salt Lake, phytoplankton only reach excessive levels under winter or hypersaline conditions where *A. franciscana* cannot flourish (Wurtsbaugh & Gliwicz, 2001; Belovsky & Larson, 2002; Belovsky et al., 2011).

Feedback mechanisms may ultimately mediate the relative strength of nutrient and grazer controls. Although *Artemia franciscana* were unable to counteract the strong stimulatory effect of N + P addition during the 2 weeks of our experiment, the increased size and reproductive output of the N + P treatment *A. franciscana* indicates a potential positive feedback mechanism in which *A. franciscana* grazing pressure would have increased in subsequent generations. Nutrient addition has been shown to increase zooplankton biomass in whole-lake fertilization experiments (e.g., Carpenter et al., 1996) and previous Great Salt Lake field studies have observed the reproductive output of *A. franciscana* to increase under food rich conditions (Wurtsbaugh & Gliwicz, 2001; Belovsky et al. 2011). Differences in food quality or the reduction of filtering rates at high food levels may also provide an energy savings that allow the female *A. franciscana* to grow larger and produce more eggs (Wurtsbaugh & Gliwicz, 2001). Male *A. franciscana* were smaller than female *A. franciscana* and likely exerted less grazing pressure on the phytoplankton. The reason that males were smaller than females is not clear, but it is possible that the former approached terminal sizes, which are smaller than those of females (Triantaphyllidis et al., 1997). In the Great Salt Lake, *A. franciscana* population growth and high grazing rates frequently drive phytoplankton to extremely low levels (<0.5 μg l^{-1}) resulting in short-term collapse of the *A. franciscana* populations and the rebound of the phytoplankton (Wurtsbaugh & Gliwicz, 2001).

Nutrient sequestration by primary producers may ultimately mediate the stimulatory effect of N + P additions. The reduction of inorganic nutrients in the N + P treatment demonstrated the significant effect that algae may have on nutrient availability. Although nutrient regeneration during the decomposition of detritus likely ameliorates this effect, in the Great Salt Lake that has a monimolimnion (Jones & Wurtsbaugh, 2014), and in thermally stratified lakes, return of mineralized nutrients from these deep layers may occur only on time-scales of seasons or years (Jellison et al., 1998). Indeed, our analysis of Great Salt Lake water indicated that DON was a significant portion of TDN. Thus, nutrient sequestration in combination with a low rate of nutrient regeneration may ultimately serve as a negative feedback mediating the effect of N + P addition. Long-term experiments that directly test the effect of algae on nutrient concentrations and measure decomposition and nutrient regeneration rates will more effectively address this potential feedback mechanism.

As urbanization and its associated impacts on the Great Salt Lake ecosystem are likely to increase in the future, efforts to preserve the Great Salt Lake ecosystem must consider both the effect of nutrient loading on phytoplankton abundance and the ability of *Artemia franciscana* to mediate and respond to high phytoplankton concentrations. As the results of our bioassay indicate, complex interactions between abiotic conditions, consumer population densities, and food web dynamics can drive ecosystem level conditions. In addition, as water diversions increase salinity levels in saline lakes worldwide (Williams, 2001), future studies should also consider how the relative strength of nutrients and grazing change across a range of salinities. Continued study that incorporates monitoring and experimental techniques will enhance our understanding of future anthropogenic impacts.

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