RESEARCH ARTICLE

Response of Lower Sacramento River phytoplankton to high-ammonium wastewater effluent

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Since the 1980s, the San Francisco Bay Delta ecosystem has experienced large declines in primary production. Hypothesized reasons for this decline include (1) suppression of nitrate (NO_3^-) uptake, and thus phytoplankton growth, due to high concentrations of ammonium (NH_4^+), and (2) wastewater NH_4^+-induced changes in phytoplankton community composition away from large-celled diatoms. These twin hypotheses implicate NH_4^+ loading from the Sacramento Regional Wastewater Treatment Plant effluent outfall in explaining declines in primary production in the region. They have been controversial within the water resources management community and have stimulated a lengthy public scientific and regulatory debate. Here, in an effort to resolve this debate, we present results from a 48-h incubation experiment with surface water from both upstream and downstream of the Sacramento Regional Wastewater Treatment Plant effluent outfall, a major source of NH_4^+ loading to the ecosystem. We amended this water with either NH_4^+, NO_3^-, or full wastewater effluent. All assays were incubated under high light (52% of incident irradiance) or low light (6% of incident irradiance). NO_3^- uptake rates were suppressed to near zero in all treatments with either added NH_4^+, added wastewater effluent, or high in situ NH_4^+ concentrations. Yet, phytoplankton uniformly grew well on all dissolved inorganic nitrogen sources, including effluent and NH_4^+. Diatom species were the most abundant taxa at all stations, and diatom cell abundances increased at greater rates than all other taxa over the course of the experiment. Among all treatments, the light treatment had the greatest effects on chlorophyll *a* accumulation and phytoplankton growth rates. Our results suggest that high NH_4^+ loading is not a driver of the lower productivity in the San Francisco Bay Delta. Although phytoplankton preferred NH_4^+ to NO_3^- when both were available in our experiment, the form of dissolved inorganic nitrogen had no effect on growth rates or species composition.

Keywords: Phytoplankton, Nutrient forms, Eutrophication, Wastewater, Nitrogen, Nutrient management

Introduction

The Sacramento River is the largest river in California and extendss 400 miles from Mount Shasta to San Francisco Bay (SFB). Along this route, it drains an area of 62,000 km² and contributes an important fraction of the freshwater supply and nutrients that fuel the food webs of the Sacramento-San Joaquin River Delta (hereafter "Delta"), as well as SFB (Schemel, 1980; Dolmagalski and Dileanis, 2000; Sobota et al., 2009; Senn and Novick, 2014). SFB is the largest estuary on the western coast of North America.

The northern portions of SFB and the Delta have experienced significant ecological changes over the past several decades (Cloern and Jassby, 2012). Most notably, phytoplankton production dropped dramatically, beginning in 1986 (Jassby et al., 2002). The decrease in productivity has cascaded up the food web (Kimmerer, 2006), leading to an overall decline in fish production throughout the northern SFB and the Delta (Sommer et al., 2007). This phenomenon is known as pelagic organism decline (POD) and has been a major focus of federal and state agency

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management efforts in the Delta region for decades. In particular, one species suffering under POD is the endangered Delta smelt (*Hypomesus transpacificus*), a federally listed species under the Endangered Species Act.

The POD presents a paradoxical puzzle because the Delta ecosystem is a high nutrient environment, receiving large inputs of nitrogen (N) from Central Valley agricultural runoff as nitrate (NO_3^-) and from wastewater treatment plant discharges as ammonium (NH_4^+). In particular, the Sacramento Regional Wastewater Treatment Plant (SRWTP) effluent outfall, located downstream of the City of Sacramento, discharges approximately 15,000 kg of NH_4^+ per day into the Lower Sacramento River (Senn and Novick, 2014). The SRWTP is the single largest point source of N to the SFB-Delta system, and NH_4^+ concentrations downstream of the effluent discharge in the Sacramento River are frequently 30–80 µM. The corresponding increase in phytoplankton biomass normally associated with such high dissolved inorganic nitrogen (DIN) inputs (often leading to eutrophication) is not observed in SFB or the Delta, and environmental managers in the region have been concerned about decreased phytoplankton productivity and POD, despite these high concentrations of DIN (Novick and Senn, 2014).

The high concentrations of DIN, in particular high concentrations of NH_4^+, have been hypothesized to reduce overall phytoplankton growth via depression in rates of NO_3^- uptake (Dugdale et al., 2007). This idea was supported by Parker et al. (2012), who found that growth on NH_4^+ suppressed carbon fixation by phytoplankton in the Lower Sacramento River, lowering overall primary productivity. Glibert et al. (2014a) argued that the decline in productivity in the Bay Delta (the portions of the Sacramento River and Suisun Bay downstream from the SRWTP) is a function of the transition away from a diatom-dominated community due to inputs of NH_4^+ from the SRWTP. Glibert (2010) specifically implicated changes in DIN loading and DIN speciation as a cause for POD, via the negative effects of high NH_4^+ concentrations on the phytoplankton community and its growth. These ideas run contrary to extensive bodies of both laboratory and field studies that show energetically favorable preferential uptake and assimilation by phytoplankton of the reduced DIN form NH_4^+ rather than NO_3^- when both are present (Ohmori et al., 1977; Cresswell and Syrett, 1979; Dortch, 1990; Boyer et al., 1994; Berges et al., 1995; He et al., 2004; Song and Ward, 2007). Yet, given the societal and economic salience of the impacts of excess DIN loading to coastal ecosystems, our understanding of how specific chemical forms of human-introduced DIN influence total phytoplankton productivity and phytoplankton community composition, and therefore ecosystem services, in human-impacted coastal aquatic ecosystems remains surprisingly uncertain and is an area of active research (Esparza et al., 2014; Glibert et al., 2016; Berg et al., 2017; Kraus et al., 2017).

As a result, resource managers have been concerned about the potential influence of NH_4^+ on the decline in phytoplankton standing stocks as well as POD. In part based on these concerns, a National Pollution Discharge
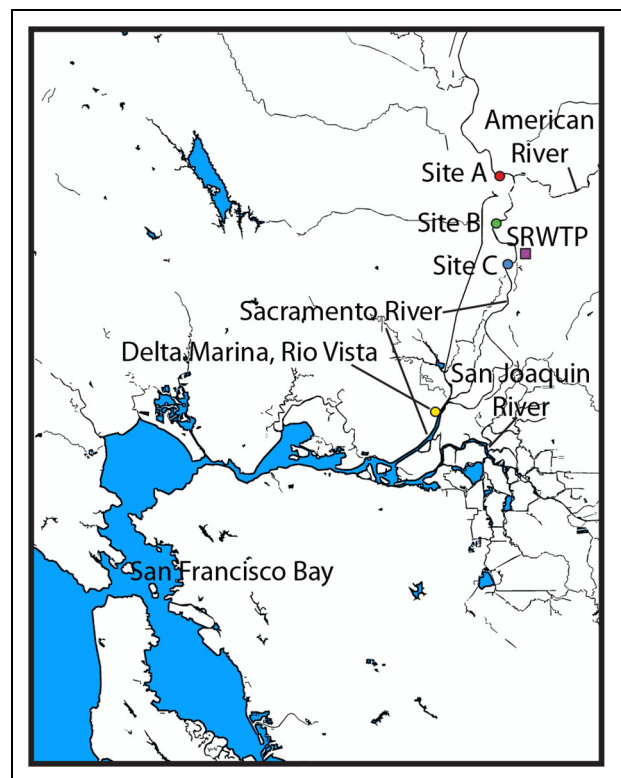

Figure 1. Map of San Francisco Bay Estuary and the Sacramento and San Joaquin rivers. Shown are the water collection sites A–C and their locations relative to the Sacramento Regional Wastewater Treatment Plant. Also shown is the Delta Marina, Rio Vista, site where the experiments were conducted. DOI: https://doi.org/10.1525/elementa.2021.040.f1

Elimination System (NPDES) permit was approved for the Sacramento Regional Sanitation District, which requires conversion of the SRWTP to a tertiary treatment system that will include both nitrification and denitrification steps (California Regional Water Board, Central Valley Region, 2014). These changes mean that the N in effluent will no longer discharge primarily as NH_4^+ and that total DIN concentrations in the discharge will decrease by 74%.

In an attempt to bring scientific clarity to nutrient management of the Lower Sacramento River, we assessed the effects of high NH_4^+ versus NO_3^- concentrations on in situ phytoplankton communities from the Lower Sacramento River using a replicated set of experimental incubations (**Figure 1**). This experiment investigates two key ecological questions about the impact of DIN species on phytoplankton growth, physiology, and community structure:

1. Are phytoplankton accumulation and physiological state at the high in situ NH_4^+ concentrations found downstream of the SWTRP reduced relative to the response at lower in situ NO_3^- concentrations?
2. How is phytoplankton community composition affected by the available DIN substrate?

Table 1. Initial NH_4^+, NO_3^-, PO_4^{3-}, and $Si(OH)_4$ concentrations ($\mu mol\ L^{-1}$) at all stations, and at time zero, in the NH_4^+, NO_3^-, and effluent treatments.[a] DOI: https://doi.org/10.1525/elementa.2021.040.t1

Station[b]	NH_4^+			NO_3^-		PO_4^{3-}		$Si(OH)_4$	
	Initial	NH_4^+	Effluent	Initial	NO_3^-	Initial	Effluent	Initial	Effluent
A	0.8	55.4	44.6	2.9	7.9	0.6	1.5	288.6	298.3
B	1.7	47.5	41.2	6.7	10.6	0.8	1.8	270.6	277.9
C	56.7	—[c]	—	2.2	—	7.8	—	278.3	—

[a]Treatments were targeted to concentrations measured using underway instrumentation during sampling transects.

[b]Stations A and B are located upstream of the SRWTP outfall (**Figure 1**); Station C is downstream from the outfall.

[c]dash indicates no amendment made.

Results from these experiments are critical to informing state and regional water quality decisions about how to manage the SFB and Delta ecosystems under future nutrient regimes, including how phytoplankton standing stocks and composition will change with future decreases in total DIN and concentrations of NH_4^+ in the Lower Sacramento River and in the context of ongoing climate change (Sinha et al., 2017). Our work also contributes new ecologically important insights into our understanding of the response of phytoplankton growth, physiology and community composition to varying nutrient forms in highly impacted riverine and estuarine systems.

Methods

Our study was designed to test the effects of high NH_4^+ on phytoplankton growth in Lower Sacramento River waters surrounding the SRWTP. As such, we used waters upstream and downstream of the SRWTP for our experiments. The upstream waters were low in NH_4^+ and represent the waters that flow southward into the effluent plume. Delta waters downstream of the SRWTP already contained effluent and thus already had elevated NH_4^+. The general design of the experiment was to amend water samples from the Sacramento River at two sites upstream and one site downstream of the SRWTP discharge location with NH_4^+, NO_3^-, or effluent taken directly from the SRWTP and to incubate the samples at two light levels. NH_4^+ and NO_3^- amendments were designed to match the amounts of added NH_4^+ and NO_3^- from the wastewater effluent. These samples were incubated for 48 h to assess phytoplankton physiological and community responses to different nitrogen forms at in situ concentrations (**Table 1**).

Sample water collection

Near-surface water was collected from the R/V *Questuary* between 07:30 and 11:30 on May 6, 2015, from three locations in the Sacramento River (**Figure 1**). Station A (the Interstate Highway 80 Bridge) was located immediately upstream of the confluence of the American and Sacramento rivers at 38.5997°N and 121.5513°W. Station B (upstream of the SRWTP effluent outfall pipe) was located at 38.5163°N and 121.5455°W downstream of the confluence of the American and Sacramento rivers, close

to USGS-Station 29: Garcia Bend. Station C (downstream of the SRWTP effluent outfall pipe) was located at 38.4348°N and 121.5163°W, near USGS-Station 26: River Mile 44. Our sites (A–C) are the same as, or in the immediate vicinity of, the sites I-80, OAK, and RM44 sampled by Parker et al. (2012).

At all stations, surface water was collected using prewashed plastic (high-density polyethylene) 5-gallon buckets deployed by hand from the deck of the ship. Sample water was poured through a funnel equipped with a 300-μm Nitex screen filter into 10-L acid-cleaned (10% hydrochloric acid [HCl] soak followed by 3 Milli-Q washes) cubitainers (ThermoScientific™ LDPE Poly-Cubitainers) to remove macrozooplankton and large detrital particles. All cubitainers were washed 3 times with sample water prior to filling. At Stations A and B, twenty-four 10-L cubitainers were filled, and at Station C, six 10-L cubitainers were filled. All cubitainers were placed in the dark during transit to the incubation site at the Delta Marina in Rio Vista, CA (38.1491°N, 121.6925°W).

Incubation experiment design

The cubitainers were incubated for 48 h (starting May 6, 2015, at 15:00 until May 8, 2015, at 15:00) in two square, white-plastic incubators placed on the dock at the Delta Marina in Rio Vista, CA. Each incubator was continuously flushed with Sacramento River surface water to maintain ambient temperature. Treatments for incubated water from Stations A and B (both upstream of the outfall) included an unamended control and amendments with NH_4^+, NO_3^-, and SRWTP effluent water obtained the day before the experiments. The effluent water was kept at ambient temperature in the dark for 24 h prior to use in experiments. NH_4^+ and effluent additions were targeted to increase NH_4^+ concentrations to approximately 55 μM, a concentration equivalent to that measured in near-surface waters at Station C on the day of sampling (**Table 1**). NO_3^- additions were targeted to achieve a concentration of 7.5 μM NO_3^-, equivalent to the concentration measured in the water column at Station C on the day of sampling (**Table 1**). Water from Station C (downstream of the outfall) was kept as an unamended control and not subjected to any nutrient additions. All treatments were run in triplicate (**Figure 2A**). Subsamples were collected
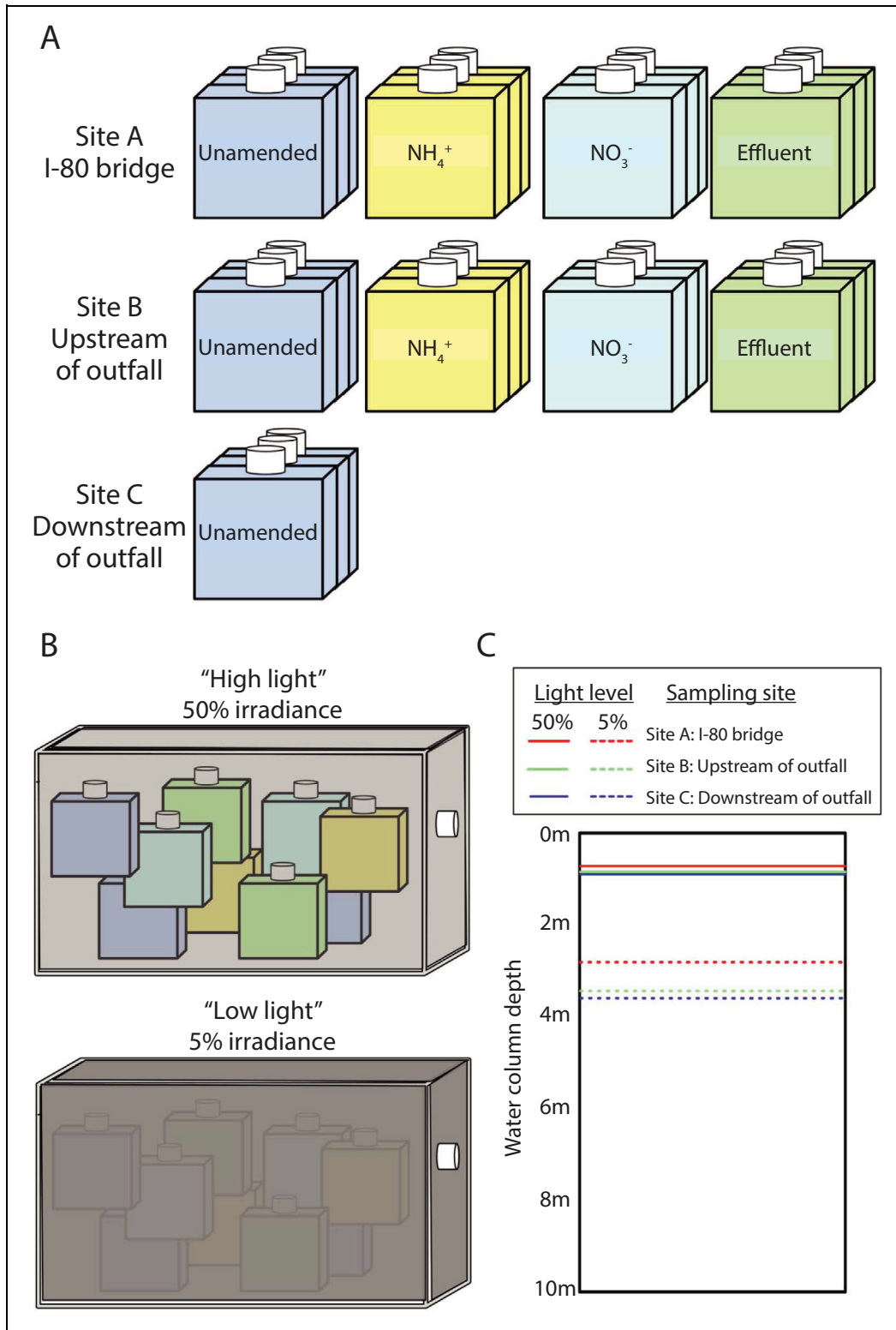
Figure 2. Experimental design of the irradiance, nutrient, and effluent experiments. Shown are the nutrient (A) and light (B) treatments and the water column depths that correspond to the light treatments (C) at each station. See **Table 1** for initial nutrient concentrations at each site and in each treatment. DOI: https://doi.org/10.1525/elementa.2021.040.f2

over the incubation period at $t = 0$ (initial), 12, 24, 36, and 48 h for analysis of concentrations of the nutrients NH_4^+, NO_3^- + nitrite (NO_2^-), phosphate (PO_4^{3-}), and dissolved silica ($Si(OH)_4$) and of particulate organic carbon (POC) and chlorophyll a (Chl a), as well as for determining

NH_4^+ and NO_3^- uptake rates and phytoplankton photophysiological state, measured as the maximum photochemical efficiency of photosystem (PS) II (Fv/Fm) and the functional absorption cross section of PSII (σ_{PSII}). Note the initial samples ($t = 0$) for the variables listed above

were collected from each cubitainer at the start of the incubation.

Light treatments

Each of the two white-plastic incubators housed 27 cubitainers, representing triplicate samples for all nine combinations of station and nutrient treatments. Each incubator was then subjected to either high light (HL) or low light (LL) using neutral density screening placed over the top of the incubators (**Figure 2B**). The HL treatment held cubitainers at approximately 52% of ambient incident photosynthetically active radiation (PAR), and the LL treatment held cubitainers at approximately 6% of ambient incident PAR. PAR was obtained from the average of multiple measurements (as percentage of PAR just below the surface water in the incubators) made prior to the start of the experiment using a scalar PAR sensor (QSL-2100, Biospherical Instruments, San Diego, CA). PAR measurements were corrected for attenuation by the plastic sides of the cubitainer. Using PAR extinction profiles from the water columns at each of our three sampling locations, we calculated the water column depths for each location represented by the HL and LL treatments (**Figure 2C**). Light within the incubators was measured throughout the duration of the experiment using a cosine luminosity detector (HOBO™ Pendant, Onset Corporation, Bourne, MA) extended from a metal fastener into the middle of each incubator. A reference cosine luminosity detector was mounted above the incubators and exposed to ambient above water irradiance.

Chl a and POC

Chl a samples collected on 25-mm Whatman GF/F filters were fluorometrically measured (Holm-Hansen et al., 1965) in triplicate at all 5 time points from each cubitainer using a Turner Fluorometer 10-AU (Turner Designs, Inc.). POC was analyzed by filtering sample water onto precombusted (450 °C for 4 h) 25-mm Whatman GF/Fs. The filters were fumed with HCl, dried at 60 °C, and packed into tin capsules (Costech Analytical Technologies, Inc.) for analysis on an Elementar Vario EL Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) at the University of California, Davis. Peach leaves and glutamic acid were used as calibration standards. Experimental results are presented as treatment concentrations at the final time ($t = 48$ h) relative to the initial time.

Nutrient analyses

Samples for NH_4^+, $NO_3^- + NO_2^-$, and PO_4^{3-} were filtered through a 0.2-μm polycarbonate syringe filter (Acrodisc® Syringe Filter with Supor Membrane, Pall Laboratory, Port Washington, NY) into plastic collection vials and frozen at −20 °C until analysis. Samples for Si(OH)$_4$ analysis were also filtered through a 0.2-μm polycarbonate syringe filter but were kept at 4 °C until analysis. NH_4^+ samples were analyzed by fluorometric development of ortho-phthalaldehyde (Holmes et al., 1999) on a FIALab 1000 ammonia analyzer. NO_3^- samples were analyzed colorimetrically using the cadmium-reduction method (Grasshoff et al.,

1999) on a SmartChem 200 discrete analyzer (Unity Scientific, Brookfield, CT). PO_4^{3-} samples were analyzed colorimetrically using the ascorbic acid method on a spectrophotometer (Towns, 1986), and dissolved Si(OH)$_4$ samples were analyzed on an inductively coupled plasma optical emission spectrometer (Thermo Scientific ICAP 6300 Duo View) after acid digestion. All analyses were performed using a standard curve at relevant concentrations, and the maximum error for replicates was 5%. All nutrient analyses were performed in laboratories at Stanford University.

Phytoplankton enumeration

Triplicate subsamples (125 mL) of the 300-μm prescreened water for the incubations were collected into amber bottles at each sampling location (Stations A–C, $n = 9$) and fixed with 2% acid Lugol's solution, then stored in the dark until analysis. Final subsamples were collected at $t = 48$ h, fixed, and stored similarly for all experimental cubitainers. For analysis, samples were filtered onto 0.2-μm polycarbonate membrane filters (Nuclepore) and enumerated using a Leica DMLB compound microscope according to McNabb (1966) as described in Beaver et al. (2013). Briefly, at least 400 natural units (colonies, filaments, and unicells) were enumerated to the lowest possible taxonomic level from each sample. The abundance of common taxa was estimated by random field counts. Rare taxa were quantified by scanning a transect of the filter. In the case of rare, large taxa, half of the filter was scanned and counted at a lower magnification. Cell volumes (biovolumes) were estimated by applying the geometric shapes that most closely matched the cell shape (Hillebrand et al., 1999). Biovolume calculations were based on measurements of 10 organisms per taxon for each sample where possible. Mean biovolume values were computed for any sampling event that included duplicate samples. Phytoplankton microscopy was performed by BSA Environmental Services, Inc. (Beachwood, OH, USA).

Phytoplankton physiology analyses

At all 5 time points during the experiment, we measured the F_v/F_m and σ_{PSII} by fast repetition rate fluorometry (FRRF, LIFT-FRR, Soliense, Santa Cruz, CA) according to Kolber et al. (1998). Briefly, the sample chamber was exposed to an FRRF excitation protocol composed of a series of microsecond-long flashlets of controlled excitation power. The saturation phase of the excitation was comprised of 100 flashlets at intervals of 2.5 ms. With the pulse excitation power of 30,000–50,000 μmol quanta m^{-2} s^{-1}, the rate of excitation delivery to PSII centers far exceeded the capacity of photosynthetic electron transport between PSII and PSI. This excess resulted in a progressive saturation of the observed fluorescence transients within the first 40–60 flashlets, at a rate proportional to σ_{PSII}. The saturation phase was followed by 90 flashlets applied at exponentially increasing time intervals starting at 20 ms, over a period of 250 ms. As the average excitation power decreased, the fluorescence signal relaxed with kinetics mostly defined by the rate of electron transport

between PSII and PSI. Each sample measurement consisted of an average of 32 transients, and each sample was measured 3 times. Recorded fluorescence transients were processed with FRRF software (http://soliense.com) to estimate F_v/F_m and σ_{PSII} for all Chl a-containing cells (excitation wavelength of 470 nm). Blanks for individual samples analyzed by FRRF were prepared by syringe filtration (0.2 μm) of the sample, and all reported values were corrected for blank effects (Cullen and Davis, 2003).

^{15}N uptake analyses

Measurements of NO_3^- and NH_4^+ uptake rates were made from all treatment cubitainers every 12 h during the experiment using the ^{15}N stable isotope tracer method (Glibert and Capone, 1993). Briefly, two subsamples from each cubitainer were taken in clean 250-mL polycarbonate square bottles, one for NH_4^+ uptake and one for NO_3^- uptake. To measure ^{15}N uptake, bottles were spiked with clean-labeled nutrients ($K^{15}NO_3$ or $^{15}NH_4Cl$) equivalent to 10% of the estimated concentration in the sample. Bottles were placed back into separate, shaded LL and HL incubators; after a 4-h incubation period, 125 mL were filtered onto a precombusted 25-mm Whatman GF/F. The δ^{15}N of particulate nitrogen (PN) was measured following the above protocol for PN on a Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, United Kingdom). Samples for analysis of NH_4^+ and NO_3^- concentrations were also taken at the beginning and end of each 4-h uptake rate incubation. Specific uptake rates (V) for each DIN species were calculated following Dugdale and Goering (1967):

$$V = (^{15}N_p - {}^{15}N_{ue})/(^{15}N_{diss} - {}^{15}N_p) * t,$$

where $^{15}N_p$ is the ^{15}N atom percentage (atom%) in the particulate fraction, $^{15}N_{ue}$ is the measured ^{15}N atom% in the unenriched sample, $^{15}N_{diss}$ is the atom% ^{15}N in the dissolved fraction after spiking, and t is the time in hours of the incubation. V is the specific uptake rate in units of h^{-1}.

Statistical analyses

Prior to analysis, all triplicate measurements of Chl a concentration were averaged. The three replicates for each nutrient and light condition were then averaged, and standard deviations were calculated for each variable of interest. Statistical analyses were organized hierarchically. The effects of the incubation itself were assessed by comparing measurements of dependent variables of interest (Chl a and POC concentrations, diatom abundance and biovolume, nutrient uptake rates) at $t = 0$ and 48 h, combining all treatments across all light and nutrient treatments. The effect of light treatments was measured by comparing changes in dependent variables of interest at $t = 48$ h between the HL and LL treatments, across all nutrient treatments. The effects of nutrient treatments were measured by comparing changes in dependent variables at $t = 48$ h between nutrient treatments, across all sampling

locations. Statistically significant effects were assessed at the α = 0.05 significance level. All tests of significance involved one-way or two-way analyses of variance followed by post hoc Tukey means comparison analysis. Standard, ordinary least squares linear regressions were performed to analyze the relationship between POC accumulation and Chl a accumulation, using the lm function in the base R statistical software.

Results

Chl a and organic carbon accumulation

At all three sites, temperature and salinity profiles indicated that the water columns were well mixed. The initial Chl a concentrations were relatively low at each site, reflecting low phytoplankton abundance. Initially, Chl a concentrations at Station A (1.3 ± 0.2 μg Chl a L^{-1}, $n = 3$) were nearly double those at Station B (0.7 ± 0.1 μg Chl a L^{-1}, $n = 3$) and more than double those at Station C (0.5 ± 0.1 μg Chl a L^{-1}, $n = 3$). After incubation for 48 h, Chl a concentration increased under all nutrient and light conditions (**Figure 3**), though increases and nutrient enrichment effects were greater in the HL treatment (**Figure 3A–C**) than the LL treatment (**Figure 3D–F**). Under HL conditions, Chl a concentrations were highest in the effluent-amended treatment, increasing by a factor of 6.4 (±2.7) and 8.9 (±1.5) at Stations A and B, respectively (Tables S1–S6). Similar increases (by factors of 5.2 ± 0.9 and 8.2 ± 1.5) were detected in the NH_4^+ treatment at both sites, respectively, and in the NO_3^- treatment (by a factor of 7.3 ± 1.7) at Site B. At Site A, the NO_3^- amendment resulted in a 2.4-fold Chl a increase (**Figure 3A, B, D**, and **E**). Finally, the increase in Chl a at Site C was greater than at either site upstream of the SRWTP, with concentrations increasing by a factor of 13.7 (±2.1) and 4.9 (±1.3) in the HL and LL treatments, respectively (**Figure 3C** and **F**).

POC concentrations under the HL treatment followed Chl a concentrations in each treatment, with the effluent and NH_4^+ amendments in the HL treatment showing the greatest increases (**Figure 3G–L**). POC and Chl a concentrations at 48 h were significantly positively correlated ($p <$ 0.05, $R^2 = 0.75$). Across all sampling locations and treatments, increases in POC were significantly greater under HL conditions (1,004 ± 283 μg C L^{-1}, $n = 3$) than under LL conditions (86 ± 42 μg C L^{-1}, $n = 3$). Just as for Chl a accumulation, POC increases under HL conditions were greatest in the effluent (3-fold) and NH_4^+ (3-fold) treatments at Station A and in the unamended water from Station C (3-fold), located downstream of the effluent outfall (**Figure 3G** and **I**; Tables S7–S12). In contrast, no significant amendment effect was observed on POC concentration in the HL treatment at Station B (**Figure 3H**). In all treatments, PO_4^{3-} remained measurable at the final time point and therefore was unlikely to have limited biomass accumulation in any of the treatments (Figure S1).

Phytoplankton community composition

The initial phytoplankton community composition in surface waters at Stations A–C was similar (**Figure 4**). Diatom species dominated initial phytoplankton cell counts
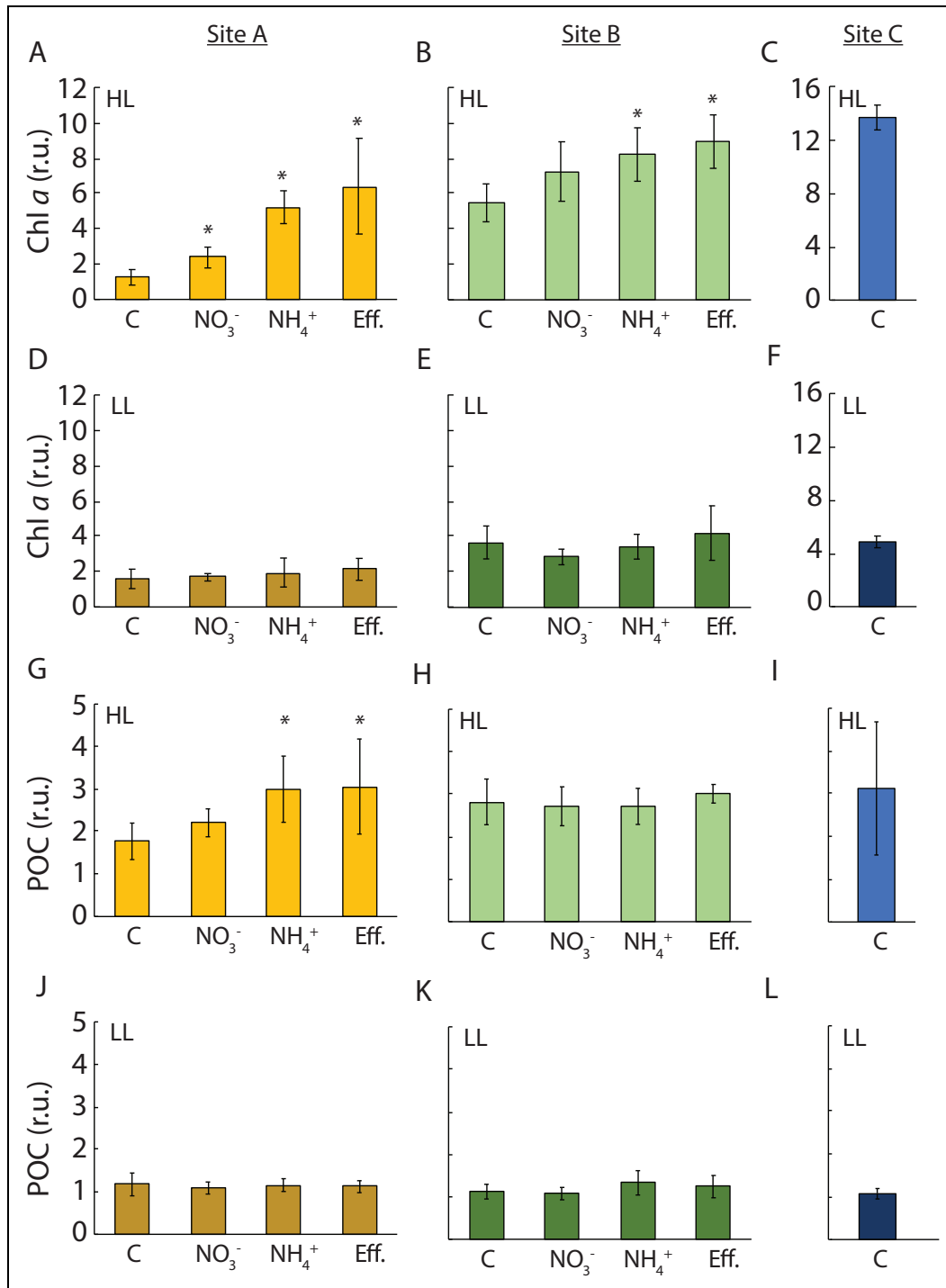
Figure 3. Relative Chl *a* and particulate organic carbon concentrations in the different treatments at the three sites. Values are calculated as treatment relative to control (*C*), where r.u. is relative units and * indicates significant difference compared with control at the $\alpha = 0.05$ significance level. Error bars indicate standard deviation of the mean ($n = 3$). DOI: https://doi.org/10.1525/elementa.2021.040.f3

(approximately 50%–60%) at all three sampling stations, with chlorophytes being the next most abundant taxon (28%–37%; **Table 2**). Cryptophytes (principally *Rhodomonas* spp.) and cyanobacteria (principally *Anabaena* spp.) were also present in initial samples, though they made up less than 15% of the total community combined. Together, these four taxonomic groups comprised over 99% of all cells in all samples. As a fraction of total

biovolume, diatoms were even more dominant, comprising 88%–95% of the total community biovolume across all stations (**Figure 5**; Table S13).

After the 48-h incubation, total phytoplankton cell densities and biovolumes were higher for all stations and under all nutrient and light treatments (**Figures 4 and 5**), consistent with observed increases in Chl *a* concentrations. After 48 h, the fraction of diatom cells significantly
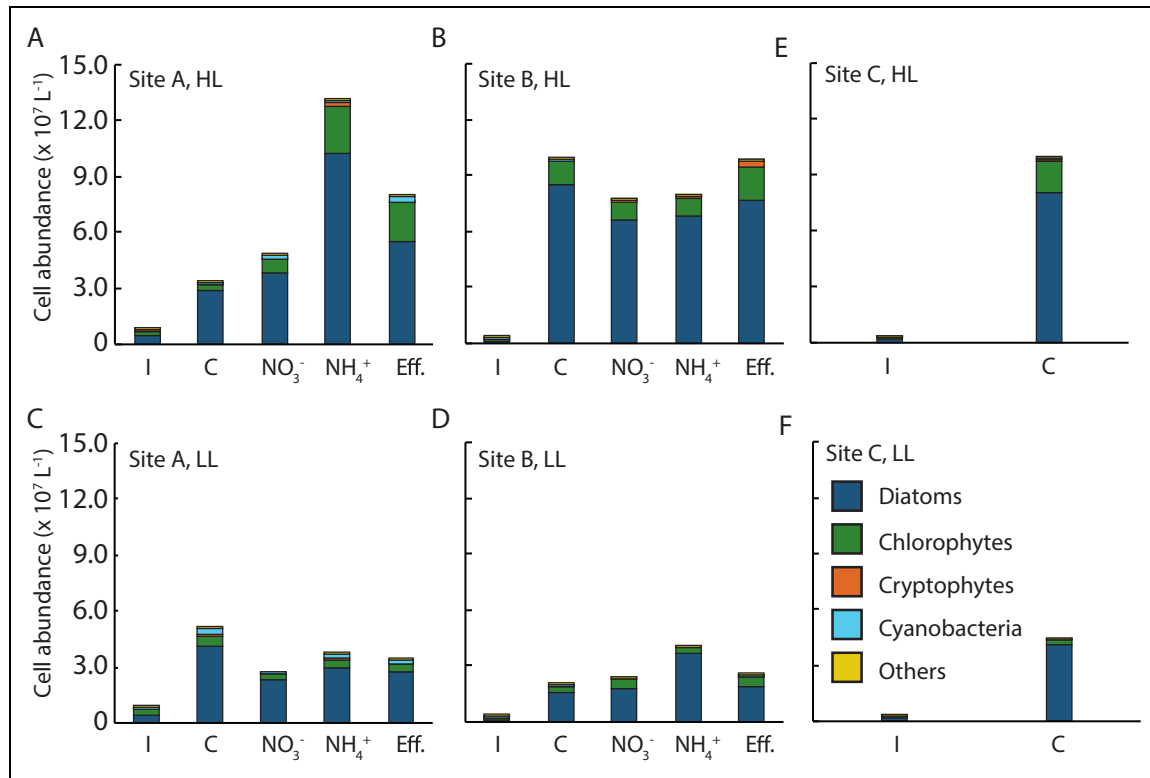
Figure 4. Cell abundance responses for the major phytoplankton taxa. Shown are initial (*I*) and final cell densities for the different phytoplankton taxa from each treatment (control [*C*] and additives NO_3^-, NH_4^+, and effluent) in each experiment under high light (upper panels) and low light (lower panels) for Sites A–C. DOI: https://doi.org/10.1525/elementa.2021.040.f4

increased from the start of the experiment under all nutrient conditions at both light levels (**Table 2**). The fraction of cell counts comprising nondiatom taxa decreased or remained unchanged during the course of the experiment across all nutrient and light treatments (**Table 2**). No significant nutrient effects on the fractional composition of the phytoplankton community were detected (**Table 2**). However, total cell abundances were higher in the NH_4^+ and effluent treatments of the HL treatment for Site A and in the NH_4^+ treatment of the LL treatment for Site B. For Site C, total cell densities increased 34- and 15-fold, in the HL and LL treatments, respectively (**Figure 4**). These increases were driven by diatoms across sites and treatments, as well as by chlorophytes in the HL treatment.

Biovolume trends (**Figure 5**) were slightly different than changes in cell densities (**Figure 4**), though for each taxonomic group, biovolume responses correlated significantly with the abundance responses across experiments (Table S14). Although diatoms dominated the total biovolume of all nutrient treatments under both light levels (diatoms were never less than 60% of total biovolume), they did not increase significantly as a percentage of total biovolume despite their density increase (Table S13). For Sites A and B, a decrease in the percentage contribution of diatoms to the total biovolume was detected in the HL control and LL + NH_4^+ treatment, respectively (Tables S15–S26). In both of these treatments, chlorophytes accounted for a greater percentage of total biovolume; however, the coefficients of variation associated with these changes were >80%. As

such, these increases did not differ significantly from initial samples or the controls. No significant change in the percentage of total biovolume for other taxa was detected across nutrient and light treatments.

POC:Chl *a*
The high initial POC:Chl *a* at all sites (**Figure 6**) indicated that concentrations of nonphytoplankton organic C (e.g., detrital) were high, and differed between sites, with POC:Chl *a* increasing downstream from Site A to Site C. At the final time point ($t = 48$ h) under the HL treatment, the POC:Chl *a* values differed from the initial values. For Site A, POC:Chl *a* increased (approximately 1.5-fold) in the control, while for Sites B and C, the POC:Chl *a* decreased dramatically by approximately 50% and 80%, respectively. Nutrient additions resulted in lower POC:Chl *a* relative to both the initials and controls, with the effluent and NH_4^+ treatments showing the greatest decline. Notably, the final POC:Chl *a* ratios of the treated waters from Sites A and B were similar in magnitude to that measured for Site C, which had a high initial NH_4^+ concentration (56.7 µmol L^{-1}; **Table 1**). Under the LL treatment, the final ($t = 48$ h) POC:Chl *a* values decreased relative to the initial values at all sites; however, there was no further decrease in the amended waters relative to the controls.

Phytoplankton physiology
The ratio of variable fluorescence to maximal fluorescence (F_v/F_m) did not vary within each light treatment across the

Table 2. Relative cell density, as a percentage (%) of total cells, by taxon at the start of the experiment and after 48 h of nutrient and light treatment. DOI: https://doi.org/10.1525/elementa.2021.040.t2

| Station | Taxon | Start of Experiment | End of Experiment ($t = 48$ h) | | | |
		Initial	Unamended	NH_4^+	NO_3^-	Effluent
High light						
A	Diatoms	52.2 ± 9.7	83.5 ± 9.4	79.0 ± 7.3	81.7 ± 6.2	70.0 ± 7.1
	Chlorophytes	37.7 ± 5.5	8.9 ± 9.4	18.4 ± 7.2	12.8 ± 13.6	26.0 ± 4.8
	Cryptophytes	4.8 ± 0.6	0.4 ± 0.5	1.7 ± 0.2	0.6 ± 0.1	0.7 ± 0.3
	Cyanobacteria	5.1 ± 6.0	6.2 ± 8.9	1.0 ± 0.8	5.1 ± 5.9	3.9 ± 4.4
B	Diatoms	50.1 ± 15.2	83.1 ± 11.4	86.2 ± 0.2	75.5 ± 25.8	78.9 ± 1.8
	Chlorophytes	35.4 ± 9.2	14.3 ± 9.2	12.3 ± 2.5	26.3 ± 24.1	18.4 ± 0.6
	Cryptophytes	6.6 ± 3.3	0.9 ± 1.0	1.1 ± 1.2	0.1 ± 0.2	0.2 ± 0.2
	Cyanobacteria	7.8 ± 8.1	1.5 ± 0	0.2 ± 0	0.5 ± 0	0.5 ± 0.5
C	Diatoms	62.7 ± 24.3	67.8 ± 25	—[a]	—	—
	Chlorophytes	28.6 ± 14.7	24.6 ± 29.5	—	—	—
	Cryptophytes	5.4 ± 3.0	1.0 ± 0.3	—	—	—
	Cyanobacteria	3.1 ± 0.0	0.6 ± 0.0	—	—	—
Low light						
A	Diatoms	52.2 ± 9.7	72.4 ± 22.1	77.4 ± 11.4	74.8 ± 22.7	78.6 ± 15.3
	Chlorophytes	37.7 ± 5.5	20.3 ± 22.9	12.5 ± 6.2	15.9 ± 10.0	13.5 ± 7.6
	Cryptophytes	4.8 ± 0.6	1.0 ± 0.3	1.8 ± 1.3	0.7 ± 0.6	1.6 ± 1.0
	Cyanobacteria	5.1 ± 6.0	6.2 ± 1.3	8.2 ± 9.2	8.7 ± 12.3	6.7 ± 8.4
B	Diatoms	50.1 ± 15.2	67.0 ± 27.2	78.9 ± 27.9	71.0 ± 22.0	63.0 ± 32.4
	Chlorophytes	35.4 ± 9.2	24.5 ± 16.2	19.7 ± 27.1	27.1 ± 22.8	31.2 ± 3.1
	Cryptophytes	6.6 ± 3.3	1.4 ± 1.4	0.9 ± 1.2	1.1 ± 1.0	2.6 ± 1.3
	Cyanobacteria	7.8 ± 8.1	6.3 ± 9.4	0.0	0.0	2.3 ± 0.6
C	Diatoms	62.7 ± 24.3	92.3 ± 2.4	—	—	—
	Chlorophytes	28.6 ± 14.7	5.8 ± 2.3	—	—	—
	Cryptophytes	5.4 ± 3.0	1.2 ± 0.5	—	—	—
	Cyanobacteria	3.1 ± 0.0	0.0	—	—	—

[a]dash indicates no amendment made.

nutrient treatments. Values in all samples were uniformly high, between 0.45 and 0.55 (**Table 3**) consistent with healthy phytoplankton populations. However, some F_v/F_m differences were detected between the light treatments. At Station A, all amendment treatments (NO_3^-, NH_4^+, and effluent) had significantly higher F_v/F_m in the HL treatment than in the LL treatment (Tables S27–S28). Functional absorption cross sections (σ_{PSII}) did not differ significantly between initial and final time points or between nutrient or light treatments at any of the sites (**Table 4**).

^{15}N uptake rates
Specific uptake rates V(h^{-1}) for NH_4^+ increased, relative to initial rates, in both the NH_4^+ and effluent treatments at

Sites A and B in the HL treatment (**Figure 7A** and **B**; Tables S29–S32). In the control and NO_3^- treatments, rates of V were more variable. No increases were detected for Site A in either treatment, while rates for Site B increased (**Figure 7A** and **B**). At the downstream Site C, the final NH_4^+-specific uptake rate was approximately 5-fold higher than the initial rate (**Figure 7C**; Tables S33–S36). In contrast, NO_3^- uptake rates were completely suppressed in the NH_4^+ and effluent treatments and in the unamended water from downstream of the outfall site (**Figure 7G–L**; Tables S37–S44). NO_3^- uptake rates were greatest in the NO_3^- treatments for Stations A and B to which no NH_4^+ was added and in which starting concentrations of NH_4^+ were <5 µM (**Figure 7G–L**, Tables S37 and S39). NO_3^- uptake rates also were low, but greater than zero, in the
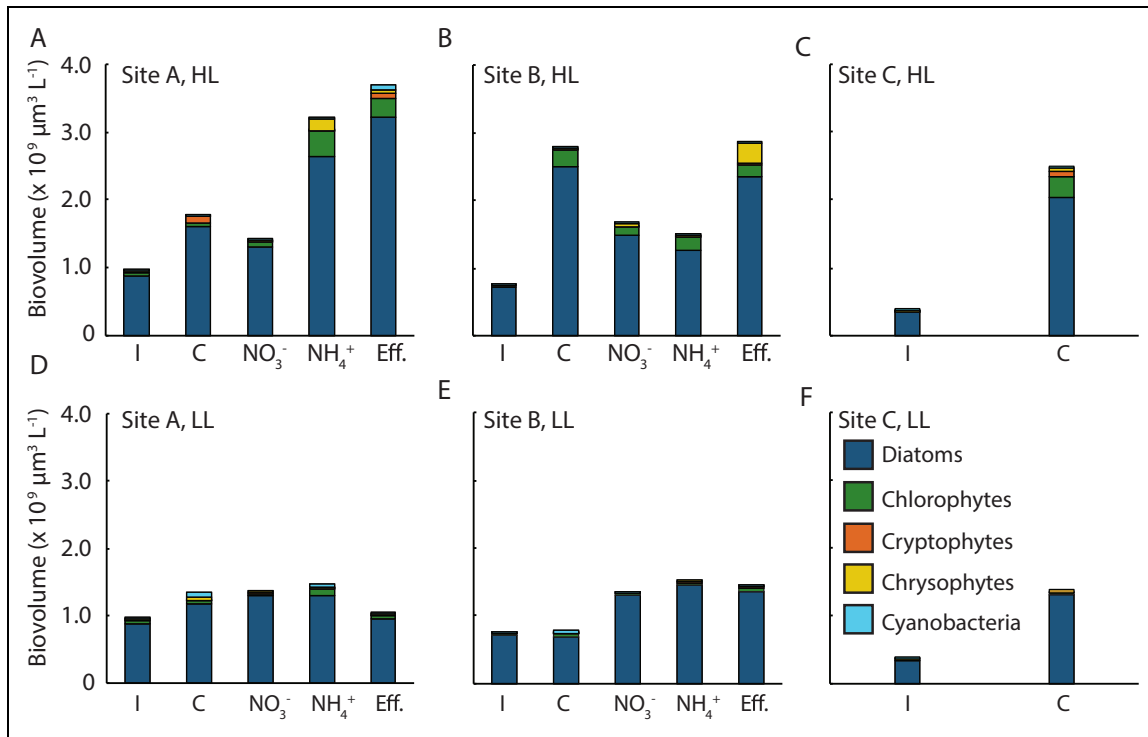
Figure 5. Biovolume responses for the major phytoplankton taxa. Shown are initial (*I*) and final biovolumes for the different phytoplankton taxa from each treatment (control [*C*] and additives NO_3^-, NH_4^+, and effluent) in each experiment under high light (upper panels) and low light (lower panels) for Sites A–C. DOI: https://doi.org/10.1525/elementa.2021.040.f5

unamended controls for Stations A and B, which had low (but nonzero) concentrations of both NH_4^+ and NO_3^- at the start of the experiment. Under LL, initial and final differences in NH_4^+-specific uptake rates were only detected for Site B in the control and NO_3^- treatments and for Site C (**Figure 7D–F**). However, little effect of nutrient or effluent additions was detected on the LL NH_4^+-specific uptake rates overall.

Discussion

Light as the limiting factor for Lower Sacramento River phytoplankton

We present clear evidence of phytoplankton growth under the HL treatments when cells were supplied with nutrients, as either individual substrates (NH_4^+ or NO_3^-) or in effluent, as well as under the high ambient nutrient concentrations (**Table 1**) of the Lower Sacramento River downstream from the wastewater effluent outfall from the SRWTP. Contrary to the findings of previous studies in the region (Dugdale et al., 2007; Parker et al., 2012), we observed phytoplankton growth (e.g., POC and Chl *a* accumulation), increased cell abundance, and evidence of bloom formation in phytoplankton exposed to high NH_4^+ concentrations (>40 μM).

Consistent with numerous laboratory (Dortch et al., 1991; Berges et al., 1995; Lomas and Glibert, 1999; Clark and Flynn, 2002; Song and Ward, 2007) and other in situ studies (McCarthy et al., 1977), as well as previous work in the Lower Sacramento River Delta (Dugdale et al., 2007), high concentrations of NH_4^+ (whether present in the

water or added as NH_4^+ or effluent) suppressed phytoplankton NO_3^- uptake rates to near zero. Yet the phytoplankton communities exposed to high NH_4^+ conditions grew well, as indicated by the increases in Chl *a* and POC in both the NH_4^+ and effluent treatments. NH_4^+ uptake rates were high and similar to NO_3^- uptake rates in samples not exposed to high NH_4^+ concentrations, and the amount of phytoplankton biomass produced from NH_4^+ uptake was the same or greater than the amount of phytoplankton biomass produced from NO_3^- uptake. Only in the NO_3^- treatments at the final time point was NO_3^- uptake higher than NH_4^+ uptake. Measurements of the photophysiological parameters F_v/F_m and σ_{PSII} made throughout the experiment showed that phytoplankton communities under these high NH_4^+ experimental conditions had well-functioning PS, which is consistent with culture studies of phytoplankton species isolated from Suisun Bay and Sacramento River demonstrating high F_v/F_m under high NH_4^+ concentrations (Berg et al., 2017, 2019). Thus, to answer our first ecological question: High concentrations of NH_4^+ similar to that found in effluent of the SRWTP did not negatively affect the growth rate or physiology of Lower Sacramento River phytoplankton in this short-term experiment.

Across all treatments, rates of DIN uptake (h^{-1}) were similar to those previously observed in this system (Parker et al., 2012). Although our experiment only lasted for 2 days, the rapid accumulation of Chl *a* and phytoplankton biomass during this time (cells were doubling at least once a day in many of our treatments under HL conditions)
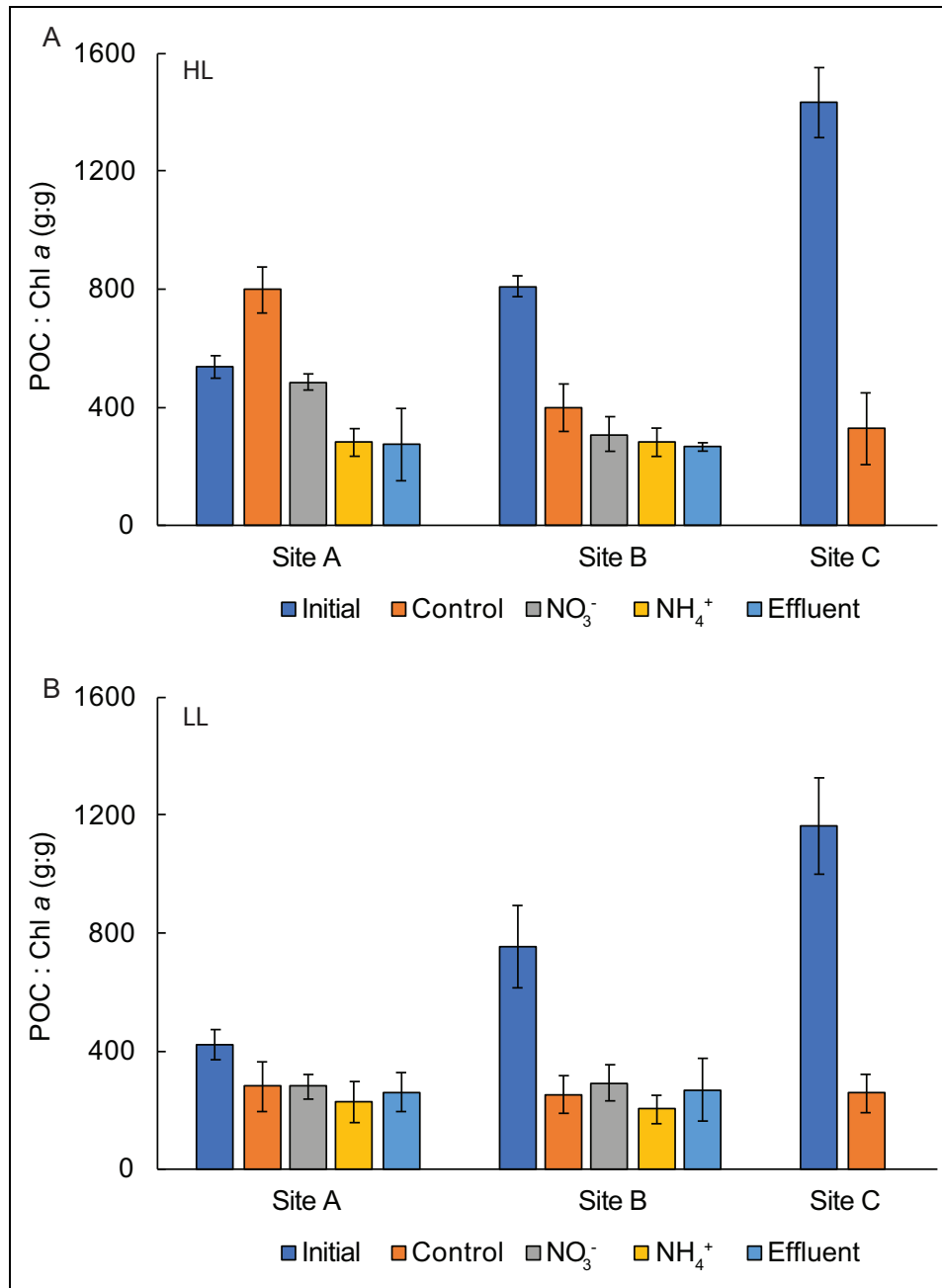
Figure 6. POC:Chl a responses in the different treatments at the three sites. Shown are the initial and final values ($t = 48$ h) for the different nutrient treatments at the three sites for the (A) high light and (B) low light treatments. Error bars indicate standard deviation of the mean ($n = 3$). DOI: https://doi.org/10.1525/elementa.2021.040.f6

suggests that bloom conditions had been initiated within our nutrient replete incubations, including those in unamended water from the Lower Sacramento River. Our experimental results indicate that the paradoxically low in situ phytoplankton growth in the Lower Sacramento River, despite relatively high ambient concentrations of DIN from human inputs, is primarily due to light limitation (at least in the absence of benthic grazers). Although nutrient effects were detected on phytoplankton growth, Chl a accumulation, nutrient drawdown, DIN uptake, and cell division, the effects were greatest in HL treatments. When held at light levels equivalent to those at depths of 3–4 m (LL treatment) in a well-mixed 10-m water column,

phytoplankton growth was a small fraction of the rates measured when cells were held at light levels equivalent to depths of 0.6–0.8 m (HL treatment).

POC:Chl a was greater under HL than under LL conditions in the controls at Stations A and B, as well as the NO_3^- treatment at Station A, consistent with a light acclimation response (Cloern et al., 1995). However, in the NH_4^+ and Effluent treatments, little difference between HL and LL was observed, suggesting that changes in POC:Chl a over the course of the experiment at Site C were not solely due to light. The greater relative increase in Chl a, compared to POC, concentrations in the NH_4^+ and Effluent treatments suggests that cells preferentially

Table 3. Ratio of variable fluorescence to maximal fluorescence (F_v/F_m) at the start of the experiment and after 48 h of nutrient and light treatment. DOI: https://doi.org/10.1525/elementa.2021.040.t3

Station	Unamended		+NO$_3^-$		+NH$_4^+$		+Effluent	
	$T = 0$	$T = 48$	$T = 0$	$T = 48$	$T = 0$	$T = 48$	$T = 0$	$T = 48$
High light								
A	0.51 ± 0.01	0.51 ± 0.02	0.51 ± 0.01	0.53 ± 0.02	0.49 ± 0.01	0.53 ± 0.01	0.49 ± 0.06	0.52 ± 0.01
B	0.5 ± 0.02	0.54 ± 0.01	0.50 ± 0.04	0.53 ± 0.01	0.51 ± 0.02	0.49 ± 0.04	0.47 ± 0.07	0.54 ± 0.01
C	0.47 ± 0.06	0.56 ± 0.02	—[a]	—	—	—	—	—
Low light								
A	0.54 ± 0.01	0.53 ± 0.02	0.52 ± 0.04	0.48 ± 0.06	0.51 ± 0.05	0.50 ± 0.02	0.50 ± 0.08	0.49 ± 0.02
B	0.52 ± 0.02	0.44 ± 0.03	0.43 ± 0.13	0.49 ± 0.05	0.52 ± 0.02	0.44 ± 0.05	0.49 ± 0.04	0.54 ± 0.03
C	0.5 ± 0.03	0.54 ± 0.01	—	—	—	—	—	—

[a]dash indicates no amendment made.

Table 4. Functional absorption cross section (σ_{PSII}) at the start of the experiment and after 48 h of nutrient and light treatment. DOI: https://doi.org/10.1525/elementa.2021.040.t4

Station	Unamended		+NO$_3^-$		+NH$_4^+$		+Effluent	
	$T = 0$	$T = 48$	$T = 0$	$T = 48$	$T = 0$	$T = 48$	$T = 0$	$T = 48$
High light								
A	460 ± 12.5	529 ± 13.1	468 ± 15.9	510 ± 50.8	467 ± 16.0	468 ± 27.4	474 ± 31.4	462 ± 31.3
B	472 ± 23.8	488 ± 9	480 ± 42.5	488 ± 12.4	454 ± 21.9	482 ± 15.1	483 ± 54.7	485 ± 12.4
C	479 ± 39.9	493 ± 16.2	—[a]	—	—	—	—	—
Low light								
A	474 ± 9.4	499 ± 3.8	478 ± 16.2	500 ± 2.2	486 ± 36.9	493 ± 15.1	459 ± 8.2	494 ± 8.2
B	474 ± 9.7	534 ± 9.5	439 ± 73.4	519 ± 17.4	482 ± 2.7	527 ± 14.6	481 ± 10.2	507 ± 6.0
C	482 ± 20.3	510 ± 5.1	—	—	—	—	—	—

[a]dash indicates no amendment made.

allocated N toward synthesis of Chl *a*. This is consistent with light limitation of phytoplankton and their need to increase capture of photons (MacIntyre et al., 2002).

Our experimental data also suggest that effluent-associated NH$_4^+$ loading to the river supports growth of most of the species within the in situ phytoplankton community. Specifically, the phytoplankton community in the Lower Sacramento River at all of our sampling locations was dominated by diatom species, in terms of both cell density and biovolume, at both the start and end of our experiment under all light and nutrient treatments. Importantly, given that POD and food web collapse have been attributed to a reduction in diatom production over the last several decades (Jassby et al., 2002; Glibert et al., 2014b), we show that diatom species grew readily on NH$_4^+$, as evidenced by increases in cell density in experimental conditions where NO$_3^-$ uptake rates were near zero.

Our analysis also shows that diatom cell densities increased at greater rates than chlorophytes, in contrast to previous findings based on pigment analyses reported by Glibert et al. (2014b) and previous observations of upper Sacramento River blooms being dominated numerically (albeit not by biovolume) by chlorophytes (Glibert et al., 2014a). Chlorophyte biovolume did increase as a percentage of total biovolume in the NH$_4^+$ and effluent treatments under HL, but increases within this group, while smaller, were also detected in the control and NO$_3^-$ treatments. Such compositional changes are expected in experiments such as these, as turbulence is substantially reduced inside experimental systems compared with conditions in the Sacramento River. Nondiatom species typically grow better than diatoms under calm and quiescent conditions, and vice versa, when nutrient concentrations are the same (Margalef, 1978; Richardson et al., 2016; Stumpner et al., 2020). Thus, with respect to our second ecological question: How is phytoplankton community composition affected by the available DIN substrate? we found that diatoms exhibited the greatest increases in abundance independently of the form of N being added
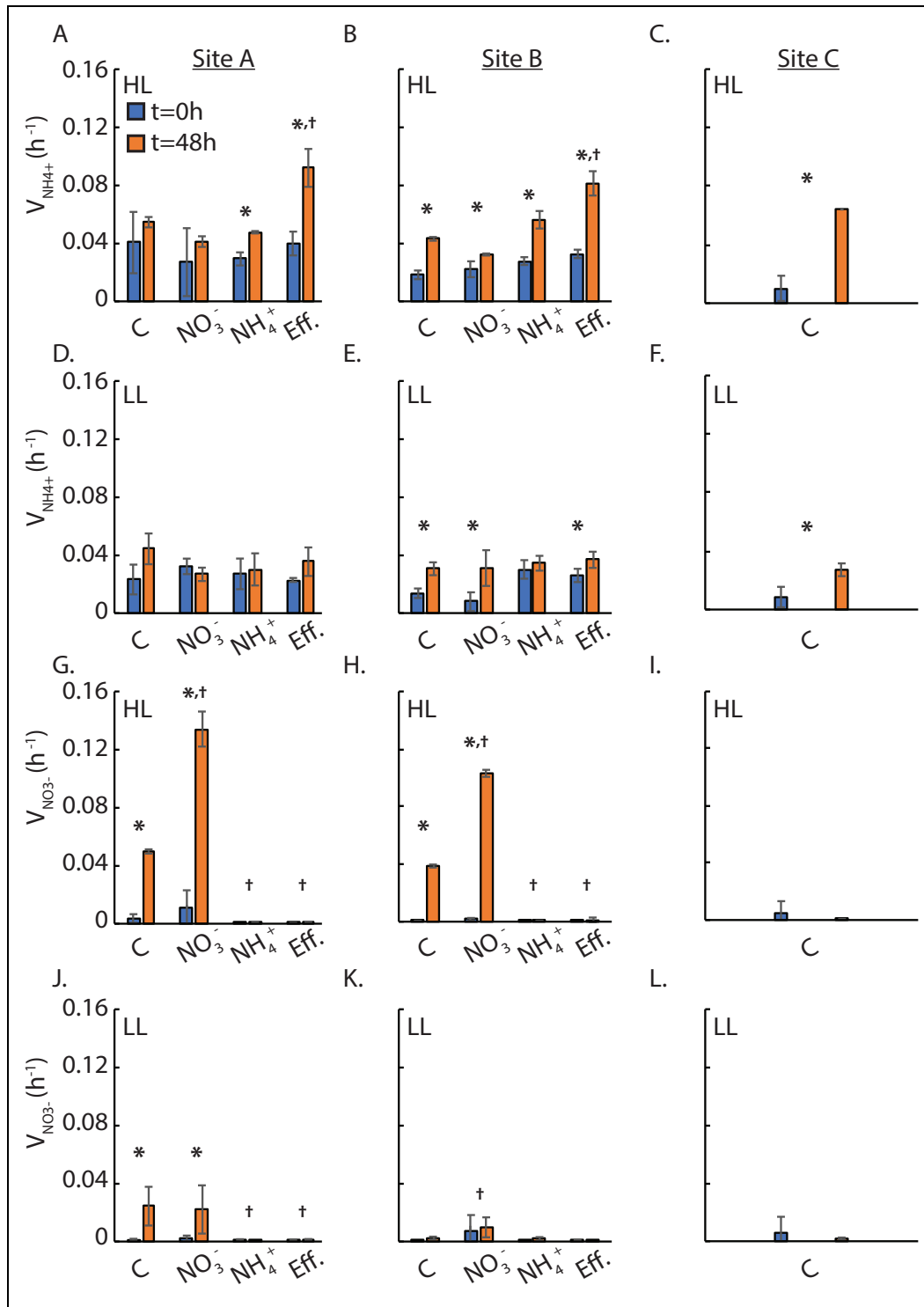
Figure 7. Specific NH_4^+ and NO_3^- uptake rates in the different treatments at the three sites. Specific uptake rates for NH_4^+ are shown for the initial and final ($t = 48$ h) times under (A–C) high light (HL) and (D–F) low light (LL) conditions for all treatments (control [C] and additives NO_3^-, NH_4^+, and effluent). Specific uptake rates for NO_3^- are similarly shown under HL (G–I) and LL (J–L) conditions for all treatments. An asterisk (*) indicates significant difference ($p < 0.05$) from the initial value; † indicates significant difference ($p < 0.05$) from the control value. Error bars indicate standard deviation of the mean ($n = 3$). DOI: https://doi.org/10.1525/elementa.2021.040.f7

(NO_3^- or NH_4^+ alone, or NH_4^+ in SRWTP effluent) and dominated the biovolumes of all treatments in all experiments. The composition of the phytoplankton community may instead depend on combinations of factors, including irradiance, temperature, and degree of turbulence and mixing (Margalef, 1978; Levasseur et al., 1984; Butterwick et al., 2005; Boyd et al., 2013; Edwards et al., 2015; Richardson et al., 2016). Recent research has shown that residence time may also be a relevant factor, as small-celled picocyanobacterial communities dominated

in regions of the the Delta with longer water residence times (Stumpner et al., 2020).

Grazing

Previous studies demonstrated that grazing by the invasive clam species *Potamocorbula amurensis*, introduced to Suisun Bay in the 1980s, played a significant role in inhibiting bloom formation in the Delta (Thompson, 2005; Hammock et al., 2019). However, at the position of X2 (defined as the distance from the mouth of the SFB to the location where estuary bottom water salinity drops to 2), there is a sharp decrease in the abundance of the clam *P. amurensis*, which is not present in the freshwater reaches of the Delta where our study was conducted. Instead, the freshwater portions of the Delta, including the lower Sacramento River location of our study, are dominated by the invasive clam *Corbicula fluminea*, which negatively impacts phytoplankton biomass and productivity in areas where it is abundant (Foe and Knight, 1985; Lopez et al., 2006). Because our experimental design relied on incubations in cubitainers with water prefiltered through a 300 μm screen, we excluded both benthic grazers and macrozooplankton from our experimental bottles. Thus, we are unable to determine whether grazing pressure was sufficient to inhibit bloom formation in the Lower Sacramento River, even if light limitation were alleviated (Lucas et al., 2002). At the very least, some of the observed increase in phytoplankton abundance and biovolume in the unamended bottles from the sites upstream of the outfall pipe is likely due to the removal of grazing pressure. Further work should emphasize the role of grazing in controlling phytoplankton biomass and production in this region. If grazing exerts further pressure on phytoplankton abundance, it would only serve to compound the light limitation we observed in our experiment.

Water quality management

In 2014, a new NPDES permit was approved for the Sacramento Regional Sanitation District, which requires conversion of the wastewater treatment plant to a tertiary treatment system that will include both nitrification and denitrification steps and that must be completed in 2021–2023 (California Regional Water Board, Central Valley Region, 2014). These changes mean that DIN loading will dramatically change in the region, as the N in effluent will no longer be discharged primarily as NH_4^+ and total DIN concentrations are expected to decrease by 74%. Given the rapid growth of diatoms that we observed on anthropogenic NH_4^+ when light limitation was alleviated, our results highlight that future reductions in NH_4^+ loading from the tertiary treatment system could decrease the productivity of phytoplankton in the Delta without concomitant increases in the light environment. Given existing agricultural N loads from the San Joaquin River, changes in the form and magnitude of SRWTP loading under new permits, our limited understanding of microbial N processing in this region, and potential changes in freshwater flows under climate change and water usage scenarios, modeling the future nutrient environment of the Delta is a key direction for future research.

Conclusions

Taken as a whole, our experimental results present evidence that high concentrations of NH_4^+ from wastewater are not likely to be the cause of POD in the Delta. When the phytoplankton in water from the river downstream of the wastewater effluent outfall was exposed to light levels greater than those experienced in situ but with no other amendments, growth (primarily of diatom species) was immediate and significant. High NH_4^+ concentrations did not prevent a diatom-dominated bloom from developing within our experiment. We conclude that high anthropogenic NH_4^+ loading from wastewater effluent is not driving the lower productivity and decline of pelagic organisms in the Delta.

Data accessibility statement

Data used for the analyses in this article are provided in the supplemental tables; any other details of interest are available from the authors.

Supplemental files

The supplemental files for this article can be found as follows:

Figure S1.
Tables S1–S44. docx.

Competing interests

The authors have no competing interests to declare.

Author contributions

Contributed equally: ALS, MMM.

Led the design of the study and managed the field work and sample analysis: ALS.

Conducted the field work: ALS, MMM, GvD, IBH, SED, GMB.

Analyzed the data: ALS, MM.

Wrote this article with input from all authors: ALS, MMM.

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