A river-scale Lagrangian experiment examining controls on phytoplankton dynamics in the presence and absence of treated wastewater effluent high in ammonium

Tamara E. C. Kraus,1* Kurt D. Carpenter,2 Brian A. Bergamaschi,1 Alexander E. Parker,3 Elizabeth B. Stumpner,1 Bryan D. Downing,1 Nicole M. Travis,4 Frances P. Wilkerson,4 Carol Kendall,5 Timothy D. Mussen6

1U.S. Geological Survey, California Water Science Center, Sacramento, California
2U.S. Geological Survey, Oregon Water Science Center, Portland, Oregon
3The California State University Maritime Academy, Vallejo, California
4Romberg Tiburon Center, San Francisco State University, Tiburon, California
5U.S. Geological Survey, National Research Program, Menlo Park, California
6Sacramento Regional County Sanitation District, Sacramento, California

Abstract

Phytoplankton are critical component of the food web in most large rivers and estuaries, and thus identifying dominant controls on phytoplankton abundance and species composition is important to scientists, managers, and policymakers. Recent studies from a variety of systems indicate that ammonium (NH$_4^+$) in treated wastewater effluent decreases primary production and alters phytoplankton species composition. However, these findings are based mainly on laboratory and enclosure studies, which may not adequately represent natural systems. To test effects of effluent high in ammonium on phytoplankton at the ecosystem scale, we conducted whole-river–scale experiments by halting discharges to the Sacramento River from the regional wastewater treatment plant (WWTP), and used a Lagrangian approach to compare changes in phytoplankton abundance and species composition in the presence (+EFF) and absence (−EFF) of effluent. Over 5 d of downstream travel from 20 km above to 50 km below the WWTP, chlorophyll concentrations declined from 15–25 to ~2.5 μg L$^{-1}$, irrespective of effluent addition. Benthic diatoms were dominant in most samples. We found no significant difference in phytoplankton abundance or species composition between +EFF and −EFF conditions. Moreover, greatest declines in chlorophyll occurred upstream of the WWTP where NH$_4^+$ concentrations were low. Grazing by clams and zooplankton could not account for observed losses, suggesting other factors such as hydrodynamics and light limitation were responsible for phytoplankton declines. These results highlight the advantages of conducting ecosystem-scale, Lagrangian-based experiments to understand the dynamic and complex interplay between physical, chemical, and biological factors that control phytoplankton populations.

Phytoplankton form the base of the food web in many aquatic ecosystems, with both their abundance and composition governing the amount and quality of food available to support higher trophic level organisms like zooplankton and fish (Vannote et al. 1980, Wehr and Descy 1998, Cloern et al. 2014). A number of factors determine phytoplankton growth and species composition, with nutrients, light, hydrodynamics, and grazing often cited as the main drivers (e.g., Cloern and Dufford 2005, Lucas et al. 2009, Scherwass et al. 2010, Cloern et al. 2014a). In many aquatic systems, these factors have been significantly altered by humans. Agriculture, industry, and urban development have increased nutrient inputs; construction of reservoirs and bank channelization have altered flow, turbulence, and residence times; changes in sediment supply, vegetation, and water depth have impacted light fields; and the introduction of invasive clams and other consumers have altered grazing pressures (Paerl et al. 2014, Cloern et al. 2016). Understanding how
these factors affect phytoplankton communities is important for the formulation of sound policies and regulations regarding water-resources management.

Worldwide, nitrogen inputs from wastewater treatment plants (WWTPs) have been identified as a major factor affecting phytoplankton, especially in the many large rivers and estuaries that support sizeable human populations (Carey and Migliaccio 2009, Paerl et al. 2016). WWTPs can contribute a significant proportion of the annual nitrogen load to these aquatic systems, and they typically discharge effluent continuously at a single point. Other sources, such as upstream watersheds, wetlands, and agriculture, represent more diffuse sources and often provide nitrogen inputs episodically, such as during storm events (Cooper et al. 2013, Saleh and Domagalski, 2015). Even if inputs from more diffuse sources represent a significant portion of the total annual load, they frequently occur during high flows and thus rapidly transit the ecosystem. Nitrogen from WWTP effluents represent a point source that can be modified via changes to treatment plant operations, and thus WWTPs are often the target of increasingly stringent regulations regarding nutrient concentrations in their discharged effluent (Carey and Migliaccio 2009). In the absence of mandates for more expensive treatment plant operations (e.g., nitrification and denitrification), treated effluent generally contains high concentrations of dissolved inorganic nitrogen (DIN) in the reduced form of ammonium ($\text{NH}_4^+$).

In many cases, higher nutrient inputs to aquatic systems result in eutrophication—the stimulation of algal growth that leads to negative consequences, such as hypoxia or nuisance algal blooms (e.g., Paerl et al. 2014). There are, however, a number of rivers and estuaries that have chronically low or declining phytoplankton populations despite non-limiting nutrient levels (e.g., Welker and Walz 1998, Cloern 2001, Yoshiyama and Sharp 2006, Scherwass et al. 2010, Sharp 2010, Cloern et al. 2014a, Rounds et al. 2015), and studies suggest that in some systems these declines result from DIN high in ammonium ($\text{NH}_4^+$), often associated with WWTP inputs (Yoshiyama and Sharp, 2006, Dugdale et al. 2007, Gilbert et al. 2016, and references therein). This concept, referred to as the “ammonium inhibition hypothesis” or the “ammonium paradox,” is based on evidence that when phytoplankton take up $\text{NH}_4^+$, their rates of total nitrogen utilization and primary productivity are low compared to growth on nitrate ($\text{NO}_3^-$). This inhibition purportedly occurs in spite of the fact that most phytoplankton species preferentially take up $\text{NH}_4^+$ over $\text{NO}_3^-$ (McCarthy 1981, Senn and Novick 2014, Gilbert et al. 2016). There is also evidence that elevated $\text{NH}_4^+$ concentrations favor the growth of cyanobacteria, dinoflagellates, and chlorophytes over diatoms (e.g., Gilbert et al. 2011, Donald et al. 2011, 2013, Gilbert et al. 2014b, Senn and Novick 2014). A decrease in diatoms is of particular concern because, being high in fatty acids, they are considered a superior food source (Caramujo et al. 2008, Galloway and Winder 2015). Cyanobacteria (blue-green algae) are less nutritious, and some species may produce toxins (Chorus and Bartram 1999, Lehman et al. 2015). However, laboratory-based studies also suggest that phytoplankton responses to the availability of $\text{NH}_4^+$ vs. $\text{NO}_3^-$ can vary among species or even within a single species, depending on growing conditions and other factors (e.g., Donald et al. 2011, Cloern and Jassby 2012, Donald et al. 2013, Zhang et al. 2013, Cloern et al. 2014b, Collos and Harrison 2014, Esparza et al. 2014).

A growing body of literature supports the idea that preferential uptake of $\text{NH}_4^+$ over $\text{NO}_3^-$ inhibits phytoplankton primary production and alters species composition, but much of the evidence so far is based on laboratory studies or multi-day enclosure studies (see recent review by Glibert et al. 2016). It is thus recognized that an understanding of whether responses measured during laboratory culture or enclosure studies translate into ecologically significant effects at the landscape level, where numerous other drivers of phytoplankton abundance and health are at play, is needed to inform ecosystem management (Cloern and Dufford 2005, Senn and Novick 2014, Gilbert et al. 2016). This is particularly important because promulgation of the ammonium inhibition hypothesis has implication for the development of nutrient criteria, ecosystem management, and modeling of phytoplankton production (Carey and Migliaccio, 2009, Cloern et al. 2014a, 2014b).

To explicitly examine the effects of elevated $\text{NH}_4^+$ concentrations from WWTPs on phytoplankton growth and species composition under field conditions, we conducted ecosystem-scale nutrient-manipulation experiments by temporarily halting WWTP effluent release into a large tidal river. In coordination with the effluent diversion, we used a Lagrangian (parcel-tracking) approach to track downstream chemical and biological changes in river-water parcels that received WWTP effluent ($+\text{EFF}$) and water parcels that did not ($−\text{EFF}$). The Lagrangian approach offered advantages over an Eulerian (fixed-point) sampling strategy by allowing us to assess the effects of effluent and its attendant high $\text{NH}_4^+$ concentrations in situ, while minimizing the confounding effects of spatial and temporal variability in constituent concentrations, bidirectional flow, irradiance, and upstream inoculum (Welker and Walz 1998, Scherwass et al. 2010, Volkmar et al. 2011).

The study site for the two Lagrangian field experiments was the lower section of the Sacramento River (California), which receives high-ammonium effluent inputs from Sacramento’s regional WWTP. Progressive downstream declines in chlorophyll $a$ ($\text{Chl}a$) concentrations in this reach of the river have been previously attributed to $\text{NH}_4^+$ inputs from the WWTP (Gilbert et al. 2011, Parker et al. 2012a, Gilbert et al. 2014a). During two effluent-hold experiments (October 2013 and June 2014), $+\text{EFF}$ and $−\text{EFF}$ water parcels were tracked.
over 5 d as they traveled downriver from about 20 km above to 50 km below the WWTP discharge location. This whole-
river–scale approach allowed us to test the hypothesis
that WWTP effluent and high NH\textsubscript{4}\textsuperscript{+} concentrations cause
(1) declines in phytoplankton abundance and (2) shifts in
phytoplankton species composition in the Sacramento River.
We also assessed the extent to which clams and zooplankton
grazing, tidal hydrodynamics, and light may have played a
role in structuring the phytoplankton community.

\textbf{Methods}

\textbf{Site description}

The Sacramento River drains a watershed of \(~70,000 \text{ km}^\text{2}\),
consisting of forested headwaters and intensively irrigated
valley cropland. The river is the main source of freshwater to
the Sacramento–San Joaquin River Delta (Delta), which expe-
rienced declines in Chl \(a\) concentrations between 1975 and
2005 despite increased nutrient inputs from agriculture,
industry, and urban development (Jassby 2008). These
decreases may also be associated with reduced populations of
diatoms (Gilbert et al. 2011, Cloern et al. 2014b). A num-
ber of studies have implicated wastewater-derived ammonium as
the primary cause of declines in primary production and a
shift away from diatoms (Dugdale et al. 2007, Parker et al.
2012a,b, Gilbert et al. 2014a, 2014b, Wilkerson et al. 2015).
Decreases in primary production and food quality are believed to be among the multiple stressors responsible for
declining fish populations in the region (Kimmerer et al.
2005, Sommer et al. 2007, Luoma et al. 2015).

Along the Sacramento River, locations are identified by
their distance in river miles (RMs) upstream of the conflu-
ence of the Sacramento and San Joaquin Rivers (Fig. 1). This
study focused on a 77-km section of the Sacramento River,
from about RM 63 (near the City of Sacramento) to RM 15.
Downstream of this section, the channelized river enters the
more hydrologically complicated Cache Slough Complex
(Fig. 1). The only significant inflows within the study stretch
are from the American River at RM 60.5 and Sacramento's
regional WWTP at RM 46.3. Upstream of the study area,
nutrients are supplied to the Sacramento River from natural
sources, agriculture, urban runoff, and other wastewater
treatment facilities (Saleh and Domagalski 2015, Schlegel
and Domagalski 2016). For the purposes of this study, we
refer to the river as effluent-free (\(–\)EFF) in the absence of dis-
charges from Sacramento's regional WWTP.

The regional WWTP serves \(~1.4\) million residential, com-
mercial, and industrial customers. Regulations specify that
the volume of effluent discharged to the Sacramento River
must be \(<6.7\%\) of the total river flow; typical effluent vol-
umes are about 1–3\%. However, tidally driven changes in
river flow can result in large ranges (0–6\%) in the river's per-
cent effluent content over the course of a tidal cycle (O'Don-
nell 2014). The treatment plant operations do not currently
include biological nitrification or denitrification; thus,
effluent N is mainly in the form of NH\textsubscript{4}\textsuperscript{+} (24–34 mg N L\textsuperscript{-1}),
and NO\textsubscript{3}\textsuperscript{-} concentrations are typically \(<0.1\) mg N L\textsuperscript{-1}. These
effluent inputs provide \(~90\%\) of the total NH\textsubscript{4}\textsuperscript{+} load to the
lower Sacramento River (Jassby 2008). Upstream of the
WWTP, river NH\textsubscript{4}\textsuperscript{+}-N concentrations are commonly \(<0.03\) mg L\textsuperscript{-1};
downstream of the effluent input, concentrations are commonly \(>0.40\) mg L\textsuperscript{-1} (Foe et al. 2010,
Kratzer et al. 2011).

\textbf{Study approach}

We employed a Lagrangian sampling approach, whereby
individual parcels of water were tracked and sampled as they
traveled downriver through the study reach (Fig. 1). Two
sampling campaigns were conducted: 24–29 October 2013
and 30 May to 4 June 2014 (hereafter referred to as the
“October” and “June” experiments). The parcel tracking was
coordinated with extended effluent diversions (no effluent
discharged to the river from the regional WWTP) during 25–
26 October 2013 (18.0 h) and 1–2 June 2014 (19.5 h). These
effluent diversions resulted in a \(\sim15\) km stretch of river that
was essentially free of effluent. During each field campaign,
one parcel of water located in the effluent-free stretch of river
(\(–\)EFF) and one parcel located downstream in the
effluent-containing parcel (+EFF) were tracked as each tra-
versed downstream until about RM 15 (Fig. 1). Two boats
were used for parcel tracking and sampling: one was a house-
boat that also served as a floating laboratory, and the 2nd
was a high-speed mapping boat equipped with a custom-
designed flow-through instrument package that continuously
displayed surface-water measurements of specific conduc-
tance (a conservative tracer), fluorescence of dissolved
organic matter (FDOM), and other water quality parameters
(Fichot et al. 2016, Downing et al., 2016). Sampling was con-
ducted during daylight hours only.

On the 1\textsuperscript{st} day of each experiment, samples were collected
at two locations well upstream of the WWTP: one in the Sac-
ramento River at RM 63.0 (880 Br) and one in the American
River, 0.25 km upstream of its confluence with the Sac-
ramento River at RM 60.5 (AmR). Water parcel tracking began
in the Sacramento River \(\sim20\) km upstream of the WWTP;
the exact starting locations and wastewater diversions were
orchestrated such that the +EFF and −EFF parcels were sam-
pared 24 h apart, with each parcel passing the effluent dis-
charge location during the ebb tide at approximately
06:00 h. This deliberate timing minimized the effects of dif-
fferences between the parcels with regard to sample location
and time of day, thus facilitating direct comparison of condi-
tions measured within each parcel. Comparisons between
parcels were made with travel time relative to passage by the
WWTP discharge location as a covariate. Travel time was
also used in figures to help visualize longitudinal trends and
facilitate comparisons between parcels.

Three methods were used to track and verify the location of
the +EFF and −EFF parcels. First, water velocity data from
U.S. Geological Survey (USGS) monitoring stations at
Fig. 1. Map of the study site showing locations where samples were collected during the October 2013 and June 2014 Lagrangian experiments. The location of the Sacramento Regional Wastewater Treatment Plant’s effluent outflow is indicated by the red arrow. Filled symbols in black indicate samples associated with water-parcels that received effluent (+EFF), while open blue symbols indicate water-parcels that did not (−EFF). Numbers in parentheses indicate the RM.
Freeport (RM 46.4) and Walnut Grove (RM 28.2) were used to estimate the parcels’ downstream progression. Second, surface current-following drifters were deployed daily within each parcel, and their movement was tracked by boat (see Supporting Information). Third, and most critical, was the information provided by the mapping boat, which periodically collected water-quality data to establish the location of the +EFF and −EFF parcels. For example, the −EFF parcel was very clearly characterized by lower surface conductance and FDOM. Data from the USGS monitoring station at Walnut Grove helped to further confirm the location of the +EFF and −EFF parcels by documenting their passage (O’Donnell 2014). Although dispersion shortened the overall −EFF parcel length by approximately 25% during transit, samples were collected near the center of the parcel, well away from the mixing front. Prior studies (Parker et al. 2012a) and data collected during a pilot effluent hold study confirmed that the river is well mixed with regard to dissolved constituents. Because the transition boundaries of the parcels were easily differentiated, confidence is high that all discrete samples were collected within their assigned parcel; laboratory measurements of elevated or reduced NH4+ concentrations further confirmed this.

River and effluent flow and water quality
River discharge, velocity, and other water-quality characteristics were measured every 15 min at two USGS monitoring stations: Freeport (at RM 46.4), 0.2 km upstream from the WWTP, and Walnut Grove (at RM 28.2), 29.2 km downstream of the plant (http://waterdata.usgs.gov/usa/nwis) (Fig. 1). The monitoring data were used to plan for and conduct the Lagrangian sampling and to document river conditions. Treated effluent water quality and discharge data (hourly averages) were provided by WWTP personnel, along with effluent daily (NH4+) and weekly (NO3−) nutrient concentrations (O’Donnell 2014; http://www.swrcb.ca.gov/centralvalley).

Water and plankton sampling, collection, and processing
Each field day between 08:00 and 17:00 h PST, three to five samples were collected from each water parcel at 2–3 h intervals. Discrete water samples were collected at a 1-m depth using a 3k Shurflo pump, then composited into an 18-liter churn splitter (USGS 2006). This step ensured particle homogenization. Subsamples for phytoplankton species composition, Chl a, nutrients, dissolved organic carbon (DOC), and total suspended sediment (TSS) were dispensed from the churn. Field measurements of temperature, specific conductance, pH, dissolved oxygen, turbidity, and FDOM were made simultaneously at all sampling locations (Yellow Springs Instruments EXO2 water-quality sonde). Each field measurement represents an average of 60 readings collected over 1 min, following a period of sensor equilibration.

Methods to measure nutrients (NH4+, NO3−, nitrite [NO2−]), and soluble reactive phosphorus [SRP]), dissolved inorganic carbon (DIC), and Chl a in the discrete water samples followed Parker et al. (2012a; see Supporting Information for details). DOC concentrations were determined by high-temperature catalytic combustion (Stumpner et al. 2015). TSS concentrations were determined by filtering known volumes of sample water through 0.3 μm glass fiber filters, then measuring the dry weight of material retained on the filter.

Rates of C and N uptake were measured on a subset of samples using stable-isotope tracer techniques (Travis 2015; see Supporting Information for details). For all samples collected upstream of the WWTP and for NO3−-uptake samples collected downstream of the WWTP, the 15N enrichments were higher than the 10% substrate “tracer” addition recommended by Dugdale and Wilkerson (1986). Because concentrations of NH4+ were much higher in water that received effluent, as well as in some of the −EFF samples collected downstream from the WWTP, NH4+ enrichments made to those samples were closer to the tracer level. The isotope-enriched bottles were incubated for 24 h, suspended at the surface of the river in a floating corral, and covered with window screening to reduce ambient light to ~50% of surface photosynthetically active radiation (PAR). Thus, these uptake results should be considered “potential” (N-saturated, high light) uptake rates. Carbon (C) and nitrogen uptake rates are reported as ρ (mg C L−1 d−1 or mg N L−1 d−1). Carbon uptake values were divided by sample Chl a concentrations to obtain a biomass-normalized C assimilation number (mg C [mg Chl a]−1 d−1).

Whole-water samples for phytoplankton enumeration were preserved with 1% Lugol’s solution and were analyzed within 12 months of collection by BSA Environmental Services. Microscope slides were prepared using membrane filtration with enumeration of 300 natural algal units (cells, filaments, or colonies) using a Leica microscope at 630X magnification. Measurements of cell biovolume were made on as many as 10 individuals per taxon. Samples of picoplankton (<0.2 μm) phytoplankton were preserved in 2.5% glutaraldehyde using an epifluorescence microscope. This size fraction accounted for an average of 4.1% of the total autotrophic community biovolume in October (n = 6) and 7.0% in June 2014 (n = 25). Given the small contribution of pico-plankton, these data were not included in the total phytoplankton biovolume calculations. The relative biovolume, expressed as percent (i.e., the biovolume of each taxon divided by the total phytoplankton biovolume in the sample, then multiplied by 100), was used to express the species composition of each sample. Qualitative observations, such as particularly high amounts of silt and empty or broken diatom frustules, were also considered in our analyses and interpretation. These enumeration data were compared to samples collected just upstream and downstream of the WWTP every 2–4 weeks between June 2013 and October 2015 to verify that conditions during the Lagrangian experiments were representative (see Supporting Information).

To qualitatively characterize phytoplankton and zooplankton assemblages, plankton were collected using 80 μm
mesh net tows. Unpreserved samples were examined using a Leica light microscope to identify the dominant plankton members, and to look for visual clues about the health of phytoplankton—specifically, chloroplast integrity and the presence of possible polyphosphate and lipid bodies.

Clam and zooplankton sampling and grazing estimates

Two weeks after both the October and June sampling campaigns, benthic trawls were conducted to survey clam populations at 11 sites within the study reach. At each site, five trawls equally spaced across the river’s width were taken, with each trawl representing about 10 m² of river bed surface area. Samples for zooplankton enumeration were collected using vertical net tows in the June +EFF and −EFF parcels, and again 2 weeks later at the 11 clam survey sites. For more details on these methods, see Supporting Information.

Clam grazing rates were estimated from clam biomass as described in Thompson et al. (2008), assuming constant grazing and 100% grazing efficiency. From this, daily Chl a losses to clams were estimated as the river turnover rate (clam grazing rates normalized by water depth measured at the time of sampling) multiplied by the measured average Chl a concentration per day. Phytoplankton losses resulting from zooplankton grazing were calculated by estimating the energy required to sustain an 11% (conservative) zooplankton growth rate per day, based on the measured growth rates of three copepod species in the low-salinity zone of the San Francisco Estuary (Kimmerer et al. 2014). Zooplankton carbon estimates were assumed to be 50% of the estimated dry weight (Latia and Salonen 1978, Beaver et al. 2013), and energy conversion efficiency was estimated to be 30% (Kimmerer and Thompson 2014). The carbon to Chl a ratio was estimated to be 35:1 (Jassby et al. 2002). Thus, the amount of Chl a grazed by zooplankton per day (µg Chl a L⁻¹ d⁻¹) was estimated by the following calculation: zooplankton biovolume × (0.5 µg zooplankton carbon biovolume⁻¹) × (0.11 growth d⁻¹) × (0.3 energy transfer) × (0.0286 µg phytoplankton Chl a carbon⁻¹). These estimated phytoplankton losses to clam and zooplankton grazing were compared to measured Chl a losses (determined by subtracting average daily Chl a concentrations from the previous day’s average). Daily phytoplankton losses due to respiration were assumed to be 1.5% of the phytoplankton population’s biomass (Jassby et al. 2002).

Statistical analyses

Longitudinal, time series, and regression plots were generated to examine general patterns in the flow, water quality, phytoplankton, and zooplankton data; these patterns in turn guided further analyses. To focus on differences between +EFF and −EFF parcels, unless otherwise mentioned, only samples collected downstream from the WWTP (i.e., travel time >0) were included in statistical tests. Upstream data (t < 0) were evaluated to help characterize initial conditions in the two water parcels and to compare ambient river conditions during the October and June experiments.

To test for statistically significant differences between individual parameters measured in the presence vs. absence of WWTP effluent, we used JMP software version 12.0 (SAS Institute 2015) to run an analysis of covariance (ANCOVA) on log-transformed data. For these models, the parameter of interest (e.g., Chl a or NH₄⁺) was the dependent variable, and parcel type (+EFF and −EFF) and date (October or June) were categorical-independent variables; travel time in hours relative to passage past the effluent outflow pipe was a continuous covariate; and a full factorial was applied to include all interaction terms.

To test for differences in species composition, the computer software package PRIMER (Plymouth Routines in Multivariate Ecological Research, Version 6; Clarke and Gorley 2006) was used to perform multivariate analyses on the phytoplankton and zooplankton data. Patterns in the phytoplankton assemblages were examined using nonmetric multidimensional scaling (NMDS) ordinations constructed from Bray–Curtis similarity matrices (square root-transformed abundance data). Potential differences between the +EFF and −EFF parcels were tested for significance using analysis of similarity (ANOSIM). Patterns in phytoplankton species composition were examined using NMDS ordinations constructed from Bray–Curtis similarity matrices using square root-transformed biovolume data. Seriation tests for downstream trends in phytoplankton assemblages were carried out on each of the four parcels (+EFF and −EFF for both June and October) to test whether community changes represent a systematic downstream change. Statistical significance for all multivariate analyses was determined using Monte Carlo simulations, with results considered significant when p values were <0.05.

Results

River conditions during wastewater-diversion experiments

The 2014 water year, during which this study took place, was a drought year. Flows during the October and June experiments were about 65% and 45%, respectively, of the 20-yr average flows for those months (1994–2004). Tidally averaged flows (~200 m³ s⁻¹) and tidally averaged river velocities (~0.18 m s⁻¹) reported for Freeport (RM 46.4) were similar during both the October and June experiments. Under these conditions, the water parcels took approximately 5 d to traverse the 77-km study reach.

Tidally driven flow reversals extended upstream of the effluent discharge location (Supporting Information Fig. S1). At Freeport, just upstream of the WWTP, instantaneous river velocities ranged from ~0.06 to ~0.40 m s⁻¹ (Supporting Information Fig. S2). At Walnut Grove (RM 28.2), 29 km downstream of the WWTP, instantaneous velocities ranged...
from $-0.11$ to $+0.46 \text{ m s}^{-1}$. Thus, over most of the study reach, particles were subjected to short-term changes in hydrodynamics.

Although river flows were similar during the autumn and summer transects, the percent effluent contribution in the $+\text{EFF}$ parcels was different, with treated wastewater constituting 4.0% and 2.7% of the river volume in October and June, respectively. In this regard, the October experiment tested greater than the typical 1–3% effluent contribution to the river flow. Water temperatures (Supporting Information Fig. S3f) were significantly lower in October ($\sim 16.5^\circ\text{C}$) than in June ($\sim 22^\circ\text{C}$).

### Dissolved inorganic nutrient concentrations

Nutrient concentrations (Fig. 2) were on the lower range of, but comparable to, those previously reported for this section of the Sacramento River (Foe et al. 2010; Kratz et al. 2011, Parker et al. 2012, Gilbert et al. 2014a, 2014b). Upstream of the WWTP, DIN (NH$_4^+$ + NO$_3^-$ + NO$_2^-$) concentrations were 0.005–0.067 mg N L$^{-1}$ (Supporting Information Fig. S4a) and SRP was 0.023–0.034 mg P L$^{-1}$ (Fig. 2c). Average NO$_3^-$ concentrations were higher in October (0.043 ± 0.06 mg N L$^{-1}$) than in June (0.011 ± 0.06 mg N L$^{-1}$), whereas average NH$_4^+$ concentrations were similar, about 0.01 mg N L$^{-1}$ (Fig. 2a,b). In June, upstream concentrations of DIN bracketed the 0.01 mg L$^{-1}$ half-saturation constant commonly used to model phytoplankton growth (Cloern and Dufford 2005) and thus may be limiting phytoplankton growth rates in some portions of the river, as suggested by Travis (2015).

WWTP effluent NH$_4^+$ concentrations were similar in October and June, $\sim 32–34$ mg N L$^{-1}$. In the $+\text{EFF}$ river parcel, NH$_4^+$ concentrations increased immediately downstream of the WWTP to about 1.3 mg N L$^{-1}$ in October and 0.8 mg N L$^{-1}$ in June (Fig. 2a); the higher concentrations in October reflect the higher percent effluent content of the river. Despite these elevated NH$_4^+$ concentrations, the relatively low pH (6.5–8.0) is not expected to result in harmful levels of unionized ammonia, NH$_3$ (Constable et al. 2003). Effluent contributions also increased river SRP (Fig. 2c), specific conductance (Supporting Information Fig. S3a), and DOC (Supporting Information Fig. S3b), and lowered dissolved oxygen and pH (likely due to nitrification of NH$_4^+$ and decomposition of residual organic matter in the effluent) (Supporting Information Fig. S3d,e). Effluent exerted no significant effect on river temperature or total suspended sediments (Table 1; Supporting Information Fig. S3c,f). As the $+\text{EFF}$ parcel traveled downstream from the WWTP, a trend of decreasing NH$_4^+$ was noted (Fig. 2a), while NO$_3^-$ (Fig. 2b) and NO$_2^-$ (Fig. S4b) increased, likely due to nitrification.

In the absence of effluent, there were increases in NO$_3^-$ and, to a lesser degree, an increases in NH$_4^+$ as water traveled downstream of the WWTP (Fig. 2). At the sites where water samples were collected, none of the in situ water-quality mapping data showed evidence of entrainment or mixing between the $+\text{EFF}$ and $-\text{EFF}$ parcels (Supporting Information Fig. S3).

### Phytoplankton biomass: Chl $a$ and algal biovolume

During both field experiments, concentrations of Chl $a$ declined progressively with transit down the river (Fig. 3a), with travel time being a significant factor related to Chl $a$ concentrations ($p = 0.007$; Table 1). There were, however, no significant differences in Chl $a$ concentrations between the $+\text{EFF}$ and $-\text{EFF}$ parcels ($p = 0.77$; Table 1). Concentrations at the most upstream sampling site were 15 mg L$^{-1}$ in October and 25 mg L$^{-1}$ in June, but declined to $\sim 5$ mg L$^{-1}$ by the time water passed the WWTP and to $\sim 2.5$ mg L$^{-1}$ at the most downstream sites. These Chl $a$ concentrations are comparable to those previously reported for this section of the river (Foe et al. 2010, Parker et al. 2012a, Gilbert et al. 2014a, 2014b) and are consistent with long-term data reported since 1980 for RM 38.6 (www.water.ca.gov; average concentration of 2.2 mg L$^{-1}$). Some of the initial Chl $a$ decrease may be attributable to dilution by the American River. However, of the total Sacramento River flow at Freeport, American River contributions constituted only $\sim 16\%$ in October and $\sim 23\%$ in June (http://waterdata.usgs.gov/nwis).

Total algal biovolumes (Fig. 3b) were much more variable than the Chl $a$ concentrations. This variability may be related to the high variability observed in species composition (discussed below) and variations in Chl $a$:total biovolume ratios and vacuole proportions. Similar to Chl $a$, biovolume sharply declined upstream of the WWTP, even over the course of a single day. During the October experiment, two of the three samples from the $+\text{EFF}$ parcel had unusually high biovolumes, owing to the high abundance of the diatom *Ulnaria ulna* (formerly *Synedra ulna*) (Compère 2001). This increase was not, however, observed in the Chl $a$ concentrations and may represent an occasional high cell biovolume of *U. ulna* relative to its Chl $a$ content.

### Phytoplankton uptake of NO$_3^-$, NH$_4^+$, and C

Potential C and N uptake ($^{15}\text{N}-\text{NH}_4^+$, $^{15}\text{N}-\text{NO}_3^-$, and $^{13}\text{C}$-DIC) were measured for a subset of samples (Supporting Information Fig. S5). In October and June, upstream of the WWTP concentrations of NH$_4^+$ were low and uptake of $^{15}\text{N}$-NH$_4^+$ was low at $<0.01$ mg N L$^{-1}$ d$^{-1}$. Following effluent addition, NH$_4^+$ uptake in the $+\text{EFF}$ parcels increased to 0.02–0.03 mg N L$^{-1}$ d$^{-1}$ during both sampling dates. These rates are comparable to those reported by Parker et al. (2012a) in March and April 2009 for the river above and below the WWTP. For the October $-\text{EFF}$ parcel, potential $^{15}\text{N}$-NH$_4^+$ uptake remained low ($<0.01$ mg N L$^{-1}$ d$^{-1}$) for all samples collected downstream of the WWTP. In the June $-\text{EFF}$ parcel, however, uptake rates for samples collected below the WWTP increased to about 0.02 mg N L$^{-1}$ d$^{-1}$, which is
Fig. 2. Nutrient concentrations during the October 2013 and June 2014 Lagrangian experiments: (a) ammonium (NH$_4^+$), (b) nitrate (NO$_3^-$), and (c) soluble reactive phosphorous (SRP). All data are plotted in relation to downriver travel time, with zero indicating the time the parcel passed the WWTP. The terms +EFF and −EFF indicate the effluent-containing and effluent-free parcels, respectively. Oct, October; AmR, American River; I80 Br, Interstate-80 Bridge at RM 63.
similar to rates observed in the +EFF parcel, despite much lower NH$_4^+$ concentrations (~0.1 vs. ~6.0 mg N L$^{-1}$).

Potential NO$_3^-$ uptake rates were ~0.013 mg N L$^{-1}$ d$^{-1}$ in October and ~0.017 mg N L$^{-1}$ d$^{-1}$ in June in the reach upstream of the WWTP discharge. Following effluent addition to the +EFF parcels, the rates dropped to near zero, which is consistent with observations by others that in the presence of NH$_4^+$, phytoplankton preferentially utilize NH$_4^+$ and NO$_3^-$ uptake is inhibited (e.g., Senn and Novick, 2014, Gilbert et al. 2016, and references therein). However, potential NO$_3^-$ uptake rates in the –EFF parcels traveling downstream from the WWTP also showed a significant decrease, though not as immediate as in the +EFF parcel. In October, these rates dropped to near zero by the 2nd day of travel past the WWTP. In June, potential NO$_3^-$ uptake remained ~0.015 mg N L$^{-1}$ d$^{-1}$ after 1 d of travel past the outfall and was still measurable at ~0.002–0.009 mg N L$^{-1}$ d$^{-1}$ after 2–3 d. The downwinder increase in potential NH$_4^+$ uptake rates and decrease in potential NO$_3^-$ uptake rates even in the absence of effluent is likely attributable to the elevated NH$_4^+$ observed downwinder of the WWTP in both the +EFF and –EFF parcels (Fig. 2a).

Primary production as measured by C uptake showed no significant difference between the +EFF and –EFF parcels ($p = 0.46$; Table 1; Fig. 3c). Uptake rates, $\rho$, were about threefold greater in June than in October, consistent with higher upstream Chl $a$ concentrations in June, greater solar insolation, and higher water temperatures. Highest $\rho$ values were measured at the most upstream sites and then declined downstream, consistent with downstream declines in Chl $a$. Assimilation numbers (C uptake normalized to Chl $a$ concentration; Fig. 3d) in June showed the opposite trend; these rates were lowest at the most upstream sites (~10–40 mg C [mg Chl $a$]$^{-1}$ d$^{-1}$) and highest at downstream sites (70–80 mg C [mg Chl $a$]$^{-1}$ d$^{-1}$). Assimilation numbers in October were much lower at 10–20 mg C (mg Chl $a$)$^{-1}$ d$^{-1}$, again likely due to lower temperatures and insolation, and showed a smaller increase with downstream travel compared with June.

**Phytoplankton species composition**

Diatoms accounted for ~90% of the total algal biovolume during the October and June experiments, with *U. ulna*, Melosira spp., Cocconeis placentula, Thalassiosira spp., and Cyclotella spp. dominating (Supporting Information Table S2; Fig. 4). Cryptophytes, chrysophytes, and green and blue-green algae were relatively minor components, constituting <1–7% of the total biovolume. These findings are similar to those found in monthly samples from 2013 to 2014 collected upstream and downstream of the WWTP (Supporting Information Fig. S6), as well as those in Greenberg (1964), Ball and Arthur (1979), Lehman et al. (2008), and Gilbert et al. (2014a).

Benthic diatoms composed 27–92% of sample biovolume (average = 67%) during our study, based on habitat preference classifications by Porter (2008). These species are typically found on bottom substrates or attached to aquatic vegetation, however, many are “facultative” meaning they can persist and even thrive in the water column. Given sufficient turbulence, colonies of filaments, ribbons, and chains of these benthic species may resuspend and entrain into flowing water (Hutchinson 1967, Reynolds and Descy 1996, Reynolds 2006). A good example of this is *U. ulna*, which occurred in the Sacramento River samples as large colonies of cells that contained lipid and polyphosphate storage structures. These structures suggest “resting” stages characteristic of phytoplankton that may have recently germinated from resting spores in the sediments (Supporting Information Fig. S7). Other facultative planktonic diatoms in the samples included *Aulacoseira, Bacillaria, Diatoma, Fragilaria, Melosira, Pseudostaurosira, Staurosira, Staurosirella, Synedra, and Tabellaria*. Many of these genera have been shown to produce resting cells or spores

**Table 1.** Subset of results from the ANCOVA, highlighting differences between the effluent-containing (+EFF) and effluent-free (–EFF) water parcels tracked during the October 2013 and June 2014 Sacramento River Lagrangian experiments. Numbers are the model parameter estimate with $p$ values indicated by * = <0.05; ** = <0.01; *** = <0.001. ns = nonsignificant. For result details, see Supporting Information Table S1.

| Parameter                  | No. of samples (n) | Date (Oct, Jun) | Parcel (+EFF, −EFF) | Travel time | Parcel x travel time |
|----------------------------|--------------------|-----------------|---------------------|-------------|---------------------|
| NH$_4^+$                    | 44                 | ns              | −0.690***           | −0.003*     | 0.004**             |
| NO$_3^-$                    | 44                 | −0.092***       | −1.140***           | 0.008***    | −0.002**            |
| Chl $a$                     | 39                 | ns              | ns                  | −0.004**    | ns                  |
| Total algal biovolume       | 37                 | ns              | ns                  | ns          | 0.006*              |
| Total algal density         | 37                 | ns              | ns                  | ns          | 0.005*              |
| Total suspended sediment    | 44                 | 0.265***        | ns                  | ns          | ns                  |
| Uptake $\rho$NH$_4^+$       | 19                 | 0.179***        | −0.136**            | ns          | ns                  |
| Uptake $\rho$NO$_3^-$       | 19                 | ns              | 0.359*              | ns          | ns                  |
| Uptake $\rho$C              | 19                 | 0.312***        | ns                  | −0.003*     | ns                  |
that allow them to repopulate (inoculate) rivers from the sediments (McQuoid and Hobson 1996).

*U. ulna* was dominant in many of the samples (Fig. 4; Supporting Information Table S2). This large, long, and narrow pennate diatom is widely distributed and is found on rocks, aquatic vegetation, and in the plankton of large rivers (Reynolds and Descy 1996, Porter 2008). In the qualitative plankton net samples, *U. ulna* colonies looked healthier in the upper part of the study reach, with vibrant, expanded chloroplasts (see photographs in Supporting Information...
Farther downstream, more of the cells appeared decrepit, with microbe swarms in some of the partially empty frustules (see photographs in Supporting Information Fig. S7c,d). This downstream decline in cell health, which was noticed in both the $-\text{EFF}$ and $+\text{EFF}$ parcels, mirrored downstream declines in Chl $\alpha$ concentrations (Fig. 3a) and primary production (Fig. 3c). The cause of this apparent decline in $U. \text{ulna}$ health is unclear, but the pattern suggests a physiological change due to light limitation or some other factor.

Phytoplankton species composition during both experiments was highly variable even within a few hours, particularly with respect to $U. \text{ulna}$ upstream of the WWTP (Fig. 4). Many samples contained high numbers of empty $U. \text{ulna}$ frustules along with detritus and sediment; these were not included in enumerations (John Beaver written comm., BSA Environmental Services, 2015). In October 2013, empty diatom frustules composed $\sim$ 40% of the total biovolume in some samples. This suggests resuspension of cells from bottom sediments is occurring, which could be an important means of exporting phytoplankton to the downstream estuary.

The ordination produced a clear separation of phytoplankton samples between October and June (Fig. 5a), which was significant ($p = 0.001$; Supporting Information Table S3) based on ANOSIM. Therefore, the October and June experiments were analyzed independently. These ordinations showed much overlap between the $+\text{EFF}$ and $-\text{EFF}$ samples (Fig. 5b,c), with no statistical difference between the parcels in October ($p = 0.55$) or June ($p = 0.30$; Supporting Information Table S3). Given the high variability among samples...
Fig. 5. Ordinations of Sacramento River phytoplankton assemblages tested for differences between (a) October 2013 and June 2014 samples, (b) October 2013 effluent-containing (+EFF) and effluent-free (−EFF) parcel samples, and (c) June 2014 +EFF and −EFF parcel samples.
from even a single day, ANOSIM was examined using the daily average species biovolumes to dampen this within-day variation. These tests were also non-significant for October \(p = 0.14\) and June \(p = 0.80\). Together these results indicate no significant difference in phytoplankton species composition between the +EFF and −EFF parcels. Although WWTP effluent was not, apparently, a primary factor in shaping phytoplankton species composition, results of the BEST analyses found TSS in October (−EFF parcel, \(p = 0.02\)) and water velocity at Walnut Grove in June (−EFF parcel, \(p = 0.02\)) were significant predictors (Supporting Information Table S4).

**Zooplankton, clams, and estimated grazing losses**

Biomass estimates for June 2014 show that zooplankton biomass, like phytoplankton, was temporally highly variable: biomass measured upstream of the WWTP at almost the same locations just 24 h apart varied over threefold from about 2 to 9 \(\mu g\ L^{-1}\) (Fig. 6, Supporting Information Fig. S9; Supporting Information Table S5). Samples collected 2 weeks later during the clam survey, under normal effluent discharge, had biomass concentrations of 0.8–2.9 \(\mu g\ L^{-1}\).

To our knowledge, this was the 1st time that quantitative sampling for bivalves was conducted this far upstream in the Sacramento River. *Corbicula fluminea*, the only bivalve species observed in the benthic trawl surveys, was distributed patchily across the river’s width and length (Fig. 6). Clam biomass at specific locations varied between the two seasons, but the overall average across the study reach was similar for October (2.4 \(g\ m^{-2}\)) and June (3.1 \(g\ m^{-2}\)). These values are slightly lower than clam biomass reported for deep channels in Suisun Bay (about 4 \(g\ m^{-2}\); Kimmerer and Thompson 2014) and much lower than clam biomass measurements made in some shallow-water habitats of the central Delta (> 100 \(g\ m^{-2}\); Lopez et al. 2006).

We compared measured Chl \(a\) losses to estimated losses due to grazing for the June experiment when we had zooplankton data (Table 2). Measured Chl \(a\) losses in June were greatest in the upstream reach (RM 63–45) during the first 2
d of downstream travel at 7–16 µg L\(^{-1}\) d\(^{-1}\), representing a daily loss of about 50% of the standing biomass. Losses during the subsequent days (RM 45–24) were <2 µg L\(^{-1}\) d\(^{-1}\) but still represented a 19–34% loss of standing biomass. By RM 24, Chl \(a\) concentrations were low enough that it was difficult to accurately measure any change; the difference between average daily Chl \(a\) concentrations was ~0.3 µg L\(^{-1}\), suggesting, if anything, a small gain in Chl \(a\) in this downstream reach.

To estimate Chl \(a\) losses due to zooplankton, we used the higher zooplankton biomass data from the +EFF parcel to ensure we were not underestimating potential losses. Even at these higher biomasses, daily Chl \(a\) losses attributable to zooplankton were <0.2 µg L\(^{-1}\) d\(^{-1}\) and thus likely had little effect on phytoplankton biomass, except possibly at the most downstream reaches where Chl \(a\) was <4 µg L\(^{-1}\) d\(^{-1}\) (Table 2). Estimated daily losses due to clams were most notable upstream between RM 63 to RM 56 (4.6 L\(^{-1}\) d\(^{-1}\)), where water depth was the shallowest (<5 m) and clam biomass was relatively high. However, even at the upstream site, clam grazing accounted for less than 30% of the measured loss of 15.7 µg Chl \(a\) L\(^{-1}\) d\(^{-1}\). Farther downstream, estimated losses to clams decreased to <0.2 µg Chl \(a\) L\(^{-1}\) d\(^{-1}\), accounting for <10% of the measured losses. Note that for these comparisons, we did not take into account phytoplankton growth from primary production; although growth rates could be estimated from the uptake data, those rates represent potential growth under high light (50% PAR) conditions and thus likely would overestimate actual rates of production.

**Discussion**

**Phytoplankton abundance and composition in the presence and absence of wastewater**

Results from this study do not support the hypothesis that wastewater effluent with high NH\(_4^+\) concentrations is the principal cause of downstream phytoplankton declines in the Sacramento River, as suggested by prior studies (Gilbert et al. 2011, Parker et al. 2012a, Gilbert et al. 2014a, 2014b). We found no significant differences in Chl \(a\) or algal biovolume between +EFF and -EFF parcels in either the October 2013 or June 2014 Lagrangian experiment (Table 1; Fig. 3). Moreover, the largest declines in phytoplankton abundance (>50% loss per day) occurred upstream of the WWTP under low NH\(_4^+\) concentrations (<0.02 mg N L\(^{-1}\); Fig. 2), and these declines occurred over short periods (~8 h), indicating that they are attributable to direct losses of phytoplankton and not inhibition of growth. In addition, although a shift in phytoplankton DIN uptake from NO\(_3^-\) to NH\(_4^+\) was observed following effluent additions of NH\(_4^+\), we did not detect a significant change in either total primary production (C uptake) or the assimilation number associated with this shift, as has been reported by others (Wilkerson et al. 2015, Gilbert et al. 2016, and references therein). In fact, higher rates of C uptake per Chl \(a\) occurred as water moved downstream, suggesting that phytoplankton production was not physiologically impaired when riverine NH\(_4^+\) concentrations were high (0.6–1.4 mg N L\(^{-1}\)).

Effluent addition also did not result in significant differences in the phytoplankton species composition compared with effluent-free conditions during either experiment (Fig. 4; Supporting Information Tables S2 and S3). Although this comparison may be confounded by the high spatial and temporal variability in phytoplankton abundance and species composition, the high variability is itself notable because it suggests that processes other than water quality are important in governing phytoplankton. While it is plausible that shifts in species composition would occur given a longer time period, significant shifts have been observed during laboratory incubations of river and estuarine water subjected to different nutrient enrichments over timeframes similar to the duration of our study (3–5 d; e.g., Sanders et al. 1987, Piehler et al. 2004, Gilbert et al. 2014a, 2014b).

The similarity in Chl \(a\) concentrations and C uptake in the presence and absence of effluent suggests that other contaminants in wastewater, such as pharmaceuticals or pesticides, are also not responsible for the observed downstream declines in phytoplankton. This finding is supported by recent enclosure experiments, conducted in conjunction with this study, which found that phytoplankton growth rates were comparable when Sacramento River water was amended with NH\(_4\)Cl, KNO\(_3\), or effluent (Travis 2015). Furthermore, under all of these amendments Chl \(a\) concentrations increased over 5 d in the enclosures whereas they decreased in the river, underscoring the conclusion that in the river phytoplankton losses overwhelms production due to factors not represented in the enclosures.

**Other factors shaping phytoplankton abundance and species composition**

Many studies have documented that physical, chemical, and biological drivers combine in various ways to affect phytoplankton abundance and species composition (e.g., Vannote et al. 1980, Lucas et al. 2009, Scherwass et al. 2010, Azevedo et al. 2014, Cloern et al. 2014a). For example, a 6d Lagrangian study along a 660 km reach of the Rhine River attributed downriver declines in phytoplankton abundance to a combination of losses from grazing and sedimentation associated with decreasing water velocities (and thus turbulence) in the downstream tidal reaches (Scherwass et al. 2010). Similarly, declines in phytoplankton in the River Spree were attributed to grazing losses, which were in turn controlled by river flow (Welker and Walz 1998). In the Hudson River, Caraco et al. (1997) found phytoplankton declines with downstream travel linked to ingestion by invasive zebra mussels, but the effect was complex because the mussels also reduced turbidity, thereby increasing light penetration and
enhancing conditions for primary productivity. In the Lower Snake River, Beaver et al. (2015) examined controls on plankton composition and determined that hydrodynamics were more important than nutrients in structuring the community. In contrast, Camacho et al. (2015) demonstrated the value of using a coupled hydrodynamic and water quality model to understand phytoplankton dynamics and investigate the impacts of different management strategies, and found that for the St Louis Bay estuary phytoplankton productivity was sensitive to nutrient concentrations.

Because the observed downriver declines in phytoplankton abundance were not explained by effluent additions and its attendant NH$_4^+$, we examined available data to assess the importance of grazing, hydrodynamics, and light during the Lagrangian experiments.

**Grazing:** Bivalve grazing by invasive species including C. fluminea has been identified as a driver of phytoplankton biomass declines in aquatic ecosystems worldwide (Cohen et al. 1984, Thompson et al. 2008, Pigneur et al. 2014). C. fluminea entered the Delta in the 1940s and is widely distributed in freshwater tidal environments. While a filter-feeder such as C. fluminea can have a strong effect on phytoplankton biomass, particularly in shallow habitats (Lopez et al. 2006, Lucas and Thompson 2012, Kimmerer et al. 2014), grazing losses by zooplankton may be of comparable importance in the lower Sacramento River (Kimmerer and Thompson 2014). We estimated that phytoplankton biomass losses in June 2014 attributable to zooplankton and clams could account for less than a third of the measured Chl a losses (Table 2). Because our calculations did not include any phytoplankton growth, the actual percent loss to grazing is likely even lower.

**Hydrodynamics:** The changes in hydrodynamic conditions as water moves from lotic to tidal environments affects characteristics such as velocity, water depths, light supply, and turbulence, all of which can have major effects on phytoplankton populations (e.g., Wetzel 1983, Reynolds 2006). Lower water velocities result in a longer residence times, which provide additional time for phytoplankton to grow and multiply but also increases exposure to grazers and other types of losses (Lucas and Thompson 2012, Lucas et al. 2016). The effects of bidirectional and slack tides in tidally-affected rivers adds another layer of complexity because it ensures there will be high short-term variability in many of these drivers.

In this reach of the Sacramento River, the dominance of benthic algal species (Fig. 4, Supporting Information Fig. S6) in the water column suggests that turbulence may be resuspending diatom cells and colonies during high-velocity periods. Although large single-celled species would normally be expected to sink, colony formation into filaments and chains—common in the Sacramento River plankton—allows for resuspension when turbulence is sufficient (Hutchinson 1967, Reynolds and Descy 1996, Reynolds 2006). The success of these facultative colonial diatoms in the Sacramento River may also include, paradoxically, their tendency to sink; true planktonic algae (“euplankton”) tend to washout at higher flows (Rounds et al. 1999). In contrast, facultative diatoms that take refuge in bottom sediments may tend to washout at higher flows (Rounds et al. 1999). Although data from this study are not sufficient to confirm these processes, there is some evidence from the continuous monitoring stations that sedimentation of particles occurs during low-flow periods. For example, measurable decreases in turbidity at Walnut Grove (RM 32) frequently occurred during slack tides (Supporting Information Fig. S10). The periodically high abundances of *U. ulna* and the presence of empty frustules, and sediments observed in cell counts is also consistent with resuspension of cells from the river bottom, particularly in the lower, tidal reach.

**Light:** Another possibility is that reduced light availability may limit photosynthesis and lead to phytoplankton declines. Low light availability is often cited as a factor limiting phytoplankton growth, and light limitation has been used to explain lower primary production in the Delta compared to other estuaries (Alpine and Cloern 1988, Jassby 2008, Cloern et al. 2014b). However, over the past decades there has been an overall decrease in Delta turbidity, with no increase in Chl a, suggesting light limitation is not the primary reason for observed long-term declines in phytoplankton production (Hestir et al. 2013, Schoellhamer et al. 2013).

In the Sacramento River, light availability to autotrophs is determined primarily by turbidity, water depth, and mixing, which dictates the amount of time cells spend in the photic zone. Parker et al. (2012a) measured light attenuation coefficients of 1.0–3.5 m$^{-1}$ for this section of the river when turbidities were similar to those measured in this study. We found no evidence that effluent inputs from the WWTP affect TSS concentrations or turbidity, and if anything, TSS declined with downstream transport (Table 1; Supporting Information Fig. S3c). However, an increase in water depth can reduce light availability by lowering the percentage of the water column in the photic zone. Water depths upstream of our study reach (above RM 63) are generally < 5 m, but in the study reach the river deepens to well over 8 m in many locations. An increase in water depth, particularly in this well-mixed, unstratified large river, would reduce the amount of time that algal cells spend in the photic zone, which may lead to light limitation (Wetzel 1983). This factor could also contribute to the observed declines of Chl a along the study reach.

**Other sources of nitrogen**

This study provided a unique opportunity to examine downstream changes in riverine nutrient concentrations in the presence and absence of effluent inputs. In the presence
of effluent, decreases in NH$_4^+$ and increases in NO$_3^-$ concentrations were observed during transit downstream, indicating nitrification of wastewater-derived NH$_4^+$ (Fig. 2; Parker et al. 2012a, O’Donnell 2014, Kendall et al. 2015). In the absence of effluent, below the WWTP discharge location both NO$_3^-$ and NH$_4^+$ steadily increased as water traveled downstream. This gradual increase suggests a diffuse or nonpoint-source of DIN, such as the benthos. Positive efflux of NO$_3^-$ from the benthos was previously shown for in the Sacramento River and the Delta (Kuwabara et al. 2009, Cornwell et al. 2014), and NH$_4^+$ oxidation by microbes likely plays a role (Kendall et al. 2015, Damashek et al. 2016). This trend of increasing NH$_4^+$ and NO$_3^-$ with downstream travel was not observed upstream of the WWTP, which suggests that increases in water column DIN downstream of the WWTP in the absence of effluent is a product of high N loading to the benthos over time from the nutrient-rich effluent.

**Implications and future studies**

Concentrations of NH$_4^+$ in aquatic ecosystems are increasing worldwide, not only as a result of increasing human populations and WWTP discharges, but also due to increasing use of urea-based vs. NO$_3^-$-based fertilizers, industrialization of animal production, and expansion of aquaculture operations (Gilbert et al. 2016, Paerl et al. 2016). A comprehensive understanding of how ammonium inputs affect food webs has ramifications for the development of nutrient regulations designed to improve water quality, which often requires investment in new management practices (Carey and Migliaccio 2009). In fact, the theory that elevated NH$_4^+$ levels inhibit phytoplankton production in the Delta was one of the key reasons for requiring Sacramento’s regional WWTP to reduce effluent NH$_4^+$-N concentrations to an average monthly concentration of 1.5–2.4 mg N L$^{-1}$ by the year 2021 (Cloern et al. 2014b, O’Donnell 2014). As a result, the WWTP is investing over 1.5 billion U.S. dollars to upgrade its treatment processes to include nitrification and denitrification. Our finding that temporary elimination of wastewater effluent did not alter phytoplankton abundance or community composition in this section of the Sacramento River suggests that the planned WWTP upgrades may not lead to the anticipated increases of phytoplankton production in the Delta.

Reductions in effluent NH$_4^+$ inputs may, however, alter estuarine phytoplankton dynamics in more downstream regions, which experience longer residence times, more complex hydrodynamic and light conditions, and stronger grazing pressures. Results from mesocosm studies combined with inferences from Eularian transects in those regions suggest that a shift in the form of DIN from NH$_4^+$ to NO$_3^-$ will lead to higher phytoplankton growth rates (Dugdale et al. 2013, Gilbert et al. 2014a, 2014b, Wilkerson et al. 2015), which under some conditions could offset losses due to other factors. However, planned upgrades to the WWTP will not only shift the predominant form of effluent DIN from NH$_4^+$ to NO$_3^-$, but will also lower effluent total DIN concentrations by > 65% (O’Donnell 2014). Monitoring water quality, phytoplankton, and grazer populations in the water column and benthos before and after the WWTP upgrade would provide important insights into how river–estuary ecosystems respond to step changes in N form and concentration.

Results from this ecosystem-scale study suggest that hydrodynamic factors may play an underappreciated role in governing phytoplankton abundance and species composition in the Sacramento River. The dominance of the phytoplankton community by benthic diatoms may also have ramifications for downstream food webs. Although benthic diatoms are considered a high-quality food for grazers, they may settle out of the water column during slack tides, thus reducing food available to pelagic species and providing increased food to the benthic community, including clams (Lucas et al. 2016). Future studies might examine hydrodynamic factors under various flow conditions, with particular emphasis on turbulence and resuspension, as well as settling during slack periods. This information could improve understanding of phytoplankton dynamics and estimates of grazing rates by benthic filter feeders and zooplankton (Sluss et al. 2008, Lucas et al. 2009, Lucas and Thompson 2012).

This study, like others, highlights the need to carefully consider methodology and scale when studying complex natural systems, particularly those subject to tidal effects (e.g., Kiemmer 2002, Duarte et al. 2009, Cloern et al. 2014b, Johnson et al., 2016). Laboratory or bottle studies can provide valuable insights into controls on phytoplankton, but extrapolating their findings to more dynamic real-world settings may require more comprehensive approaches. Landscape-level experimental treatments such as the one used in this study are logistically challenging and expensive to conduct. In addition, as in many field studies, interpretations and inferences may be confounded by simultaneous changes in multiple drivers. Our results demonstrate the importance of conducting ecosystem-scale manipulations, combined with Lagrangian-based sampling, to shed light on the dynamic and complex interplay between physical, chemical, and biological factors.

**References**

Alpine, J. E., and J. E. Cloern. 1988. Phytoplankton growth rates in light-limited environment, San Francisco Bay. Mar. Ecol. Prog. Ser. 44: 167–173. doi:10.3354/meps044167

Azevedo, I. C., A. A. Bordalo, and P. Duarte. 2014. Influence of freshwater inflow variability on the Douro estuary primary productivity: A modelling study. Ecol. Model. 272: 1–15. doi:10.1016/j.ecolmodel.2013.09.010

Ball, M. D., and J. F. Arthur. 1979. Planktonic chlorophyll dynamics in the northern San Francisco Bay and Delta, p.
265–285. The Urbanized Estuary Pacific Division, American Association for the Advancement of Science.

Beaver, J. R., and others. 2013. Response of phytoplankton and zooplankton communities in six reservoirs of the middle Missouri River (USA) to drought conditions and a major flood event. Hydrobiologia 705: 173–189. doi:10.1007/s10750-012-1397-1

Beaver, J. R., K. C. Scotese, E. E. Manis, S. T. J. Juul, J. Carroll, and T. R. Renicker. 2015. Variation in water residence time is the primary determinant of phytoplankton and zooplankton composition in a Pacific Northwest reservoir ecosystem (Lower Snake River, USA). River Syst. 21: 241–255. doi:10.1127/rs/2015/0100

Camacho, R. A., J. L. Martin, B. Watson, M. J. Paul, L. Zheng, and J. B. Stribling. 2015. Modeling the factors controlling phytoplankton in the St. Louis Bay Estuary, Mississippi and evaluating estuarine responses to nutrient load modifications. J. Environ. Eng. 141: 04014067. doi: 10.1061/(ASCE)EE.1943-7870.0000892

Caraco, N. F., and others. 1997. Zebra mussel invasion in a large, turbid river: Phytoplankton response to increased grazing. Ecology 78: 588–602. doi:10.1890/0012-9658(1997)078[0588:ZMIJAL]2.0.CO;2

Caramujo, M. J., H. T. S. Boschker, and W. Admiraal. 2008. Fatty acid profiles of algae mark the development and composition of harpacticoid copepods. Freshwater Biol. 53: 77–90. doi:10.1111/j.1365-2427.2007.01868.x

Carey, R. O., and K. W. Migliaccio. 2009. Contribution of wastewater treatment plant effluents to nutrient dynamics in aquatic systems: A review. Environ. Manage. 44: 205–217. doi:10.1007/s00267-009-9309-5

Chorus, I., and J. Bartram. 1999. Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management. E & FN Spon.

Clarke, K. R., and R.N. Gorley. 2006. PRIMER v. 6, User manual: Plymouth, U.K., Primer-E-Ltd., 190 p.

Cloern, J. E. 2001. Our evolving conceptual model of the coastal eutrophication problem. Mar. Ecol. Prog. Ser. 210: 223–253. doi:10.3354/meps210223

Cloern, J. E., and R. Dufford. 2005. Phytoplankton community ecology: Principles applied in San Francisco Bay. Mar. Ecol. Prog. Ser. 285: 11–28. doi:10.3354/meps285011

Cloern, J. E., and A. D. Jassby. 2012. Drivers of change in estuarine-coastal ecosystems: Discoveries from four decades of study in San Francisco Bay. Rev. Geophys. 50: RG4001. doi:10.1029/2012RG000397

Cloern, J. E., S. Q. Foster, and A. E. Kleckner. 2014a. Phytoplankton primary production in the world’s estuarine-coastal ecosystems. Biogeosciences 11: 2477–2501. doi:10.5194/bg-11-2477-2014

Cloern, J. E., and others. 2014b. The Suisun Bay problem: Food quality or food quantity? IEP NewsL. 27: 15–23.

Cloern, J. E., and others. 2016. Human activities and climate variability drive fast-paced change across the world’s estuarine–coastal ecosystems. Global Change Biol. 22: 513–529. doi:10.1111/gcb.13059

Cohen, R. R., P. V. Dresler, E. J. Phillips, and R. L. Cory. 1984. The effect of the Asiatic clam, Corbicula fluminea, on phytoplankton of the Potomac River, Maryland. Limnol. Oceanogr. 29: 170–180. doi:10.4319/lo.1984.29.1.0170

Collos, Y., and P. J. Harrison. 2014. Acclimation and toxicity of high ammonium concentrations to unicellular algae. Mar. Pollut. Bull. 80: 8–23. doi:10.1016/j.marpolbul.2014.01.006

Compère, P. 2001. A new genus name for Fragilaria subgen. Altersasyndra lange-bertalot with comments on the typification of synedra ehrenberg., p. 97–101. In R. Jahn, J. P. Kociolek, A. Witkowski, and P. Compère [eds.], Studies on Diatoms. A.R.G. Gantnter Verlag K.G. Ruggell.

Constable, M., M. Charlton, F. Jensen, K. McDonald, G. Craig, and K. W. Taylor. 2003. An ecological risk assessment of ammonia in the aquatic environment. Human Ecol. Risk Assess. 9: 527–548. doi:10.1080/713609921

Cooper, S. D., P. S. Lake, S. Sabater, J. M. Melack, and J. L. Sabo. 2013. The effects of land use changes on streams and rivers in Mediterranean climates. Hydrobiologia 719: 383–425. doi:10.1007/s10750-012-1333-4

Cornwell, J. C., P. M. Glibert, and M. S. Owens. 2014. Nutrient fluxes from sediments in the San Francisco Bay Delta. Estuaries Coasts 37: 1120–1133. doi:10.1007/s12237-013-9755-4

Damashke, J., K. L. Casciotti, and C. A. Francis. 2016. Variable nitrification rates across environmental gradients in turbid, nutrient-rich estuary waters of San Francisco Bay. Estuaries Coasts 39: 1050–1071. doi:10.1007/s12237-016-0071-7

Donald, D. B., M. J. Bogard, K. Finlay, and P. R. Leavitt. 2011. Comparative effects of urea, ammonium, and nitrate on phytoplankton abundance, community composition, and toxicity in hypereutrophic freshwaters. Limnol. Oceanogr. 56: 2161–2175. doi:10.4319/lo.2011.56.6.2161

Donald, D. B., M. J. Bogard, K. Finlay, L. Bunting, and P. R. Leavitt. 2013. Phytoplankton-specific response to enrichment of phosphorus-rich surface waters with ammonium, nitrate, and urea. PLoS One. 8: e53277. doi:10.1371/journal.pone.0053277

Downing, B. D., B. A. Bergamaschi, C. Kendall, T. E. C. Kraus, and T. S. Von Dessonneck. 2016. Using continuous water isotope measurements to map water residence time in hydrodynamically complex tidal environments. Environ. Sci. Technol. 50: 13387–13396. doi:10.1021/acs.est.6b05745

Duarte, C. M., D. J. Conley, J. Carstensen, and M. Sánchez-Camacho. 2009. Return to Neverland: Shifting baselines affect eutrophication restoration targets. Estuaries Coasts. 32: 29–36. doi:10.1007/s12237-008-9111-2

Dugdale, R. C., and F. P. Wilkerson. 1986. The use of 15N to measure nitrogen uptake in eutrophic oceans: Experimental considerations. Limnol. Oceanogr. 31: 673–689. doi:10.4319/lo.1986.31.4.0673
Jassby, A. D. 2008. Phytoplankton in the upper San Francisco Estuary: Recent biomass trends, their causes and their trophic significance. San Francisco Estuary Watershed Sci. 6: 1–24. http://escholarship.org/uc/item/71h077r1

Jassby, A. D., J. E. Cloern, and B. E. Cole. 2002. Annual primary production: Patterns and mechanisms of change in a nutrient-rich tidal ecosystem. Limnol. Oceanogr. 47: 698–712. doi:10.4319/lo.2002.47.3.0698

Johnson, D. S., R. S. Warren, L. A. Deegan, and T. J. Mozdzer. 2016. Saltmarsh plant responses to eutrophication. Ecol. Appl. 26: 2649–2661. doi:10.1002/eco.1402

Kendall, C., M. B. Young, S. R. Silva, T. E. C. Kraus, S. Peek, and M. Guerin. 2015. Tracing nutrient and organic matter sources and biogeochemical processes in the Sacramento River and Northern Delta: Proof of concept using stable isotope data. U.S. Geological Survey, Data Release. doi:10.5066/F7Q7FCM

Kimmerer, W. J. 2002. Effects of freshwater flow on abundance of estuarine organisms: Physical effects or trophic linkages? Mar. Ecol. Prog. Ser. 243: 39–55. doi:10.3354/meps243039

Kimmerer, W. J., N. Ferm, M. H. Nicollini, and C. Penalva. 2005. Chronic food limitation of egg production in populations of copepods of the genus Acartia in the San Francisco Estuary. Estuaries 28: 541–550. doi:10.1007/BF02696065

Kimmerer, W. J., T. R. Ignoffo, A. M. Slaughter, and A. L. Gould. 2014. Food-limited reproduction and growth of three copepod species in the low-salinity zone of the San Francisco Estuary. J. Plankton Res. 36: 722–735. doi:10.1093/plankt/fbt128

Kimmerer, W. J., and J. K. Thompson. 2014. Phytoplankton growth balanced by clam and zooplankton grazing and net transport into the low-salinity zone of the San Francisco Estuary. Estuaries Coasts. 37: 1202–1218. doi:10.1007/s12237-013-9753-6

Krater, C. R., R. H. Kent, D. K. Saleh, D. L. Knifong, P. D. Dileanis, and J. L. Orlando. 2011. Trends in nutrient concentrations, loads, and yields in streams in the Sacramento, San Joaquin, and Santa Ana Basins, California, 1975–2004. U.S. Geological Survey Scientific Investigations Report 2010-5228. Scientific Investigations Report. U.S. Geological Survey [accessed 15 January 2016]. Available from http://pubs.usgs.gov/sir/2010/5228/.

Kuwabara, J. S., B. R. Topping, F. Parchaso, A. C. Engelstad, and V. E. Greene. 2009. Benthic flux of nutrients and trace metals in the northern component of San Francisco Bay, California. U.S. Geological Survey Open-File Report 2009-1286, 14 p. U.S. Geological Survey [accessed 15 January 2016]. Available from http://pubs.usgs.gov/of/2009/1286/.

Latjá, R., and K. Salonen. 1978. Carbon analysis for the determination of individual biomass of planktonic animals. Verhandlungen der Internationalen Vereinigung für
The influence of environmental conditions on the seasonal variation of Microcystis cell density and microcysts concentration in San Francisco Estuary. Hydrobiologia 600: 187–204. doi:10.1007/s10750-007-9231-x

Lehman, P. W., C. Kendall, M. A. Guerin, M. B. Young, S. R. Silva, G. L. Boyer, and S. J. Teh. 2015. Characterization of the microcystis bloom and its nitrogen supply in San Francisco Estuary using stable isotopes. Estuaries Coasts. 38: 165–178. doi:10.1007/s12237-014-9811-8

Lucas, L. V., and J. R. Koseff. 2009. Shallow water processes govern system-wide phytoplankton bloom dynamics: A modeling study. J. Mar. Syst. 75: 70–86. doi:10.1016/j.jmarsys.2008.07.011

Lucas, L. V., and J. K. Thompson. 2012. Changing restoration rules: Exotic bivalves interact with residence time and depth to control phytoplankton productivity. Ecosphere 3: 1–26. doi:10.1890/ES12-00251.1

Lucas, L. V., J. E. Cloern, J. K. Thompson, M. T. Stacey, and J. R. Koseff. 2016. Bivalve grazing can shape phytoplankton communities. Frontiers Mar. Sci. 3: 14. doi:10.3389/fmars.2016.00014

Luoma, S. N., C. N. Dahm, M. Healey, and J. N. Moore. 2015. Water and the Sacramento-San Joaquin Delta: Complex, chaotic, or simply cantankerous? San Francisco Estuary Watershed Sci. 13: 1–25. doi:10.15447/sfews.2015v13iss3art7

McCarthy, J. J. 1981. The kinetics of nutrient utilization. Can. J. Fish. Aquat. Sci. Bull. 210: 211–213.

McQuoid, M. R., and L. A. Hobson. 1996. Diatom resting stages. J. Phycol. 32: 889–902. doi:10.1111/j.0022-3646.1996.00089.x

O’Donnell, K. 2014. Nitrogen sources and transformations along the Sacramento River: Linking wastewater effluent releases to downstream nitrate. Master’s thesis. California State Univ.

Paerl, H. W., N. S. Hall, B. L. Peierls, and K. L. Rossignol. 2014. Evolving paradigms and challenges in estuarine and coastal eutrophication dynamics in a culturally and climatically stressed world. Estuaries Coasts. 37: 243–258. doi:10.1007/s12237-014-9773-x

Paerl, H. W., and others. 2016. It takes two to tango: When and where dual nutrient (N & P) reductions are needed to protect lakes and downstream ecosystems. Environ. Sci. Technol. 50: 10805–10813. doi:10.1021/acs.est.6b02575

Parker, A. E., R. C. Dugdale, and F. P. Wilkerson. 2012a. Elevated ammonium concentrations from wastewater discharge depress primary productivity in the Sacramento River and the Northern San Francisco Estuary. Mar. Pollut. Bull. 64: 574–586. doi:10.1016/j.marpolbul.2011.12.016

Parker, A. E., V. E. Hogue, F. P. Wilkerson, and R. C. Dugdale. 2012b. The effect of inorganic nitrogen speciation on primary production in the San Francisco Estuary. Estuarine Coastal Shelf Sci. 104-105: 91–101. doi:10.1016/j.ecss.2012.04.001

Piehler, M. F., L. J. Twomey, N. S. Hall, and H. W. Paerl. 2004. Impacts of inorganic nutrient enrichment on phytoplankton community structure and function in Pamlico Sound, NC, USA. Estuarine Coastal Shelf Sci. 61: 197–209. doi:10.1016/j.ecss.2004.05.001

Pigneur, L. M., and others. 2014. Impact of invasive Asian clams, Corbicula spp., on a large river ecosystem. Freshwater Biol. 59: 573–583. doi:10.1111/fwb.12286

Reynolds, C. S. 2006. Ecology of phytoplankton. Cambridge Univ. Press.

Reynolds, C. S., and J. P. Descy. 1996. The production, biomass and structure of phytoplankton in large rivers. Arch. Hydrobiol. (suppl.) 113(Large Rivers 10): 161–187. doi:10.1127/lr/10/1996/161

Rounds, S. A., T. M. Wood, and D. D. Lynch. 1999. Modeling discharge, temperature, and water quality in the Tualatin River, Oregon. U.S. Geological Survey Water-Supply Paper 2465-B, 121 p. U.S. Geological Survey [accessed 15 January 2016]. Available from https://pubs.usgs.gov/publication/wsp2465B

Rounds, S. A., K. D. Carpenter, K. J. Fesler, and J. L. Dorsey. 2015. Upstream factors affecting Tualatin River algae—tracking the 2008 Anabaena algae bloom to Wapato Lake, Oregon. U.S. Geological Survey Scientific Investigations Report 2015–5178, 41 p. U.S. Geological Survey. doi:10.3133/sir20155178

Saleh, D., and J. Domagalski. 2015. SPARROW modeling of nitrogen sources and transport in rivers and streams of California and adjacent states, U.S. JAWRA J. Am. Water Resour. Assoc. 51: 1487–1507. doi:10.1111/1752-1688.12325

Sanders, J. G., S. J. Cibik, C. F. D’elia, and W. R. Boynton. 1987. Nutrient enrichment studies in a coastal plain estuary: Changes in phytoplankton species composition. Can. J. Fish. Aquat. Sci. 44: 83–90. doi:10.1139/f87-010

Scherwass, A., T. Bergfeld, A. Schol, M. Weitere, and H. Arndt. 2010. Changes in the plankton community along the length of the River Rhine: Lagrangian sampling during a spring situation. J. Plankton Res. 32: 491–502. doi:10.1093/plankt/fbp149

Schlegel, B., and J. L. Domagalski. 2016. Riverine nutrient trends in the Sacramento and San Joaquin Basins, California: A comparison to state and regional water quality policies. San Francisco Estuary Watershed Sci. 13, 1–30. doi:1015447/sfews.2015v13iss4art2

Schoellhamer, D. H., S. A. Wright, and J. Z. Drexler. 2013. Adjustment of the San Francisco estuary and watershed to decreasing sediment supply in the 20th century. Mar. Geol. 345: 63–71. doi:10.1016/j.margeo.2013.04.007
Senn, D., and E. Novick. 2014. Suisun Bay ammonium synthesis report. Contribution No. 706. San Francisco Estuary Institute.

Sharp, J. H. 2010. Estuarine oxygen dynamics: What can we learn about hypoxia from long-time records in the Delaware Estuary? Limnol. Oceanogr. 55:535–548. doi: 10.4319/lo.2009.55.2.0535

Sluss, T. D., G. A. Cobbs, and J. H. Thorp. 2008. Impact of turbulence on riverine zooplankton: A mesocosm experiment. Freshwater Biol. 53:1999–2010. doi:10.1111/j.1365-2427.2008.02023.x

Sommer, T., and others. 2007. The collapse of pelagic fishes in the upper San Francisco estuary. Fisheries 32:270–277. doi:10.1577/1548-8446(2007)32[270:TCOPFI]2.0.CO;2

Stumpner, E. B., and others. 2015. Mercury, monomethyl mercury, and dissolved organic carbon concentrations in surface water entering and exiting constructed wetlands treated with metal-based coagulants, Twitchell Island, California. U.S. Geological Survey Data Series 950, p. 26. U.S. Geological Survey.

Thompson, J. K., J. R. Koseff, S. G. Monismith, and L. V. Lucas. 2008. Shallow water processes govern system-wide phytoplankton bloom dynamics: A field study. J. Mar. Syst. 74:153–166. doi:10.1016/j.jmarsys.2007.12.006

Travis, N. M. 2015. Phytoplankton communities in the wastewater plume of the lower Sacramento River. Master’s thesis. San Francisco State Univ.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37:130–137. doi:10.1139/f80-017

Volkmar, E. C., R. A. Dahlgran, W. T. Stringfellow, S. S. Henson, S. E. Borglin, C. Kendall, and E. E. Van Nieuwenhuyse. 2011. Using Lagrangian sampling to study water quality during downstream transport in the San Luis Drain, California, USA. Chem. Geol. 283:68–77. doi:10.1016/j.chemgeo.2011.01.029

Wehr, J. D., and J. P. Descy. 1998. Use of phytoplankton in large river management. J. Phycol. 34:741–749. doi:10.1046/j.1529-8817.1998.340741.x

Welker, M., and N. Walz. 1998. Can mussels control the plankton in rivers?—a planktological approach applying a Lagrangian sampling strategy. Limnol. Oceanogr. 43:753–762. doi:10.4319/lo.1998.43.5.0753

Wetzel, R. G. 1983. Limnology, 2nd ed. Saunders College Publishing.

Wilkerson, F. P., R. C. Dugdale, A. E. Parker, S. B. Blaser, and A. Pimenta. 2015. Nutrient uptake and primary productivity in an urban estuary: Using rate measurements to evaluate phytoplankton response to different hydrological and nutrient conditions. Aquat. Ecol. 49:211–233. doi:10.1007/s10452-015-9516-5

Yoshiyama, K., and J. H. Sharp. 2006. Phytoplankton response to nutrient enrichment in an urbanized estuary: Apparent inhibition of primary production by overeutrophication. Limnol. Oceanogr. 51:424–434. doi:10.4319/lo.2006.51.1_part_2.0424

Zhang, J. Y., W. M. Ni, Y. M. Zhu, and Y. D. Pan. 2013. Effects of different nitrogen species on sensitivity and photosynthetic stress of three common freshwater diatoms. Aquat. Ecol. 47:25–35. doi:10.1007/s10452-012-9422-z

Acknowledgments

Field, laboratory, and instrumentation support was provided by Katy O’Donnell, Angela Hansen, Sarah Blaser, Edmund Antell, Jamie Lee, Scott Nagel, Amy Story, Paul Kreun, Travis von Dosseneck, Brandon Oldham, Erica Schmidt, Thi Pham, Matt Richter, Chris Tai, and Andrew Pedersen. We thank Regional San’s laboratory staff, especially Srividhya Ramamooorthy, for providing chemical and biological analyses on monthly river samples. We thank Janet Thompson for guiding our clam biomass, grazing, and turnover estimations; Lisa Thompson for supporting the project at critical junctures; and Richard Dugdale, Joe Domagalski, and two anonymous reviewers for providing input on earlier versions of this manuscript. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Geological Survey. The authors gratefully acknowledge funding support from the Interagency Ecological Program (IEP) for the San Francisco Estuary, Sacramento County Regional Sanitation District, the United States Bureau of Reclamation, and the USGS Cooperative Water Program. Marianne Guerin, supported by funding from the Contra Costa Regional Sanitation District, modeled flows in advance of the field studies to help plan the Lagrangian experiments. The funding from Interagency Ecological Program for the San Francisco Estuary (Grant Number: 13WSCA4600010038/4600010038), Sacramento County Regional Sanitation District (Grant Number: 13WSCA60000947/90000080), and US Bureau of Reclamation (Grant Number: R15PG00085) are greatly acknowledged.

Conflict of Interest

None declared.

Submitted 15 April 2016
Revised 05 August 2016; 15 November 2016
Accepted 21 November 2016

Associate editor: Anssi Vähätalo