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To Regional San,

The attached pdf of the manuscript submitted to the Journal of Limnology and Oceanography serves as the final deliverable for Sacramento Regional County Sanitation District (SRCSD) Agreement #90000080, for the project titled *Nitrogen Dynamics along the Sacramento River and Links to Phytoplankton Dynamics: Resolving Spatial and Temporal Variability Using In Situ, High Frequency Measurements and Other Tools.* The study took place from April 1, 2013 to March 31, 2016, and was also partially supported by funding provided by the California Department of Water Resources under the Interagency Ecology Program (IEP), Agreement 13WSCA600000947, as well as USGS Cooperative Matching Funds.

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Controls on riverine phytoplankton dynamics in the presence and absence of treated wastewater effluent high in ammonium—A Lagrangian based study

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Abstract:	Studies suggest that ammonium in treated wastewater effluent entering rivers and estuaries negatively affects phytoplankton growth and alters species composition, but much of this evidence comes from enclosure studies and has not been explicitly evaluated in-situ. In the Sacramento–San Joaquin River Delta of California (USA), ammonium from a large wastewater treatment plant (WWTP) discharges to the Sacramento River, the main source of water entering the estuary. To assess effluent effects on phytoplankton, in October 2013 and June 2014 we diverted WWTP discharges to the river and used a Lagrangian approach to compare changes in nutrients and phytoplankton in the absence (-EFF) and presence (+EFF) of effluent as water traveled downstream. Changes in phytoplankton chlorophyll-a, species composition, and productivity were tracked, along with nutrients, zooplankton, and benthic grazer abundances. Over 5 days of travel, chlorophyll-a concentrations declined from 15–25 µg L-1 to ~2.5 µg L-1, with the greatest decline occurring upstream of the WWTP. There was no statistical difference in phytoplankton chlorophyll-a or species composition between the +EFF and -EFF parcels during either experimental periods, indicating that declines in phytoplankton were not attributable to effluent effects, including elevated ammonium. These results, together with the prevalence of benthic and facultative planktonic diatoms, suggest that hydrodynamic factors may play an underappreciated role in phytoplankton losses through settling during slack periods. Our results highlight the advantages of in-situ, whole-river scale, Lagrangian experiments to understand the dynamic and complex interplay between physical, chemical, and biological factors that control phytoplankton

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Controls on riverine phytoplankton dynamics in the presence and absence of treated wastewater effluent high in ammonium—A Lagrangian based study

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Running Head: Effluent effects on river phytoplankton

Key Words: phytoplankton, wastewater, ammonium, nitrogen, river, Lagrangian, Sacramento River

ABSTRACT

Studies suggest that ammonium in treated wastewater effluent entering rivers and estuaries negatively affects phytoplankton growth and alters species composition, but much of this evidence comes from enclosure studies and has not been explicitly evaluated in-situ. In the Sacramento–San Joaquin River Delta of California (USA), ammonium from a large wastewater treatment plant (WWTP) discharges to the Sacramento River, the main source of water entering the estuary. To assess effluent effects on phytoplankton, in October 2013 and June 2014 we diverted WWTP discharges to the river and used a Lagrangian approach to compare changes in nutrients and phytoplankton in the absence (-EFF) and presence (+EFF) of effluent as water traveled downstream. Changes in phytoplankton chlorophyll-*a*, species composition, and productivity were tracked, along with nutrients, zooplankton, and benthic grazer abundances. Over 5 days of travel, chlorophyll-*a* concentrations declined from 15–25 μg L^{-1} to ~2.5 μg L^{-1}, with the greatest decline occurring upstream of the WWTP. There was no statistical difference in phytoplankton chlorophyll-*a* or species composition between the +EFF and -EFF parcels during either experimental periods, indicating that declines in phytoplankton were not attributable to effluent effects, including elevated ammonium. Estimated losses from zooplankton and clam grazing could not account for the measured declines. These results, together with the prevalence of benthic and facultative planktonic diatoms, suggest that hydrodynamic factors may play an underappreciated role in phytoplankton losses through settling during slack periods. Our results highlight the advantages of in-situ, whole-river scale, Lagrangian experiments to understand the dynamic and complex interplay between physical, chemical, and biological factors that control phytoplankton populations.

INTRODUCTION

Large rivers serve multiple ecosystem functions, supporting aquatic organisms living within the river-riparian-floodplain corridor and providing important nutrient and biological inputs to downstream

estuaries and coasts that can drive food-web dynamics (Cloern et al. 2014a). In many river-estuary systems phytoplankton form the base of the food web, with both their abundance and composition governing the amount and quality of carbon available to upper trophic organisms (Vannote et al. 1980; Wehr and Descy 1998). Because downstream reaches of large rivers serve as a key freshwater resource and provide geographic advantages, they are typically impacted by anthropogenic activities; they receive nutrients, sediment, and other contaminants, and they experience alterations to flows, channel morphology, and habitat. In many cases, anthropogenic nutrient inputs result in eutrophication—stimulation of phytoplankton production resulting in 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 primary production despite non-limiting nutrient levels (e.g., Cloern 2001; Yoshiyama and Sharp, 2006; Sharp et al. 2010; Rounds and Carpenter 2013; Cloern et al. 2014a), and the reduced export of plankton may have direct consequences for downstream food webs.

Such is the case for the Sacramento–San Joaquin River Delta system of central California (USA) and the northern San Francisco Estuary (hereafter referred to as the "Delta" and "Estuary"); despite increased nutrient inputs from agricultural, industrial, and urban development, chlorophyll-a (Chl-*a*) concentrations measured between 1975 and 2005 show a significant decline across much of the region (Jassby 2008). The phytoplankton decline is of great concern because of co-occurring observations of food limitation in other parts of the food web (Muller-Solger et al. 2002; Kimmerer et al. 2005) and declines in the abundance of several fish species (Sommer et al. 2007). It is widely believed that one of the main stresses on these fish species is a reduction of food resources resulting from declines in phytoplankton production (Jassby et al. 2002; Sobzak et al. 2005).

In addition to changes in phytoplankton abundance, shifts in phytoplankton species composition may occur in response to environmental factors (Reynolds 2006; Beaver et al. 2012, 2015). A decrease in the abundance of diatoms is of particular concern as these algae are considered a superior food source

owing to their high fatty acid content compared with other types of phytoplankton (Caramujo et al. 2008). An associated increase in cyanobacteria that are less palatable and nutritious, and may produce toxins, is another concern of water managers (Chorus and Bartram 1999; Lehman et al. 2015). In the Delta, the decline in phytoplankton primary production has been associated with reduced populations of diatoms. Long-term data from state natural resource agencies analyzed by Glibert et al. (2011) suggested that shifts in species composition, with fewer diatoms and more small flagellates and cyanobacteria, may have occurred. Subsequently, Cloern et al. (2014b) concluded that, because of changes in enumeration methods, these historic data may not be accurate enough to discern whether increases in the abundance of small flagellates and cyanobacteria have occurred, but the reduced abundance of diatoms is believed to be valid.

Although there are likely a number of factors that play a role in phytoplankton declines and shifts in species composition in the Delta, including changes in streamflow (amount and source), light availability, landscape changes, introduced species such as phytoplankton-grazing clams, and contaminants (Luoma et al. 2015), a number of studies suggest that high ammonium (NH_4) concentrations repress phytoplankton growth by inhibiting nitrate (NO_3) uptake, leading to lower rates of productivity in some systems (e.g., Dugdale et al. 2007; Glibert et al. 2015 and references therein), and that NH_4 favors the growth of small flagellates and cyanobacteria (Glibert et al. 2011). Studies showing NH_4 suppression of phytoplankton production included river-estuarine transect surveys, short-term enclosures of river and estuarine water, and multi-day enclosures. We note that reviews and studies examining the effects of dissolved inorganic nitrogen (DIN) form (NO_3 versus NH_4) on phytoplankton dynamics present an inconsistent picture, indicating that responses to NH_4 versus NO_3 vary among phytoplankton species, as well as for the same species when grown under different conditions (e.g., Cloern et al. 2012, 2014b; Donald et al. 2011; 2013; Collos and Harrison 2014; Esparza et al. 2014; Glibert et al. 2015). Furthermore, an understanding of whether responses measured during laboratory culture studies or

enclosure studies translate into ecologically significant effects under field conditions, where numerous other drivers of phytoplankton abundance and health are at play, is needed in order to inform ecosystem management (Cloern and Dufford 2005; Senn and Novick 2014; Wilkerson et al. 2015).

The Sacramento River is a primary source of water and nutrients to the Delta (Jassby 2008). One of the main sources of nutrients to the Sacramento River is the Sacramento Regional Wastewater Treatment Plant (SRWTP). The effluent typically makes up 1–3% of the total river flow, and under current treatment operations effluent contains a high amount of N, predominantly in the form of NH_4 (~35 mg N L^{-1}). Parker et al. (2012) examined phytoplankton biomass and primary production along a transect of the Sacramento River beginning about 20 km upstream of the WWTP and documented progressive declines in phytoplankton abundance and primary production, which were attributed to high NH_4 inputs from SRWTP effluent. Glibert et al. (2011, 2014a) similarly identified the zone immediately downstream of the WWTP as potentially having a strong negative impact on phytoplankton due to high NH_4 concentrations. However, these studies also documented declines in Chl-*a* upstream of the treatment plant, prior to SRWTP effluent additions, where NH_4 concentrations were low (<0.01 mg N L^{-1}).

This study was designed to test whether the WWTP effluent additions that lead to elevated riverine NH_4 concentrations are responsible for (i) declines in phytoplankton abundance and (ii) a shift in phytoplankton species composition in the Sacramento River. A secondary objective was to evaluate other possible factors affecting phytoplankton abundance and species composition, including clam and zooplankton grazing, hydrodynamics, and light availability, using available information. Full-scale river experiments were conducted in the lower Sacramento River in October 2013 and June 2014 by temporarily ceasing effluent release to the river, producing a ~15-km effluent-free parcel of river water. The effluent-free river parcel and a matching parcel containing effluent were tracked during transit to permit Lagrangian sampling of phytoplankton biomass and species composition, nutrients, and other key factors during 5 days of travel from about 20 km above to 50 km below the effluent outflow

location. The Lagrangian approach allowed us to assess the effects of effluent and its attendant high NH_4 concentrations while minimizing the confounding effects of spatial and temporal variability in constituent concentrations, flow and irradiance (e.g., Welker and Waltz 1998; Scherwass et al. 2010).

METHODS

Study reach

The Sacramento River drains a watershed of ~70,000 km^2, consisting of forested headwaters and intensively irrigated cropland in the valley. The river provides ~80% of the annual freshwater inflows to the Delta (Jassby 2008). Sacramento River flows are primarily regulated by reservoir releases from Shasta, Oroville, and Folsom Lakes, with minor inputs from tributaries, agricultural return flows, and precipitation. To identify locations on the river, river miles (RM) are calculated as the distance in miles upstream of the confluence between the Sacramento and San Joaquin Rivers (Figure 1). This study focused on the lower section of the Sacramento River, extending from the City of Sacramento near RM 63 downstream to RM 15, where the channelized river enters the more hydrologically complex region known as the Cache Slough Complex. Within the study reach the only significant inflow to the Sacramento River is the American River at RM 60.5.

Just below Freeport at RM 46.3, the Sacramento River receives treated wastewater effluent from the SRWTP, which serves approximately 1.4 million residential, commercial, and industrial customers (Figure 1). Effluent inputs are estimated to provide ~90% of the total NH_4 load to the lower Sacramento River (Jassby 2008). Most of the N discharged within SRWTP effluent is in the form of NH_4 (24–34 mg N L^{-1}), whereas NO_3 concentrations are typically less than 0.1 mg N L^{-1} (O'Donnell 2014). Effluent discharge must remain less than 6.7 % of the total river flow (river to effluent ratio >14:1) and must cease when the Sacramento River's instantaneous flow is <36.8 m^3 s^{-1} (1,300 cfs), which occurs regularly when tidally averaged flow drops below ~280 m^3 s^{-1} (10,000 ft^2 s^{-1}), causing flow reversals at

151 Freeport. SRWTP discharge typically accounts for 1–3 % of the total river flow, but tidally driven changes
152 in river flow rates can result in large differences in the river's dissolved effluent concentrations (within
153 the permitted range) over the course of a tidal cycle (O'Donnell 2014). Upstream of the study area,
154 nutrients are supplied to the Sacramento River from agriculture, urban runoff, and other wastewater
155 treatment facilities (Saleh and Domagalski 2015), but for the purposes of this study, we refer to the river
156 as effluent free (-EFF) in the absence of SRWTP discharge.

157 River flow, velocity, and other water-quality characteristics (Figure 2, Fig. S-6) were measured
158 every 15 minutes at the U.S. Geological Survey (USGS) monitoring stations near Freeport (RM 46.3) and
159 Walnut Grove (RM 28.2), 0.2 km upstream and 29.2 km downstream from the wastewater treatment
160 plant (WWTP), respectively (*http://waterdata.usgs.gov/usa/nwis*). These data were used to plan and
161 conduct sample collection and to document river conditions above and below the WWTP's discharge
162 location. Treated effluent quality and flow data (hourly average) were provided by SRWTP, along with
163 weekly effluent nutrient concentrations monitored as part of their discharge permit (O'Donnell
164 2014; *http://www.swrcb.ca.gov/centralvalley*).

165

166 ***Study approach***

167 We employed a Lagrangian sampling approach, whereby individual parcels of water were
168 tracked and sampled as they traveled through the study reach (Fig. S-5). Two sampling campaigns were
169 conducted, from October 24 to 29, 2013, and May 30 to June 4, 2014 (hereafter referred to as the
170 "October" and "June" experiments). The Lagrangian parcel tracking was coordinated with extended
171 effluent diversions (no effluent discharged to the river) during October 25–26, 2013, (18 hours) and June
172 1–2, 2014 (19.5 hours). These effluent diversions resulted in approximately a 15 km stretch of river that
173 was essentially free of SRWTP effluent. During each experiment, one parcel of water located in the
174 effluent free stretch of river (-EFF) and one parcel located downstream in the effluent containing parcel

(+EFF) was tracked as each traversed downstream until about RM 15, prior to mixing into the Cache Slough Complex (Figure 1). In addition, to better understand algal and nutrient conditions entering the study reach, samples were collected on the first day of each experiment well upstream of the WWTP at the I-80 Bridge (RM 63.0) (Figure 1), as well as at the American River 0.25 km upstream of its confluence (RM 60.5, Figure 1).

Parcel tracking began approximately 20 km upstream of the WWTP; the exact parcel upstream starting locations and wastewater diversions were orchestrated such that +EFF and -EFF parcels were sampled approximately 24 hours apart with each parcel passing the effluent discharge location during the ebb tide at approximately 6 a.m. This minimized the effect of any differences between parcels with regard to sample location and time of day, thus facilitating direct comparison of conditions measured within each parcel. Comparisons between parcels were made with travel time relative to passage by the WWTP discharge location as a covariate, and travel time was also used in figures to help visualize longitudinal trends and to facilitate comparisons between parcels.

Three methods were used to track and verify the location of each parcel: (1) velocity data from USGS monitoring stations at Freeport (RM 46.4) and Walnut Grove (RM 28.2) were used to estimate the distance that the water parcels traveled between sampling events; (2) neutrally buoyant drifters were deployed in the river daily, and their movement was tracked; (3) changes in specific conductivity, fluorescence of dissolved organic matter (FDOM), and other water-quality parameters were measured in real-time using a boat-based flow-through instrument package to identify the +EFF and -EFF parcel locations (Fichot et al. 2015). Data from the USGS monitoring station at Walnut Grove further helped to confirm the location of the +EFF and -EFF parcels by documenting their passage (O'Donnell 2014). Because the transition boundaries of the parcels were easily differentiated, confidence is high that all discrete samples were collected within their respective parcels; laboratory measurements of elevated or reduced NH_4 concentrations further confirmed this.

199

200 ***Water and plankton sampling, collection, and processing***

201 Two boats were used during the study; one tracked and collected samples in the +EFF parcel while the
202 other tracked the -EFF parcel. Each day between 8 a.m. and 5 p.m. Pacific Standard Time, 3–5 samples
203 were collected from each parcel at approximately 2- to 3-hour intervals. Field measurements of
204 temperature, specific conductivity, pH, dissolved oxygen, turbidity, and fluorescence of dissolved
205 organic matter (FDOM) were made simultaneously at all sampling locations using a Yellow Springs
206 Instruments (YSI) EXO2 water-quality sonde. Field data represent an average of 60 readings collected
207 over 1 minute following a period of sensor equilibration.

208 Discrete water samples were collected at a 1-meter depth using a clean 3k Shurflo pump with
209 clear ½" tubing using USGS protocols (USGS 2006). Samples were either pumped into 8-L Teflon Jerri
210 cans and transferred into, or pumped directly into a 20-L churn splitter (USGS 2006). Samples were
211 processed within 1 hour of collection for nutrients, dissolved organic carbon (DOC), Chl-*a*, total
212 suspended sediment (TSS), plankton enumeration. Whole-water samples for phytoplankton
213 identification and enumeration were preserved with 1% Lugol's solution and analyzed within 12 months
214 of collection.

215 Methods to determine nutrient (NH_4, NO_3, nitrite [NO_2] and soluble reactive phosphate [SRP]),
216 dissolved inorganic carbon (DIC), and Chl-*a* concentrations followed those described in Parker et al.
217 (2012a) (see Supporting Information for details). Rates of C and N uptake were measured on a subset of
218 samples collected in the +EFF and -EFF parcels using stable isotope tracer techniques (Travis 2015; see
219 Supporting Information for details). The ^{15}N enrichments were higher than the 10% substrate "tracer"
220 addition recommended by Dugdale and Wilkerson (1986) for all samples collected upstream of the
221 WWTP discharge, as well as NO_3 uptake downstream from the WWTP discharge. Because of much
222 higher riverine concentrations of NH_4 in +EFF and -EFF samples collected downstream from the WWTP,

223 NH_4 enrichments made to those samples were closer to the tracer level. The isotope-enriched bottles
224 were incubated for 24 hr, suspended at the surface of the river in a floating corral, and covered with
225 window screening to reduce ambient light to ~50% of surface photosynthetically active radiation (PAR).
226 Thus these uptake results should be considered "potential" (N-saturated, high light) uptake rates.
227 Carbon and nitrogen uptake rates are reported as ρ (mg C L^{-1} d^{-1} or mg N L^{-1} d^{-1}). Carbon uptake data
228 were divided by sample Chl-*a* concentration to express a biomass normalized assimilation number (mg C
229 [mg Chl-*a*]$^{-1}$ d^{-1}).
230 DOC concentrations were determined by high-temperature catalytic combustion as described in
231 Stumpner et al. (2015). Concentrations of TSS were determined by filtering a known volume of sample
232 water through a 0.3-μm glass fiber filter and determining the dry weight of material retained on the
233 filter.
234 Phytoplankton enumerations were performed by BSA Environmental Services, Inc., Cleveland,
235 Ohio. Microscope slides were prepared using membrane filtration with enumeration of 300 natural algal
236 units (cells, filaments, or colonies) using a Leica microscope at 630X. Measurements of cell biovolume were
237 made on as many as 10 individuals per taxon. Pico-sized (<0.2 μM) phytoplankton, analyzed in separate
238 enumerations by BSA Environmental Services for a subset of the samples preserved in 2.5%
239 gluteraldehyde using an epifluorescence microscope, were found to comprise on average 4.1% (*n*=6) of
240 the total autotrophic community biovolume in October and 7.0% (*n*=25) in June 2014. Given the small
241 contribution of picoplankton, these data were not included in the total biovolume calculations. The
242 relative biovolume (RBV), expressed as percent—calculated as the biovolume of each species divided by the
243 total sample biovolume multiplied by 100—was used to express the species composition of each sample.
244 Qualitative observations from these enumerations, particularly high amounts of silt and empty or broken
245 diatom frustules, were also considered in our analyses.

Samples of plankton from 80-µm-mesh net tows were used to qualitatively characterize the phytoplankton and zooplankton assemblages during each Lagrangian experiment. Unpreserved samples were examined using a Leica microscope to provide initial insight into the dominant plankton and to look for visual clues about the health of the cells on the basis of chloroplast integrity, presence of possible polyphosphate, and lipid bodies. These observations were used in tandem with the preserved plankton sample counts to characterize the phytoplankton and zooplankton assemblages.

Monthly phytoplankton data

In addition to samples collected during the Lagrangian experiments, the WWTP collected water samples every 2-4 weeks from RM 46.4 (Station R1), just upstream of the effluent discharge location at Freeport Bridge and just downstream at RM 45.6 or RM 44.4 (Station R4), or both, depending on the constituent sampled (Figure 1). All samples were collected during periods of downstream flow. Whole-water samples were preserved with Lugol's solution and analyzed for phytoplankton enumeration as described above. These data provide insight into how phytoplankton populations varied over time and allow us to place our Lagrangian experiments into a larger context; the data also provide information about potential immediate effects of effluent addition on phytoplankton Chl-*a* concentrations or species composition. Nutrient data (NH_4, NO_3, NO_2) were collected, and basic field water-quality measurements (temperature, pH, conductivity, dissolved oxygen, turbidity) were made, as part of the WWTPs permit requirements.

Zooplankton and clam sampling and grazing estimates

Samples for zooplankton enumeration were collected during the June Lagrangian in the +EFF and -EFF parcels (4 locations per parcel) and again 2 weeks later at 11 sites in coordination with a benthic clam abundance survey (see below). Vertical tows using nets with 35- and 153-µm mesh sizes to capture

small- and large-size classes, respectively, were pulled at 0.3 m s^{-1} from 0.5 m above the bottom of the channel to the surface. At each site, three replicate samples were collected for each net size, and each of these replicates was a composite of three separate vertical tows. Zooplankton samples were immediately preserved in 5% Lugol's solution; enumerations were conducted by BSA Environmental using three 1-mL aliquots examined using a Wilovert inverted microscope. Two 200-organism tallies, one for each mesh size, were made, and tallies were summed at each sampling location. Biomass estimates of each taxon were based on measurements of as many as 10 individuals per sample using established length/width relations (Beaver et al. 2013).

To survey clam populations, sampling locations were selected to match the average river mile locations sampled during each day of the Lagrangian experiment. The benthic trawl used a 35-cm-long rake with 8-mm-wide metal teeth spaced 2.5 cm apart, which projected into the sediment a maximum of 6.4 cm. Scraped sediment and clams were collected in a 5-mm mesh basket, which could hold 0.2 m^3 of scrapings. This mesh size likely allowed most clams with shells < 5 mm long to pass through the basket, but some small individuals were retained and included in the analysis. The trawl was dragged into to the river flow, and 5 transects equally spaced across the river's width per location were trawled. Trawls were conducted for 1 minute at a speed of 0.66 m s^{-1}, resulting in an average 10.4-m^2 area sampled per trawl. River depths were recorded for each sampling location using an acoustic depth finder (Lowrance, Elite-5 DSI) and ranged from 1.9 to 9.7 m across all locations. Clams were fixed in buffered 10% formalin and transferred into 70% ethanol within 2 weeks of collection for storage. Clam shell widths were measured individually using digital calipers (INSIZE, 6 in-USB). Shell lengths were converted to estimated ash-free dry weight (afdw) biomass by using an empirical conversion formula based on previous *Corbicula fluminea* (Asian clam) collections in the Sacramento River (Thompson et al. 2008) and are reported as dry weight in g m^{-2}. Although our methods selected for larger-bodied clams and juvenile clams can make up a large percentage of the total population by number, the small-bodied clams

294 commonly account for a small (2%) percentage of the population's total biovolume and therefore
295 provide only a small proportion of the total grazing pressure.
296 Phytoplankton losses resulting from zooplankton grazing were calculated by estimating the
297 energy required to sustain an 11% (conservative) growth rate per day, based the measured growth rates
298 of three copepod species in the low-salinity zone of the San Francisco Estuary (Kimmerer et al. 2014).
299 Zooplankton carbon estimates were assumed to be 50% of the estimated dry weight (Latja and Salonen
300 1978, Beaver et al. 2010), and the energy conversion efficiency was estimated to be 30% (Kimmerer and
301 Thompson 2014). Carbon to Chl-*a* ratio was estimated to be 35:1 (Jassby et al. 2002). Thus, the amount
302 of Chl-*a* grazed by zooplankton per day (μg d^{-1}) was estimated by the following calculation: zooplankton
303 biovolume x (0.5 μg zooplankton carbon $biovolume^{-1}$) x (0.11 growth d^{-1}) x (0.3 energy transfer) x
304 (0.0286 μg phytoplankton Chl-*a* $carbon^{-1}$). Clam grazing rates were estimated from clam biomass as
305 described in Thompson et al. (2008), assuming constant grazing and 100% grazing efficiency. From this,
306 daily Chl-*a* losses to clams were estimated as the river turnover rate (clam grazing rates normalized by
307 water depth measured at the time of sampling) multiplied by that day's average Chl-*a* concentration.
308 These estimated losses to zooplankton and clams were compared to measured Chl-a losses determined
309 by subtracting average daily Chl-*a* concentrations from the previous day's average values.
310
311 ***Statistical analyses***
312 Longitudinal, time-series, and regression plots were generated to examine general patterns in the flow,
313 water quality, phytoplankton, and zooplankton data, which guided further analyses. To focus on
314 differences between -EFF and +EFF parcels, unless otherwise mentioned, only samples collected
315 downstream from the WWTP (i.e. travel time > 0) were included in statistical tests. Upstream data were
316 evaluated to help characterize the initial conditions in the two parcels and to compare conditions
317 between the October and June transects.

To test for statistically significant differences between individual parameters measured in the presence rather than the absence of effluent, we ran a linear mixed-effects model using JMP software version 12.0 (SAS Institute, Inc. 2015). For these models, the parameter of interest (e.g., NH_4, Chl-*a*, cell biovolume) was the dependent variable, and parcel type (-EFF and +EFF) and date (October and June) were fixed factors, travel time in hours relative to passage past the effluent outflow pipe was a covariate, and a full factorial was applied to include all interaction terms. These analyses were run on log transformed data; for phytoplankton enumeration parameters that included zero values (i.e. no cells counted) data were log (x+1) transformed.

Multivariate statistical analyses were run on the phytoplankton and zooplankton enumeration data to test for differences in plankton composition using the computer software package PRIMER (Plymouth Routines In Multivariate Ecological Research, Version 6; Clarke and Gorley 2006). Potential differences in the phytoplankton species composition between -EFF and +EFF parcels, and between the 2013-2015 data from Stations R1 and R4, were tested using Analysis of Similarity (ANOSIM). Patterns in the phytoplankton assemblage data, particularly between the -EFF and +EFF parcels, were examined using non-metric multidimensional scaling (NMDS) ordinations of phytoplankton samples constructed from Bray-Curtis similarity matrices based on square-root transformed algal biovolume data. The ordination algorithm works iteratively to optimize a solution whereby samples having higher similarity are plotted close together and samples with lower similarity are plotted farther apart. Seriation tests for downstream trends in phytoplankton assemblage structure were also carried out on each of the four parcels (+EFF and -EFF for both experiments) to test whether possible community changes represent a systematic downstream change. Similar tests were performed on the 2013–15 data for Stations R1 and R4 (Figure 1) to test for possible differences between samples collected upstream and downstream from the WWTP.

Finally, to understand potential factors structuring the phytoplankton assemblages, available environmental data (river discharge, water velocity, DOC, NH_4, NO_3, NO_2, SRP, water temperature, and specific conductance) were examined for possible correlation with the phytoplankton species matrix using PRIMER's Bio-Env+Stepwise (BEST) procedure. Environmental data were log x+1 transformed and standardized to a normal distribution (mean of 0, standard deviation of 1) in PRIMER for these analyses. Through an iterative process, unimportant and redundant variables were removed until a final solution for 1 or more variables was obtained. The strength of each variable, or combination of variables, is represented by the Rho and associated P value. PRIMER determines the statistical significance of the selected combination of variables (global rho (R) statistic) or individual rho value using a Monte-Carlo permutation simulation. Results were considered significant when P values were < 0.05.

Zooplankton abundance data, which were available only for the June transects, and collected only once per day, were examined separately to observe relationships between phytoplankton biomass and total zooplankton density, as well as total density of cladoceran, copepod, and rotifer populations.

RESULTS

River conditions during wastewater-diversion experiments

This study took place during a drought year; flows in October 2013 were about 65% of average flow for this month between 1994-2014, and flows in June 2014 were about 45% of the average flow for this month over the same time period. Tidally averaged flows (~200 $m^3 s^{-1}$) and tidally averaged river velocities (~0.18 m s^{-1}) reported for Freeport were similar during the October and June experiments. Under these low-flow conditions, it took approximately 5 days for water to travel the 75 km study reach, and tidally driven flow reversals occurred upstream of the WWTP discharge location at Freeport (Figure 2; Fig. S-5). At Freeport, instantaneous river velocities ranged from -0.06 m s^{-1} to +0.40 m s^{-1}, whereas

farther downstream at Walnut Grove, instantaneous river velocities ranged from -0.11 m s^{-1} to +0.46 m s^{-1}. Water temperatures were significantly higher in June (~22˚C) than October (~16.5˚C, Fig. S-1).

Dissolved inorganic nutrient concentrations

Nutrient concentrations were on the lower range of, but comparable to, those previously reported for this section of the Sacramento River (Foe et al. 2010; Kratzer et al. 2011; Parker et al. 2012a; Glibert et al. 2014a,b). During the October and June experiments upstream of the WWTP, total DIN (NH_4 + NO_3 + NO_2) was 0.005–0.067 mg N L^{-1} (Fig. S-2), and SRP was 0.023–0.034 mg-P L^{-1} (Figure 3). Upstream average NO_3 concentrations were greater in October (0.043 ± 0.06 mg N L^{-1}) than June (0.011 ± 0.06 mg N L^{-1}), while average NH_4 concentrations were about 0.01 mg N L^{-1} in both October and June (Figure 3). Silica was > 5.6 mg-Si L^{-1}. Nitrogen concentrations in the American River were similar to those in the Sacramento River mainstem, whereas SRP concentrations were lower at about 0.006 mg-P L^{-1} during both transect dates. In June, upstream concentrations of DIN bracketed the 0.01 mg L^{-1} half-saturation constants 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) (Fig. S-2).

Effluent NH_4 concentrations were 33.8 ± 1.3 mg N L^{-1} (*n*=6) in October and 32 ± 1 mg N L^{-1} (*n*=6) in June. Although river flows were similar during the October and June transects, effluent concentrations in the +EFF parcels were 4.0% and 2.7% of the total river volume, respectively. In this regard, the October experiment tested double the average effluent concentration in the river.

Immediately downstream from the WWTP, NH_4 concentrations in the +EFF parcel increased to about 1.3 mg N L^{-1} in October and 0.8 mg N L^{-1} in June (Figure 3); the higher concentrations in October reflect the higher effluent content of the river. In addition to causing higher DIN concentrations, effluent discharges increased SRP (Figure 3), specific conductivity and DOC, and also lowered dissolved oxygen and

pH downstream of the WWTP (likely due to nitrification of NH_4 and decomposition of organic matter in the effluent). There was no significant effect of effluent on temperature or total suspended sediment (Table 1, Fig. S-1). As the +EFF parcel traveled downstream from Freeport, there was a general trend of decreasing NH_4, whereas NO_3 and NO_2 concentrations increased (Figure 3; Fig. S-2), likely reflecting the net result of nitrification of wastewater-derived NH_4, phytoplankton uptake and benthic release of inorganic nitrogen (Parker et al. 2012; O'Donnell 2014; Kendall et al. 2015).

In the absence of effluent, there were downstream increases in NO_3 and, to a lesser degree, an increase in NH_4 (Figure 3 and Fig. S-2). Neither specific conductance, DOC, nor any other in situ water-quality mapping data showed evidence of entrainment or mixing between +EFF and -EFF parcels (Fig. S-1).

Phytoplankton biomass—Chlorophyll-a and algal biovolume

Phytoplankton Chl-*a* was highest in the upstream part of the study reach where concentrations at the I-80 Bridge were 15 and 25 μg L^{-1} in October and June, respectively. Downstream at Freeport, concentrations were about 5 μg L^{-1}, and by the time water reached RM15 near Isleton, Chl-*a* had decreased to about 2 μg L^{-1} (Figure 4). These concentrations are comparable to those previously reported for this section of the river (Foe et al. 2010; Parker et al. 2012a; Glibert et al. 2014a,b); the high 25 μg L^{-1} measured at the I-80 bridge in June 2014 is similar to the 22 μg L^{-1} reported by Glibert et al. (2014b) during low-flow conditions in March 2014. The lower downstream Chl-*a* concentrations in the Sacramento River match the average values reported by California Department of Water Resources' long-term monitoring program. Since 1980, monthly Chl-*a* samples collected at RM 38.6 (Hood, Figure 1) have had consistently low Chl-*a*, with an average concentration of 2.2 μg L^{-1} (*www.water.ca.gov*).

Although Chl-*a* concentrations became progressively lower with downstream travel in both the -EFF and +EFF parcels on both dates, the most precipitous declines (> 50% loss in Chl-*a* d^{-1}) occurred between the I-80 Bridge and Freeport, upstream of the WWTP. Again, these declines have been

412 observed by other studies under various flow and nutrient conditions (Foe et al. 2010; Parker et al.
413 2012a; Glibert et al. 2014a,b). Downstream of the WWTP travel time was a significant factor affecting
414 Chl-*a* concentrations ($P = 0.007$), however there was no significant difference in Chl-*a* concentrations
415 between the +EFF and -EFF parcels ($P = 0.77$; Table 1).
416 Downstream from the WWTP, algal biovolume was significantly correlated with Chl-*a* ($P < 0.01$),
417 considering both experimental dates, more so in October ($P < 0.05$) than in June ($P > 0.05$). There was no
418 significant difference in algal biovolume between the +EFF and -EFF parcels downstream from the
419 WWTP (Table 1). Compared with Chl-*a* concentrations, cell biovolumes were more variable (Figure 4).
420 This may be related to the high variability in species composition (see below) and subsequent variations
421 in Chl-*a*:total biovolume ratios, even within a taxon. Declines in biovolume were also most notable in the
422 reach just upstream of the WWTP; a 2–3 fold decline occurred in the total algal biovolume of each
423 tracked parcel over the course of a single day. During the October experiment, 2 of 3 samples in the
424 +EFF parcel had notably high biovolume downstream from the WWTP, owing to the high abundance of
425 *Ulnaria ulna* (formerly *Synedra ulna* [Compère 2001]). This increase was not, however, observed in the
426 Chl-*a* concentrations and may represent an artefact caused by the high cell biovolume of *U. ulna* relative
427 to its Chl-*a* content.
428
429 ***Phytoplankton uptake of NO_3, NH_4 and C***
430 Potential carbon and nitrogen uptake measurements (^{15}N-NO_3, ^{15}N-NH_4, ^{13}C-DIC) were made on a subset
431 of samples collected in the +EFF and -EFF parcels. On the basis of prior uptake studies (e.g. Dugdale et al.
432 2007; Parker et al. 2012 a, b; Glibert et al. 2015), following the addition of wastewater derived NH_4, we
433 expected to see a shift in phytoplankton DIN uptake from predominantly NO_3 to NH_4. In October and
434 June, concentrations of NH_4 were low upstream of the WWTP outflow, and uptake of ^{15}N-NH_4 was low
435 at < 0.01 mg N L^{-1} d^{-1}. Following effluent addition, NH_4 uptake increased to 0.02–0.03 mg N L^{-1} d^{-1} and

436 stayed at similar levels for the remainder of the experiments during both sampling dates (Fig. S-3). These
437 rates are comparable to those reported by Parker et al. (2012a) in March and April 2009 for the river
438 above and below the WWTP.

439 In the -EFF parcel, potential ^{15}N-NH_4 uptake remained low (< 0.01 mg N L^{-1} d^{1}) downstream from
440 the WWTP in October. However, in June, the rate increased in the -EFF parcel below the WWTP to about
441 0.02 mg N L^{-1} d^{-1}, which is similar to rates observed in the +EFF parcel, despite much lower NH_4
442 concentrations (~0.1 vs >6.0 mg N/L; Figure 3).

443 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
444 the reach upstream of the WWTP discharge**.** Following effluent addition to the +EFF parcels, NO_3 uptake
445 rates dropped to near zero, which is consistent with observations by others that, in the presence of NH_4,
446 phytoplankton preferably take up NH_4 and NO_3 uptake is inhibited (e.g. Dugdale et al. 2007; Parker et
447 al. 2012 a, b; Glibert et al. 2014b; 2015). However, NO_3 uptake rates in the -EFF parcels traveling
448 downstream from the WWTP also showed a significant decrease, though not as immediate as in the
449 +EFF parcel. In October, NO_3 uptake dropped to near zero by the second day of travel past the WWTP.
450 In June, NO_3 uptake remained at about 0.015 mg N L^{-1} d^{-1} after 1 day of travel past the outfall and after
451 2–3 days was still measurable at about 0.002–0.009 mg N L^{-1} d^{-1}. The increase in NH_4 uptake rates and
452 decrease in NO_3 uptake rates even in the absence of effluent is likely attributable to the elevated NH_4
453 observed in both +EFF and -EFF parcels. The NH_4 may be entering the water column from other sources,
454 such as benthic release and/or degradation of organic N. By the time the -EFF parcels reached the most
455 downstream location, NH_4 concentrations increased to about 0.08 mg N L^{-1} (Figure 3).

456 Primary production as measured by C uptake showed no significant difference between the +EFF
457 and -EFF parcels (P value = 0.46; Table 1, Figure 4). Uptake rates, ρ, were about three fold greater in
458 June than in October, consistent with higher upstream Chl-*a* concentrations in June (Figure 2), greater
459 solar insolation, and higher water temperatures. Highest ρ values were measured at the most upstream

sites then declined downstream, consistent with declines in Chl-*a*. Assimilation numbers (C uptake normalized to Chl-*a* concentration, (Figure 4) showed the opposite trend in June; 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 much 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.

Phytoplankton species composition

Of the 275 algal taxa identified in the 57 samples collected during the October and June Lagrangian experiments diatoms accounted for about 90% of the total biovolume, followed by flagellate cryptophytes (7%, mostly *Rhodomonas* sp.) and some green algae (2%). Blue-green algae and chrysophytes contributed <1%. The most abundant diatoms included *U. ulna*, *Melosira* spp., *Cocconeis placentula*, *Thalassiosira* sp., and *Cyclotella* spp. (Table 2, Figure 5), similar to findings of prior studies in this region (Greenberg 1964; Ball and Arthur 1979; Lehman et al. 2008; Glibert et al. 2014a).

Benthic diatoms averaged 67% of the phytoplankton sample biovolumes (range 27–92%). These organisms tend to live on the bottom, attached to substrates (and aquatic vegetation), and dwell within or on top of bottom sediments (Porter 2008; Diatoms of the United States web page at https://westerndiatoms.colorado.edu/). Many of these benthic diatoms also fall under the classification of "facultative" planktonic diatoms, meaning they can exist and even thrive in the water column. Many were observed forming colonies (filaments, ribbons, and chains), which allows resuspension and entrainment into the water column when turbulence is sufficient (Hutchinson 1967, Reynolds and Descy 1996; Reynolds 2006). Prominent among these facultative planktonic diatoms was *U. ulna* (dominant in 50% of samples), which formed large multi-cellular colonies (Fig. S-8). Many of the *U. ulna* cells contained structures that indicate they were resting cells; perhaps these had recently

484 germinated from the sediments. Other facultative planktonic diatoms included taxa within the genera
485 *Aulacoseira*, *Bacillaria*, *Diatoma*, *Fragilaria*, *Melosira*, *Pseudostaurosira*, *Staurosira*, *Staurosirella*,
486 *Synedra*, and *Tabellaria*, and many of these have been shown to make resting cells or spores that allow
487 them to survive in and repopulate (inoculate) rivers from the sediments when conditions are
488 favorable—this
489 life-cycle trait thus avoids hydraulic "washout," a major source of loss for solely planktonic algae
490 (McQuoid and Hobson 1996).
491 Phytoplankton species composition was highly variable even among samples collected within a
492 parcel just a few hours apart, particularly with respect to *U. ulna* (Figure 5). During enumeration it was
493 noted that many samples also contained empty and broken diatom frustules (particularly those from *U.*
494 *ulna* but also *Diatoma*, *Gyrosigma*, and others) along with appreciable detritus and (or) sediment (John
495 Beaver, BSA Environmental Services, written commun. 2015). During the October 2013 experiment,
496 empty frustules composed nearly 40% of the total biovolume (equivalent) in some samples. Sediment
497 and empty frustules in the water column points to resuspension of these particles from the bottom,
498 which could be an important process affecting phytoplankton export to the estuary.
499 As described above, U*. ulna*, a large, long and narrow facultatively planktonic pennate diatom
500 (Fig. S-10), was often the most abundant alga in the lower Sacramento River, being dominant in about
501 50% of samples. *U. ulna* is ubiquitous and cosmopolitan in its distribution, growing among periphyton on
502 rocks (Porter 2008), as an epiphyte on aquatic vegetation, and in the plankton of large rivers (Reynolds
503 and Descy 1996). In qualitative plankton net samples, *U. ulna* colonies were generally healthier looking
504 in the upper part of the reach, with vibrant, expanded chloroplasts, and vesicles of polyphosphate and
505 lipid, resources stored for future growth (see photographs Fig. S-10 A-B). Farther downstream, more of
506 the cells appeared decrepit, with small microbes swarming within some of the partially empty frustules
507 (see photographs in Fig. S-10 C-D). Although the cause of this was not verified or investigated in any

detail, McQuoid and Hobson (1996) previously reported that rejuvenation of viable dormant diatoms is initially accompanied by the accumulation of lipids and polyphosphates, so our observations of energy-packed *U. ulna* colonies may reflect the occurrence of freshly germinated *U. ulna* cells in the upstream reach. This gradient in cell health was noticed with downstream travel in both the -EFF and +EFF parcels and mirrored the pattern in Chl-*a* and primary production (Figure 4). It is unclear what caused this apparent decline in health, but it may suggest light limitation, some kind of physiological change such as nutrient deficiency, or some other effect.

Samples collected upstream of the WWTP showed considerable variation in species composition during the October and June experiments (Figure 5). Although time of day, turbidity, water velocity, and other factors were queried to explain patterns in these variations, their effects on biovolume or species composition were not clear with our limited dataset. Although there was also high variability in species composition downstream from the WWTP, the ordination of samples produced a clear separation between the October and June samples (Figure 6), and ANOSIM revealed a significant difference between experiments ($P = 0.001$; Table 3). Based on these results, the October and June experiments were analyzed separately to test for possible effects of wastewater on the phytoplankton assemblages.

Separate ordination of the October and June phytoplankton samples showed much overlap in the -EFF and +EFF samples (Figure 6B-C), and ANOSIM found no statistically significant difference between the parcels ($P = 0.55$ and 0.30, respectively). Given the high variability among samples collected on the same day (Figure 5), additional ANOSIM runs were performed on the daily average species biovolumes to dampen the within-day variations. Nonetheless these comparisons were also non-significant for October ($P = 0.14$) and June ($P = 0.80$). Together these results suggest there were no statistical difference in phytoplankton species composition between the experimental parcels.

Results of the BEST analyses to identify potential factors (flow, velocity, and water-quality) structuring the phytoplankton assemblages indicate that only TSS in October (+EFF parcel, $P = 0.02$) and

532 water velocity at Walnut Grove in June (-EFF parcel, P = 0.02) were significant predictors of species
533 composition (Table S-1).
534
535 ***Phytoplankton biomass and species composition, 2013-2015***
536 Phytoplankton time-series enumeration data collected from 2013 to 2015 at Stations R1 (just upstream
537 of the WWTP) and R4 (several km downstream of the WWTP) helped to put our Lagrangian experiments
538 into context and showed that, for example, the October and June experiments occurred during periods
539 when the river supported intermediate-size phytoplankton populations, not as large as the blooms of
540 early 2014 but larger than many other times of the year (Figure 7). In addition, the longer-term data
541 show a period of relatively abundant, although potentially declining, population of *U. ulna* during the
542 two experiments.
543 Minimal differences in Chl-*a* concentration were observed between Stations R1 and R4 despite
544 the input of treated wastewater effluent between these two stations (Figure 1). Chl-*a* concentrations
545 were typically between 1 and 5 μg L^{-1}, except in early February 2014 and mid-April 2014 and 2015 when
546 concentrations increased to 10–15 μg L^{-1} (Figure 2). Higher Chl-a concentrations were attributed to small
547 centric diatoms (*Cyclotella spp.*, *Cyclostephanos invisitatus*) (Fig. S-8) and larger facultative planktonic
548 diatoms (*Melosira* spp. and *U. ulna*) (Figure 5).
549 Benthic diatoms typically accounted for most of the total biovolume at both the R1 and R4 sites,
550 and facultative planktonic species dominated 58% of the samples at R1 and 52% at R4 (Fig. S-9).
551 Ordination of phytoplankton samples produced substantial overlap between the two sites, and ANOSIM
552 found no statistically significant differences in algal assemblages based on biovolume or density (P >0.9
553 for both), although once again a high degree of variability, particularly in the total biovolume but also in
554 species composition, was seen at both sites (Figure 7). For example, peaks in the biovolume of
555 *Cyclostephanos invisitatus* were measured at R4 but not R1 in January, and two weeks later at R1 but

not R4. At R1 the abundance of *Melosira* spp. during March and April 2014 was highly variable, and occasional spikes in green algae (e.g., *Spirogyra* sp.) were also common. This degree of variability could mask statistical detection of species composition differences between these sites.

BEST analyses identified instantaneous streamflow as the single influential variable structuring algal assemblages in the Sacramento River at Freeport during 2013–15, but it was not significant (P = 0.07). No other variable or combination of variables produced a significant correlation. ANOSIM tests for potential differences in the phytoplankton assemblages between Station R1 (-EFF) and R4 (+EFF) was non-significant (P = 0.87). There were, however, significant trends over time at both sites (seriation trends were P = 0.003 for Station R1 and P = 0.001 for Station R4), indicating seasonal trends over time that are probably related to the annual growth cycles of algal populations.

Zooplankton data, clam data, and estimated grazing losses

Zooplankton biomasses measured in June 2014 were highly variable: biomass in the +EFF parcel ranged from 7–12 μg L^{-1}, whereas the -EFF parcel, which was sampled at almost the same sites just 24 hours later, had one-third of the biomass, ranging from 1.5 to 3 μg L^{-1} (Figure 8). Samples collected 2 weeks later during the clam survey, under normal effluent discharge, had biomass concentrations similar to those in the -EFF parcel. All of these transects showed a longitudinal decline in the rotifer population, with an increasing proportion of copepods and cladocerans, from upstream to downstream (Fig. S-10).

Corbicula fluminea was the only bivalve species observed in the benthic trawl surveys and was distributed patchily across the river's width. *C. fluminea,* an invasive species that entered the Delta in the 1940s, is known to be widespread in freshwater, tidal reaches of the Delta and has been shown to have a strong effect on phytoplankton biomass in shallow freshwater areas (Lopez et al. 2006; Lucas and Thompson 2012; Kimmerer at al. 2014). To our knowledge this was the first time that quantitative sampling was conducted this far upstream in the Sacramento River. 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). In general, clam biomass was patchy; it was highest in the upstream and downstream regions sampled, although maximum densities occurred in the middle of the study reach (Figure 8).

Zooplankton data were only collected in June 2014, thus we only compared measured Chl-a losses to estimated losses due to grazing for the June Lagrangian experiment (Table 4). Measured Chl-*a* losses (the percent change in average Chl-*a* concentrations between days) in June were greatest in the upstream reach (RM 63 to 45) during the first 3 days of downstream travel; the rate of loss was about 60% of the standing biomass per day. Losses during the subsequent days (RM 45 to 24) were much less, representing 34% and 19%, respectively, of the standing biomass. By RM24, 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 there was a small gain in Chl-*a* at 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 4). 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.

DISCUSSION

Phytoplankton abundance and composition in the presence and absence of wastewater

Our study found that wastewater effluent and its attendant high NH_4 concentrations were not directly related to the progressive decline in phytoplankton abundance in this stretch of the Sacramento River. We found no significant differences in Chl-*a* or algal biovolume between parcels which contained effluent and those that were effluent free, and the largest declines in phytoplankton (> 50% loss per day) occurred well upstream of the WWTP where effluent was not present (Figure 3, Table 1). Similar declines in Chl-*a* have been previously measured in this reach upstream of the WWTP under different conditions of flow, Chl-*a* and nutrient concentrations (Foe et al. 2010; Parker et al. 2012; Glibert et al. 2014a,b).

Effluent addition to the river also did not result in statistically significant differences in the phytoplankton community composition compared with effluent free conditions during either the October 2013 or June 2014 Lagrangian experiment (Figure 6, Table 3). Although this comparison may be confounded by the high spatial and temporal variability in phytoplankton populations that we observed in this study (Figure 5 and Table 2), the high variability is in itself notable because it indicates that processes other than water quality are important factors affecting phytoplankton species composition (see below).

Studies that link elevated NH_4 concentrations to depressed phytoplankton biomass and declines in primary productivity often point to inhibition of NO_3 uptake (Yoshiyama and Sharp 2006; Glibert et al. 2015; Wilkerson et al. 2015 and references therein). Although a shift in phytoplankton DIN uptake from NO_3 to NH_4 was observed following effluent addition of NH_4, we did not find a corresponding decline in primary productivity (Figure 4). In fact, higher rates of C uptake per Chl-*a* occurred as water moved downstream, suggesting phytoplankton production was not physiologically impaired by the presence of

effluent when riverine NH_4 concentrations were high (0.6 – 1.4 mg L^{-1}) . Moreover, the largest declines in phytoplankton abundance observed upstream of the WWTP under low NH_4 concentrations occurred over short periods (~8 hours), indicating that they are attributable to direct *losses* of phytoplankton and not *inhibition* of growth. Similar conclusions were made by Foe et al. (2010) based on data collected along this river reach in 2009 and 2010.

The similarity in C uptake in the presence versus absence of effluent also suggests that other contaminants in wastewater, such as pharmaceuticals or pesticides, are not responsible for observed declines in phytoplankton in this stretch of the river. 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_4Cl, KNO_3, or effluent (Travis 2015). Moreover, in the enclosure experiments Chl-*a* concentrations increased over 5 days under these amendments, whereas they decreased in the river, underscoring the conclusion that in the river losses overwhelm biomass production due to factors not represented in the enclosures.

Other factors shaping phytoplankton abundance and species composition

Many studies have found that a variety of physical, chemical, and biological drivers combine in various ways to affect phytoplankton abundance and species composition (e.g., Vannote et al. 1980; Cloern and Dufford 2005; Scherwass et al. 2010; Lucas et al. 2009; Cloern et al. 2014a). Because declines in phytoplankton abundance were not explained by effluent additions and its attendant NH_4, we examined other available data to gain insight into the importance of grazing, hydrodynamics, and light.

Grazing Pressure: Grazing by invasive clams and mussels has been identified as a primary factor responsible for decreases in phytoplankton biomass in aquatic systems worldwide (Cohen et al. 1984; Pigneur et al. 2014, Karatayev et al. 2015), and grazing losses by zooplankton may be of comparable importance (Kimmerer and Thompson 2014). To examine whether estimated losses to grazing or

respiration could reasonably account for the losses of Chl-*a* observed during our experiments, we compared these estimated losses to measured Chl-*a* losses during June 2014; grazing losses in October were expected to be lower due to lower water temperatures. 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. Respiration losses were assumed to be 1.5% of the phytoplankton population's biomass (Jassby et al. 2002). Overall, estimated phytoplankton biomass losses owing to zooplankton, clams, and respiration accounted for less than 30% of the observed Chl-*a* losses (Table 4). The percent loss to grazing would be even lower if phytoplankton growth were taken into account.

These loss estimates do not take into account microzooplankton grazing that have been suggested to consume as much as half of the Chl-*a* standing stock per day (Calbet and Landry 2004; Kimmerer and Thompson 2014). However, these grazing rate estimates were developed under laboratory conditions and thus may overestimate in situ rates. Future studies which verify the accuracy of microzooplankton grazing rates under different environmental conditions are needed (York et al. 2013).

Hydrodynamics: It is likely that the change in hydrodynamics from lotic to tidal conditions along this river reach impacts both phytoplankton abundance and composition. Characteristics like velocity, residence time, flushing rates, water depths, and turbulence all vary in direct response to changes in discharge, and all have a major effect on phytoplankton populations (e.g. Wetzel 1983; Reynolds and Descy 1996; Reynolds 2006). Lower water velocities result in a longer residence time, which provides more time for phytoplankton to grow and multiply but also increases exposure to grazers and other types of losses (Lucas and Thompson 2012). The effects of bidirectional flow 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 species within the phytoplankton population in the water column suggests that hydrodynamic factors may affect species composition. Although many single-celled benthic species are large and would be expected to sink, colony formation into filaments and chains—common in the Sacramento River plankton—fosters resuspension when turbulence is sufficient (Hutchinson 1967, Reynolds and Descy 1996; Reynolds 2006). The tendency to sink, combined with an ability to survive in sediments, helps these species avoid downstream advection or "washout" during higher flows (Rounds et al. 1999). Their persistence in the sediment also gives these benthic species opportunities to subsequently inoculate the water column (McQuoid and Hobson 1996; Reynolds 2006).

In contrast, some of the features that likely favor benthic species can also provide avenues for their losses once they enter the tidal reach where instantaneous flows are variable and eventually include slack periods and tidal reversals. These conditions favor settling of the larger particles to the bottom where they may be ingested by clams (Lucas and Thompson 2012; Lucas et al. 2016). Larger and heavier benthic species, which dominated the Sacramento River phytoplankton biomass, are reliant on turbulent flow for transport and are particularly susceptible to settling losses, especially during slack tide. 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 measureable decreases in turbidity at Walnut Grove (RM 32) frequently occurred during slack tides (Fig. S-7). The apparent decline in the health of *U. ulna* with downstream travel, in both the presence and absence of effluent, also suggests conditions for these facultative planktonic benthic species were becoming less favorable farther into the tidal reach (Fig. S-8). These same processes may explain the observed decline in zooplankton abundance (Fig. S-10).

The highly variable, patchy nature of both phytoplankton and zooplankton populations in the Sacramento River also suggests that physical dynamics, which vary over short periods (i.e., hours) play a

key role. In addition to the periodically high abundances of *U. ulna* colonies, the presence of empty frustules and associated silt particles observed in cell counts point to resuspension of diatoms from the riverbed, a process that could contribute to the observed variability in assemblages, particularly considering the tidal effects. The presence of benthic organisms in the plankton, such as *Acanthocyclops vernalis*, an exclusively benthic copepod species, along with very large benthic diatoms (*Surirella* and others) is also consistent with sediment resuspension processes.

Light: Another possibility is that reduced light availability, resulting from an increase in river depth, may limit photosynthesis. Light availability is often cited as the main factor limiting phytoplankton growth, and light limitation has historically been linked to low primary production in the Delta compared to other estuaries (e.g., Alpine and Cloern, 1988; Jassby 2008; Cloern et al. 2014a). In the Sacramento River, light availability to aquatic organisms is determined primarily by turbidity, water depth, and mixing. Over the last few decades there has been an overall decrease in turbidity in the Delta without an observed increase in Chl-*a* (Schoellhamer et al. 2013; Hestir et al. 2013), suggesting light limitation is not the primary reason for observed long-term declines in phytoplankton production (Schoellhamer 2011). In this study, there was no evidence that effluent inputs from the WWTP affect TSS concentrations or turbidity, and if anything, TSS declined with downstream transport (Table 2; Figure S-1). However, a change in water depth can effectively change light availability by altering the percentage of the water column that is in the photic zone. Water depths upstream of the I-80 Bridge are generally <5 m, but downstream the river deepens to well over 8 m in many locations. An increase in water depth, particularly in a well-mixed, unstratified large river, would reduce the amount of time algal cells spend in the photic zone, which may lead to light limitation (Wetzel, 1983). This could also explain the loss of Chl-*a* along the study reach.

Controls on nitrogen

This study provided a unique opportunity to examine differences in nutrient concentrations in the presence versus absence of effluent nitrogen inputs. During the October 2013 and June 2014 Lagrangian experiments, effluent from the WWTP directly contributed the bulk of DIN to the river. However, we observed monotonic increases of NO_3 and NH_4 to the river downstream from the WWTP in the absence of effluent (Figure 3A-B). The gradual increase in NO_3 and NH_4 measured in the -EFF parcel with downstream transport, rather than a step increase associated with a particular location, suggests these nutrients likely have a non-point source origin. The most likely source is release from the benthos. Positive efflux of NO_3 from the benthos in the Sacramento River and the Delta has been reported (Kuwabara et al. 2009; Cornwell et al. 2014), and NH_4 oxidation by benthic microbes likely plays a role in this process (Damaschek et al. 2016). Studies have also shown that the presence of bivalves, including *C. fluminea*, can drive benthic DIN efflux both directly through excretion of their waste and indirectly by increasing rates of microbial mineralization (Zhang et al. 2011; Tureck and Hoelein 2015).

The increase in NH_4 concentrations in the river in the absence of effluent during both the October and June experiments are of particular interest because concentrations neared or exceeded the approximate threshold of 0.01 - 0.05 mg N L^{-1}, that has been reported to inhibit NO_3 uptake (Dugdale et al. 2007). Although our data suggest the NH_4 and NO_3 being released into the water column are not associated with recent effluent inputs, because there is no evidence of mixing with +EFF water, it is likely that this N is a product of high N loading to the system over time from WWTP discharges. This is supported by the fact that there were low concentrations of NO_3 and NH_4 just upstream of the WWTP (Figure 3). Note that conditions in the -EFF parcel represent water that is not affected by recent effluent inputs, but is likely still affected by the long-term high nutrient inputs to this river reach. In the future, when nutrients discharged from the WWTP are reduced, the resupply of nutrients to the benthos will likely diminish, thus altering the amount of N released from the benthos.

747

748 ***Implications and Future Studies***

749 The finding that temporary elimination of wastewater effluent discharges to the Sacramento River did
750 not alter phytoplankton productivity rates or community composition suggests that future WWTP
751 upgrades – nitrification and denitrification resulting in reduced NH_4 discharges – are unlikely to lead to
752 greater phytoplankton export from the Sacramento River. However, this study did not test how effluent
753 inputs dominated by NO_3 might affect phytoplankton dynamics; it is possible that under higher NO_3
754 concentrations, expected with the WWTP upgrade, there will be greater phytoplankton growth rates
755 that could off-set losses, particularly during periods when upstream DIN concentrations limit growth
756 (Dugdale et al. 2013). Likewise, this study did not test how a reduction in NH_4 inputs will affect
757 phytoplankton dynamics farther downstream in the estuary that experience longer residence times,
758 more complex hydrodynamic and light conditions, and more grazing pressure compared with the
759 upstream river. A number of studies predict that a shift to a NO_3-dominated system without inhibitory
760 NH_4 concentrations will promote the growth of phytoplankton in the northern Estuary (Wilkerson et al.
761 2015 and references therein). However, future upgrades to the WWTP will not only shift the
762 predominant source of DIN from NH_4 to NO_3, but will also substantially lower effluent DIN
763 concentrations by > 65% (O'Donnell 2014). Considering the Sacramento River is a major source of N to
764 the Delta and the northern Estuary, there is increased potential for some downstream regions to
765 become nutrient limited during phytoplankton blooms. Monitoring water quality and biological
766 populations in the water column and benthos before and after the WWTP upgrade would enhance our
767 understanding of how river-estuary ecosystems respond to step change in N form and concentration.
768 The loss of phytoplankton during transport and the dominance of the phytoplankton community
769 by benthic species also has ramifications for downstream food webs; although benthic diatoms are
770 generally considered a good source of high quality food for zooplankton and other planktivores, they

771 may settle out of the water column, reducing food available to pelagic species and providing increased
772 food to the benthic community, including clams (Lucas et al. 2016). Signs of hydrodynamic effects on
773 particles were apparent in the longitudinal pattern of phytoplankton biovolumes during our study, and
774 other indicators such as turbidity at the Walnut Grove continuous monitoring station (described above)
775 suggest that low water velocities promote settling of particles. Future studies could examine
776 hydrodynamic affects with a focus on phytoplankton sedimentation and resuspension under various
777 flow conditions, with particular emphasis on the effects of tidal cycles and slack periods. This
778 information can be used not only to model sedimentation and resuspension but also to improve
779 estimates of grazing rates by benthic filter feeders and zooplankton (Sluss et al. 2008; Lucas et al. 2009;
780 Lucas and Thompson 2012).

781 This study took place during a period of drought conditions when streamflows and water
782 velocities in the Sacramento River were below average. This resulted in strong tidal effects, including
783 flow reversals extending upstream of Freeport. Changes in flow and sources of water in the upstream
784 part of the river may also have affected the phytoplankton inocula (amount and type) entering the
785 stream reach. A recent study in the Missouri River found that long-term hydrologic conditions can have
786 profound effects on plankton biomass and species assemblages (Beaver et al. 2012), which the authors
787 postulated was related to in-stream sedimentation. Continued monitoring of phytoplankton during dry
788 and wet periods will allow resource managers to better understand the inter-annual variability in algal
789 production leading to more realistic expectations of how populations may change given different
790 management actions.

791 Although laboratory studies can provide many valuable insights into controls on phytoplankton
792 production, our results highlight the need to include more variable and dynamic factors before
793 extrapolations can be made to the complex and dynamic nature of real world conditions (Cloern et al.
794 2014b; Esparza et al., 2014). This study demonstrates the benefits gained from conducting in situ

Lagrangian-based sampling combined with large-scale river manipulations to shed light on the dynamic and complex interplay between physical, chemical, and biological factors.

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FIGURE CAPTIONS

Figure 1. Map of the Sacramento River including the study reach for Lagrangian experiments. Sacramento Regional Wastewater Treatment Plant's (SRWTP) effluent outflow location is indicated by the red arrow. Stations R1 (RM46.4) and R4 (RM 44.4), where the SRWTP collected monthly samples, are indicated in green. +EFF and -EFF indicate effluent containing and effluent-free sampling locations, respectively. Numbers in parentheses indicate the river mile (RM).

Figure 2. Instantaneous discharge, tidally filtered discharge, and phytoplankton chlorophyll-*a* in the Sacramento River, California, 2013-15, and the timing for the Lagrangian experiments (red arrows). Tidally filtered discharge (dark blue) and instantaneous discharge (light blue indicates downstream, light red indicates upstream). Flow data are from the U.S. Geological Survey continuous monitoring station at Freeport (11447650). Laboratory chlorophyll-*a* (Chl-*a*) data (green symbols) are reported by Sacramento Regional County Sanitation District for station R1 (RM46.4) and R4 (RM44.4).

Figure 3. Nutrient concentrations including (A) NH_4 (B) NO_3, and (C) soluble reactive phosphorus (SRP) during the October 2013 and June 2014 Lagrangian experiments plotted in relation to travel time where zero time indicates passage past the wastewater treatment plant (WWTP). +EFF and -EFF indicate effluent containing and effluent free parcels, respectively. Oct, October; AmR, American River; I80 Br, Interstate-80 Bridge.

Figure 4. Algal population indicators including (A) chlorophyll-*a* (Chl-*a*), (B) total algal biovolume, (C) carbon uptake, and (D) assimilation number during the October 2013 and June 2014 Lagrangian experiments plotted in relation to travel time where zero time indicates passage past the wastewater treatment plant (WWTP). +EFF and -EFF indicate effluent containing and effluent free parcels, respectively. Oct, October; AmR, American River; I80 Br, Interstate-80 Bridge.

Figure 5. Downstream pattern in phytoplankton assemblage during the October 2013 and June 2014 Lagrangian experiments, including conditions upstream from the experimental reach and in the effluent free (-EFF) and effluent containing (+EFF) parcels. Vertical lines separate days. Note different scale for y-axis in the upstream sites plots for June. AR, American River.

Figure 6. Ordinations of phytoplankton assemblages in the Sacramento River, California, tested for differences (A) between October 2013 and June 2014 samples, (B) October 2013 samples between effluent containing (+EFF) and effluent free (-EFF) parcels, and (C) June 2014 samples between +EFF and -EFF parcels.

Figure 7. Seasonal patterns in phytoplankton in the Sacramento River, California, 2013-15, showing biovolume of major algal groups at (A) Freeport (R1) and (B) RM44 (R4). Algae groups are mutually exclusive, and although *Ulnaria ulna* and *Melosira* spp. are both facultative planktonic diatoms, they are not included in the "Other facultative planktonic diatoms" group. Red arrows and dashed lines indicate October 2013 and June 2014 Lagrangian-based experiments. Tidally averaged discharge at Freeport is also shown in panel A. RM, river mile.

Figure 8. Zooplankton and clam (*Corbicula fluminea*) biomass data collected for the Sacramento River, California, June 2014. Error bars indicate standard error. +EFF, effluent containing parcel; -EFF, effluent free parcel.

Table 1. Statistical comparison of the data associated with the effluent containing (+EFF) and effluent free (-EFF) water parcels tracked during the October 2013 and June 2014 Lagrangian experiments (Sacramento River, CA) using analysis of variance (ANOVA).

Parameter	sample number (*n*)	Date (October, June)	Parcel (+EFF, -EFF)	Travel Time	Date x Parcel	Date x Travel Time	Parcel x Travel Time	Date x Parcel x Travel Time
NH_4	*44*	0.1006	**<0.0001**	**0.0352**	0.0511	0.9472	**0.0041**	0.8919
NO_3	*44*	**<0.0001**	**<0.0001**	**<0.0001**	**0.0181**	0.9105	**0.0096**	0.0936
Chlorophyll-*a*	*39*	0.0701	0.7725	**0.0007**	0.2257	0.6578	0.4732	0.7153
Total Algal Biovolume	*37*	0.8275	0.2031	0.1541	0.7977	0.8922	**0.0285**	0.7816
Total Algal Density	*37*	0.7267	0.1682	0.1440	0.6914	0.4878	**0.0184**	0.8159
Total Suspended Sediment	*44*	**<0.0001**	0.2434	0.7934	0.6570	0.5214	0.3272	0.2913
Uptake ρ NH_4	*19*	**0.0002**	**0.0014**	0.7009	0.1159	0.5507	0.9507	0.2302
Uptake ρ NO_3	*19*	**0.3835**	**0.0104**	0.5200	**0.0119**	0.5989	0.1035	0.7577
Uptake ρ C	*19*	**<.0001**	**0.4624**	**0.0480**	0.7075	0.2377	0.9444	0.7178

Table 2. Average biovolume data ($\mu m^3 L^{-1}$) for the most abundant phytoplankton taxa in effluent containing (+EFF) and effluent free (-EFF) water parcels tracked during the October 2013 and June 2014 Lagrangian experiments in the Sacramento River, California.

		October 2013		June 2014	
Algal taxa	**Taxon description/growth habit**	**-EFF**	**+EFF**	**-EFF**	**+EFF**
*Ulnaria ulna**	Facultative planktonic diatom	308,370,263	390,194,854	280,704,939	150,029,557
Melosira varians	Facultative planktonic diatom	24,639,552	15,540,499	116,695,906	259,993,617
Rhodomonas spp.	Planktonic Cryptophye	76,024,031	52,441,777	23,980,037	27,398,572
Cocconeis placentula	Benthic diatom	27,840,894	12,949,966	43,205,068	26,934,440
Bacillaria paxillifer	Facultative planktonic diatom	67,629,841	34,427,066	4,541,892	876,708
Cyclotella meneghiniana	Planktonic diatom	102,975	551,757	71,443,203	18,286,836
Pseudostaurosira brevistriata	Facultative planktonic diatom	15,294,924	19,409,934	31,790,466	21,484,773
Cyclotella spp.	Planktonic diatom	29,562,102	35,131,325	0	1,194,618
Diatoma vulgaris	Benthic diatom	14,334,320	24,286,966	13,867,476	12,661,170
Aulacoseira granulata	Facultative planktonic diatom	0	4,797,372	1,042,011	54,821,499
Thalassiosira sp.	Planktonic diatom	7,764,993	9,851,573	16,647,653	17,951,472
Gyrosigma sp.	Benthic diatom	3,721,995	23,467,042	11,146,383	3,368,858
Cyclostephanos invisitatus	Planktonic diatom	26,108,733	9,833,098	0	0
Fragilaria crotonensis	Facultative planktonic diatom	1,809,745	0	27,104,444	6,709,046
Gomphoneis minuta	Benthic diatom	0	29,040,591	1,673,108	1,528,668
Cymbella mexicana	Benthic diatom	16,384,586	2,929,228	11,157,241	0
Anabaena sp.	Planktonic blue-green alga	0	0	1,321,801	28,866,730
Chlorella minutissima	Planktonic green alga	14,573,499	11,093,371	0	0
Navicula capitatoradiata	Benthic diatom	9,490,168	3,866,254	5,155,760	6,780,944
Surirella sp.	Benthic diatom	20,566,420	2,519,315	--	730,044
Diatoma moniliformis	Benthic diatom	2,243,242	1,557,171	10,583,749	8,272,079
Cryptomonas erosa	Planktonic Cryptophye	895,964	705,157	7,109,351	5,701,300
Aulacoseira alpigena	Facultative planktonic diatom	7,769,412	2,128,622	4,000,823	177,995

* Formerly called *Synedra ulna*

Table 3. Statistical comparison of the data associated with the effluent containing (+EFF) and effluent free (-EFF) water parcels tracked during the October 2013 and June 2014 Lagrangian experiments (Sacramento River, CA) using analysis of similarity (ANOSIM).

Experiment/ Time period	ANOSIM tests	Rho value	P value	Sample number (*n*)
October and June	Algal biovolumes, between synoptics	0.486	0.001	34
October	Algal biovolume, between +EFF and -EFF parcels	-0.016	0.552	20
June	Algal biovolume, between +EFF and -EFF parcels	0.056	0.3	14
October	Percent algal biovolume, between +EFF and -EFF parcels	0.002	0.428	20
June	Percent algal biovolume, between +EFF and -EFF parcels	0.029	0.374	14
October	Algal cell density, between +EFF and -EFF parcels	0.026	0.242	20
June	Algal cell density, between +EFF and -EFF parcels	0.045	0.298	14
2013-15	Algal biovolume, between Freeport and RM 44	-0.019	0.88	32
2013-15	Algal cell density, between Freeport and RM 44	-0.026	0.98	32

Table 4. Observed and estimated chlorophyll-*a* losses for the June 2014 Lagrangian experiments in the Sacramento River, California.

Experimental Day	Day0*	Day1	Day2	Day3	Day4	Day5
River Mile	**63.0**	**55.6**	**44.6**	**33.7**	**23.5**	**18.0**
River Depth (m)	**2.3**	**6.4**	**6.7**	**7.3**	**7.1**	**4.0**
Measured Chl-*a*						
Chl-*a* Concentration ($\mu g\ L^{-1}$)	25.5	13.4	5.8	3.8	3.1	3.4
Chl-*a* Losses ($\mu g\ L^{-1}\ d^{-1}$)	**15.7**	**7.7**	**2.0**	**0.7**	**-0.3**	
Estimated Chl-*a* Losses ($\mu g\ L^{-1}\ d^{-1}$)						
clam grazing	4.60	0.50	0.19	0.05	0.12	
mesozooplankton grazing **	no data	0.11	0.20	0.12	0.12	
respiration	0.38	0.20	0.09	0.06	0.05	
Total Estimated Chl-*a* loss ($\mu g\ L^{-1}\ d^{-1}$)	**4.98**	**0.81**	**0.48**	**0.22**	**0.29**	

*Day 0 conditions estimated from samples collected at I80 bridge (RM63). Travel time from this location to the Day 1 sampling reach (12 km) was estimated to be 0.77 d; loss rates estimated over 24 hours (d^{-1})

**mesozooplankton grazing rates were estimated from zoolankton biomass associated with the June 2014 +EFF parcel.

FIGURES

Controls on riverine phytoplankton dynamics in the presence and absence of treated wastewater effluent high in ammonium—A Lagrangian based study

Tamara E.C. Kraus[1*], Kurt D. Carpenter[2], Brian A. Bergamaschi[1], Alex Parker[3], Elizabeth B. Stumpner[1], Bryan D. Downing[1], Nicole M. Travis[4], Frances P. Wilkerson[4], Timothy D. Mussen[5]

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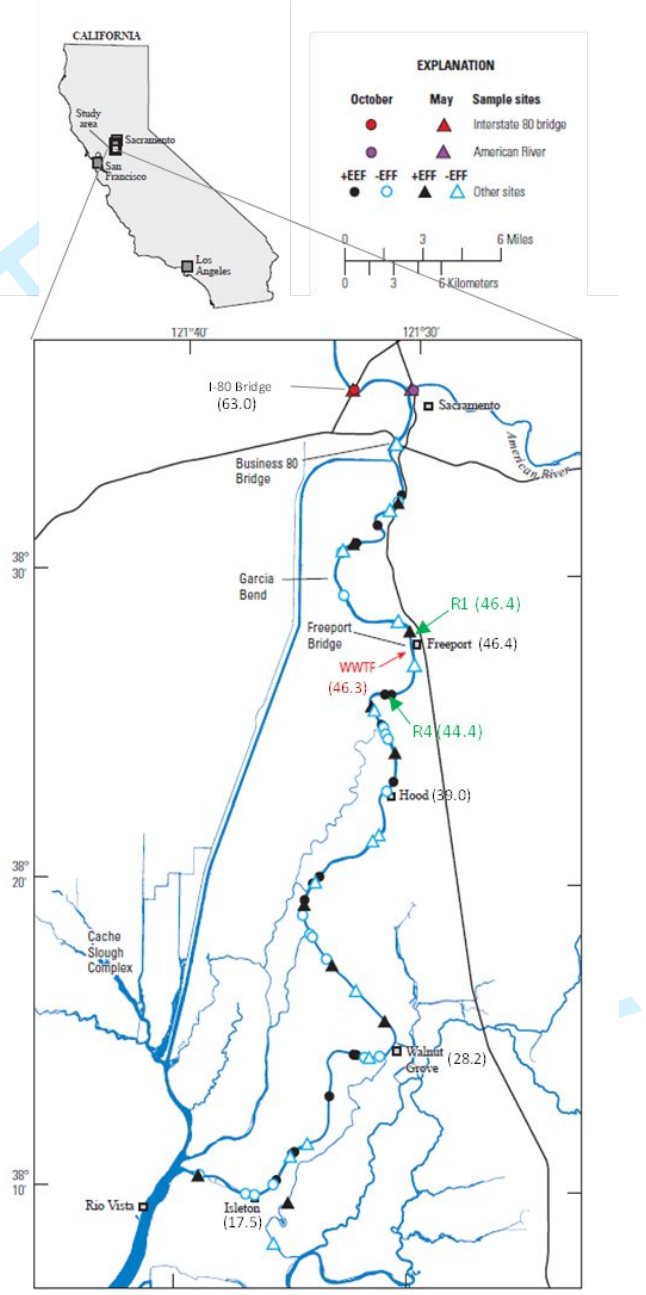

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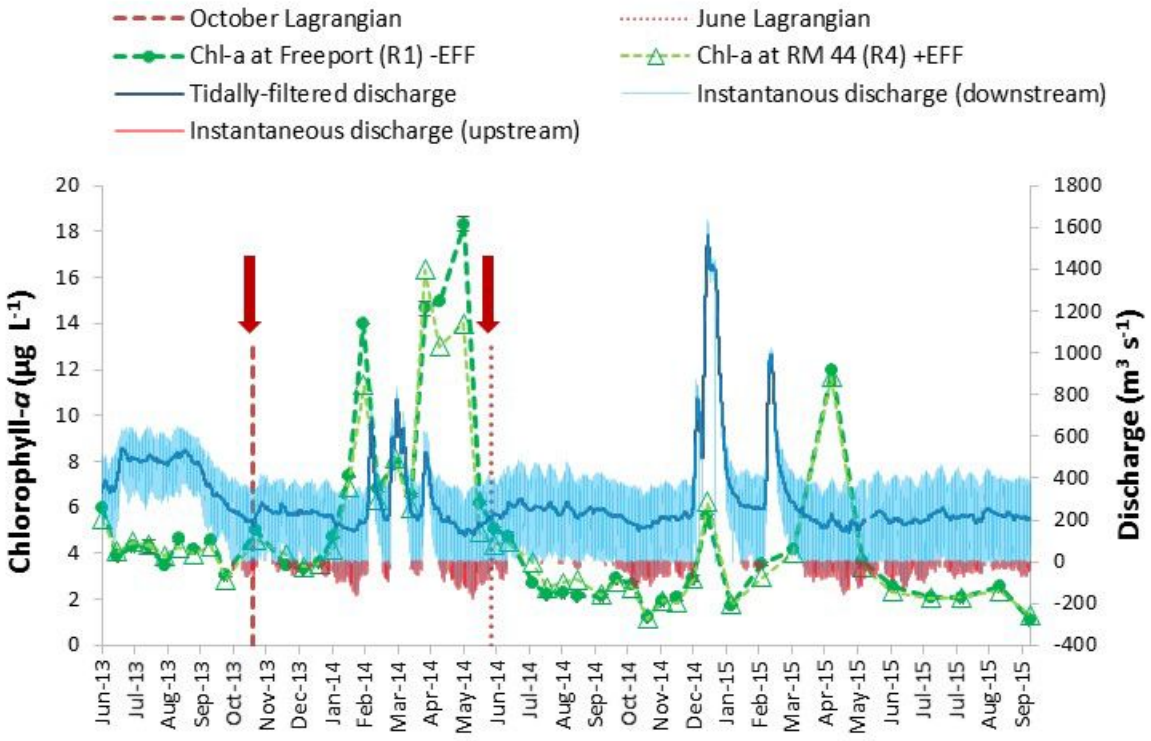

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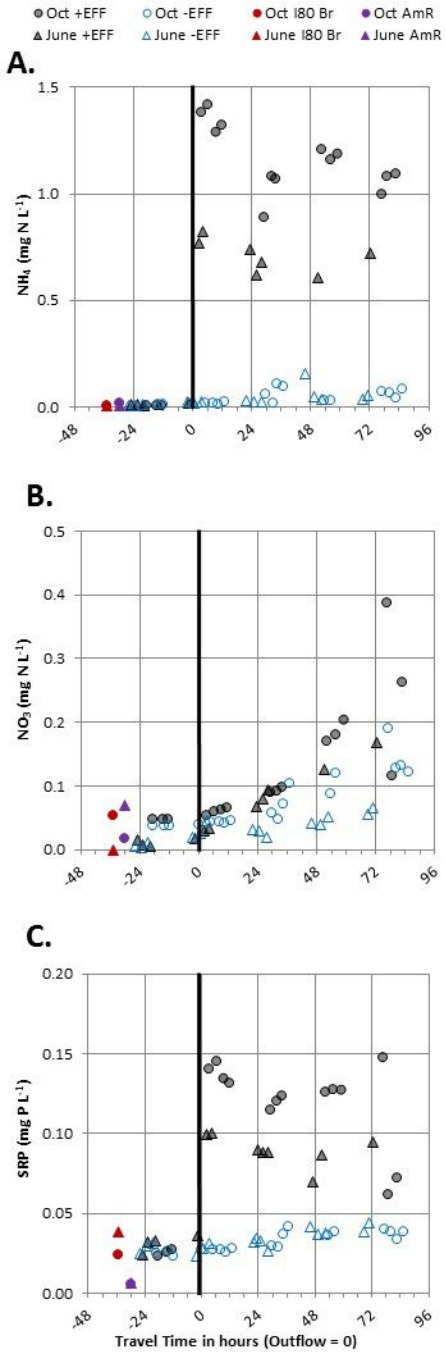

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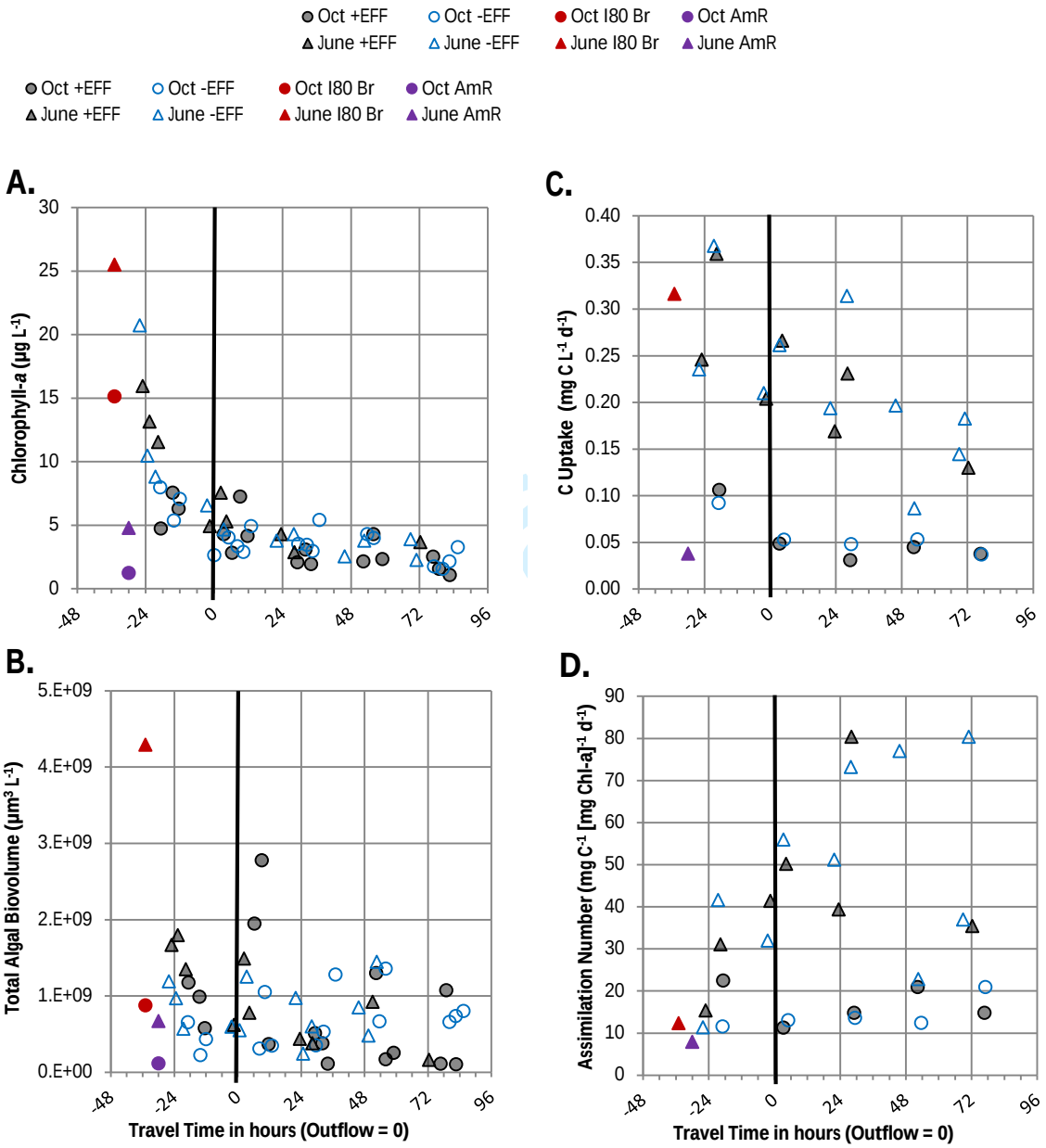

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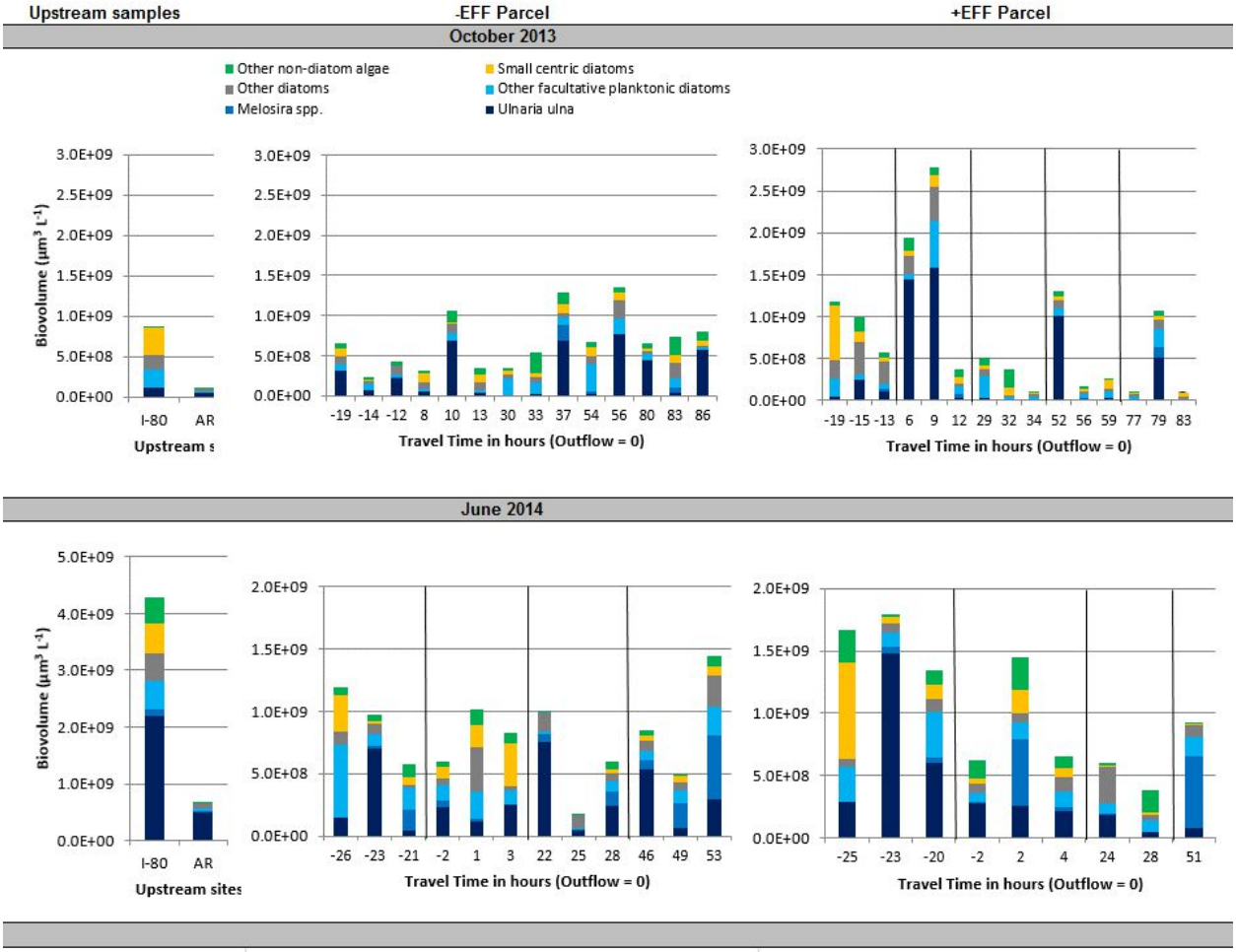

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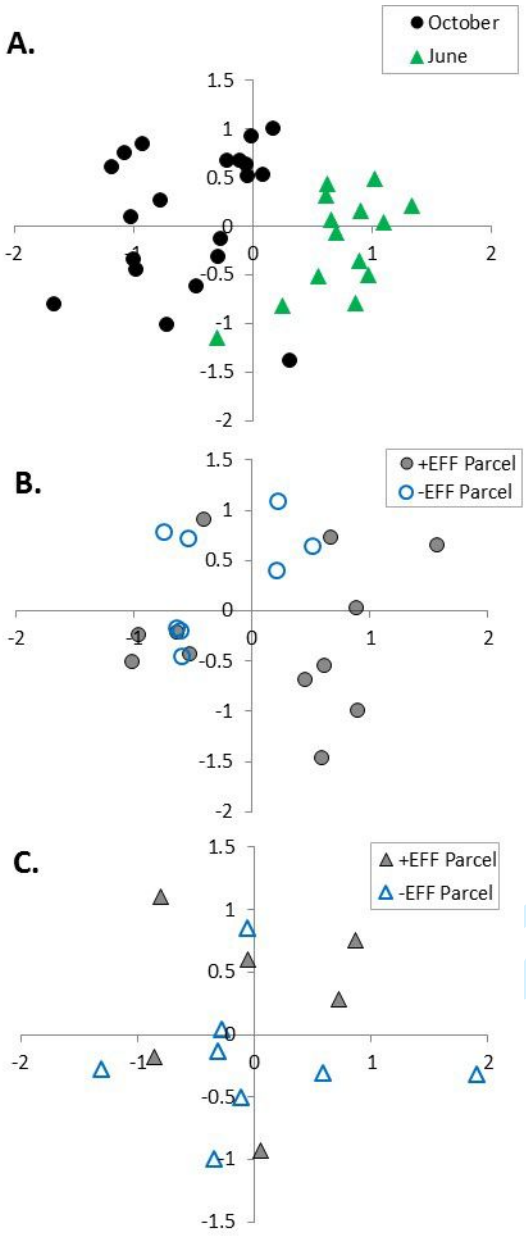

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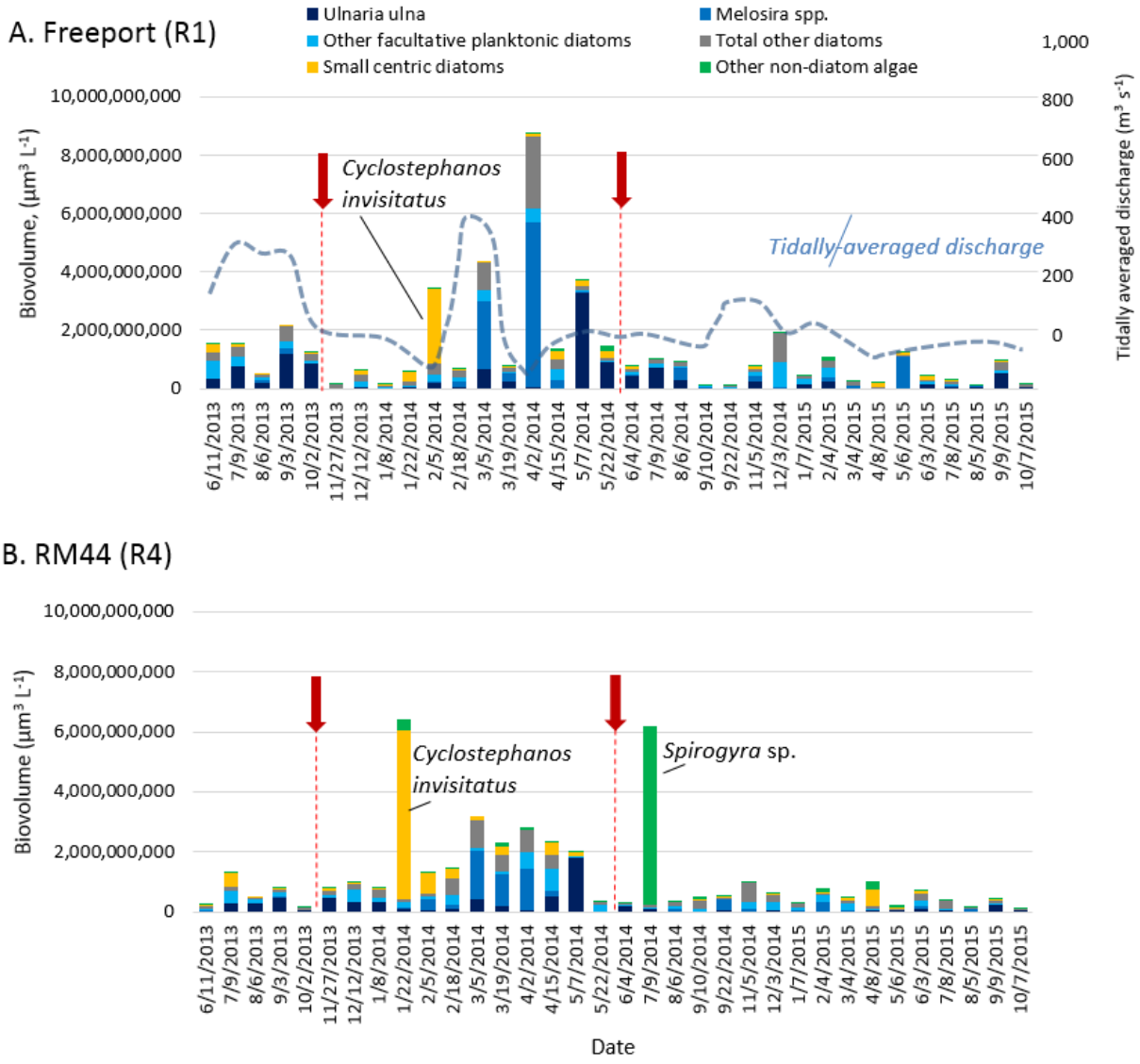

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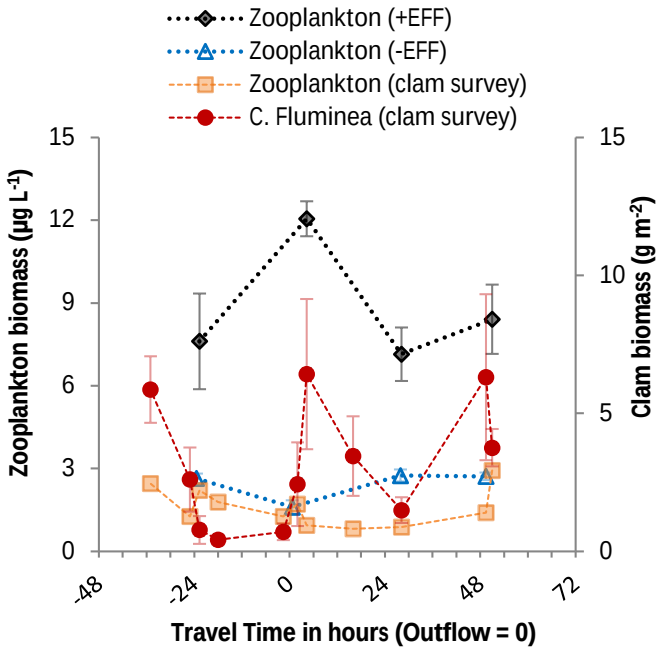

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SUPPORTING INFORMATION

Controls on riverine phytoplankton dynamics in the presence and absence of treated wastewater effluent high in ammonium—A Lagrangian based study

Tamara E.C. Kraus[1*], Kurt D. Carpenter[2], Brian A. Bergamaschi[1], Alex Parker[3], Elizabeth B. Stumpner[1], Bryan D. Downing[1], Nicole M. Travis[4], Frances P. Wilkerson[4], Timothy D. Mussen[5]

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SUPPORTING INFORMATION – METHOD DETAILS

Methods to determine nutrient, dissolved inorganic carbon (DIC), and chlorophyll-*a* (Chl-*a*) concentrations followed those described in Parker et al. (2012a) and Travis (2015).

Briefly, for nutrients sample water was filtered through a Whatman GF/F filter using a 50-ml syringe and stored frozen at -20°C for up to three months prior to analysis. Analysis of all nutrients except ammonium (NH_4) was performed on a Bran and Luebbe AutoAnalyzer II using the methods of Whitledge et al. (1981) for nitrate, nitrite, and phosphate (NO_3 + NO_2, and PO_4, respectively) and Bran and Luebbe Inc. (1999) and Macdonald et al. (1986) for silicic acid [$Si(OH)_4$]. NH_4 concentrations were measured spectrophotometrically using a 10-cm path length cell according to Solorzano (1969). DIC concentrations, necessary for the calculation of primary production, were determined using an MBARI-clone DIC analyzer (Friederich et al. 2002; Parker et al. 2006). Chl-*a* concentrations were determined for particulates from 100 mL water samples captured on 25-mm Whatman GF/F filters (nominally cells >0.7-µm). Filters were stored at -20°C until analysis. Samples were extracted at -20°C in 8 ml of 90% v/v acetone over 24 hours, and in vitro fluorometric analysis of Chl-*a* pigment was performed using a Turner Designs Model 10 fluorometer (Arar & Collins 1992) using 10% hydrochloric acid to correct for and measure phaeophytin (Holm-Hansen and Riemann 1978). Calibration was conducted with commercially available Chl-*a* standards obtained from Turner Designs.

Rates of carbon (C) and nitrogen (N) uptake were measured on a subset of samples collected in the +EFF and –EFF water parcels using stable isotope tracer techniques (Legendre and Gosselin, 1996; Parker 2005). Two 160-ml clear polycarbonate incubation bottles were filled with sample water from each parcel, and either $NaH^{13}CO_3$ and $^{15}NH_4Cl$ or $NaH^{13}CO_3$ and $K^{15}NO_3$ were added (Il stable isotope stocks contained 99 atom% heavy isotope; Cambridge Isotope Laboratories). Carbon-13 additions were

112 µM-C, representing roughly 11% and 14% substrate concentration in October 2013 and June 2014, respectively. NH4-15 additions were 0.1 µM-N for all samples collected from the -EFF parcels upstream and downstream from the WWTP discharge, as well as samples from the +EFF parcels that were upstream from the WWTF discharge. These NH_4 additions represented a roughly 31% NH_4 substrate increase in both parcels upstream from and a 7% increase in NH_4 substrate in the -EFF parcel downstream from the WWTF. $^{15}NH_4$ additions were 8.8 µM-N in samples collected from the +EFF parcel downstream from the WWTF discharge, representing a 15% enrichment. $^{15}NO_3$ enrichments were 1.2 µM-N for all samples collected from both parcels, representing an average of 58% and 27% substrate enrichment for samples collected upstream and downstream from the WWTF discharge, respectively. In this way, the N enrichments were higher than the 10% substrate "tracer" addition recommended by Dugdale and Wilkerson (1986) for all samples collected upstream from the WWTF discharge, as well as NO_3 uptake downstream from the WWTF discharge. NH_4 enrichments made to samples of +EFF and -EFF water collected downstream from the WWTF were closer to the tracer level. The spiked bottles were incubated for 24 hr, suspended at the surface of the river in a floating corral, and covered with window screening to reduce ambient light to ~50% of surface PAR. Thus these results are meant to be considered "potential" uptake rates. Incubations were terminated by filtration onto pre-combusted (450°C for 4 hr) 25-mm diameter GF/F filters. Determination of concentrations and isotopic enrichment of particulate carbon and nitrogen were measured on a PDZ Europa 20/20 gas chromatograph–mass spectrometer. Carbon and nitrogen uptake rates were calculated according to Dugdale and Wilkerson (1986) and Legendre and Gosselin (1996) and are reported as ρ (mg-N L^{-1} d^{-1} or mg-C L^{-1} d^{-1}).

55 ## SUPPORTING INFORMATION –TABLES
56

57 **Table S-1.** Summary of multivariate analyses relating phytoplankton assemblages and BEST
58 environmental factors for effluent containing (+EFF) and effluent free (-EFF) water parcels during the
59 Lagrangian experiments and during 2013-15 at Freeport, Sacramento River, California. Only samples
60 from the experimental reach downstream of the WWTF were included.
61

Transect Period	Samples Included	Number of Samples	BEST Variables	P Value
October and June	All samples	**34**	Water temperature Water velocity at Walnut Grove NO_3 concentration	P = 0.001
October 2013	All samples	20	Total suspended sediment	P = 0.02
	+EFF parcel	12	Total suspended sediment	P = 0.01
	-EFF parcel	8	Specific conductance	P = 0.25
June 2014	All samples	14	Travel time	P = 0.17
	+EFF parcel	6	Water velocity (Walnut Grove)	P = 0.03
	-EFF parcel	8	Dissolved organic nitrogen	P = 0.17
2013-2015	Freeport	32	Streamflow	P = 0.07
	RM44	32		

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Table S-2. Dominant zooplankton taxa in the Sacramento River, California, June 2014, during Lagrangian-based experiments and clam survey. Zooplankton samples were collected as part of the clam survey on June 18-19 (see Methods).

Parcel/ Sample#	Travel time past outflow	Dominant/sub-dominant zooplankton
		May 31-June 3, 2014
+EFF	-23	Moina sp._cl/cyclopoid copepodid_co
+EFF	4	Moina sp._cl/cyclopoid copepodid_co/Bosmina longirostris_cl
+EFF	28	Moina sp._cl/cyclopoid copepodid_co
+EFF	51	calanoid copepodid_co/cyclopoid copepodid_co/Moina sp._cl/nauplii
-EFF	-23	cyclopoid copepodid_co/Eurycercus longirostris_cl
-EFF	1	Eurycercus longirostris_cl
-EFF	28	nauoplii/cyclopoid copepodid_co/Bosmina longirostris_cl
-EFF	49	cyclopoid copepodid_co/nauplii/Acanthocyclops vernalis_co
		June 18-19, 2014 (clam survey)
1	-35	cyclopoid copepodid_co
2	-25	cyclopoid copepodid_co/Brachionus calyciflorus_r/Polyarthra vulgaris_r
3	-23	Acanthocyclops vernalis_co/cyclopoid copepodid_co
4	-18	cyclopoid copepodid_co/Acanthocyclops vernalis_co
5	-2	cyclopoid copepodid_co/Acanthocyclops vernalis_co
6	2	cyclopoid copepodid_co/nauplii
7	4	cyclopoid copepodid_co/Acanthocyclops vernalis_co
8	16	Pseudodiaptomus forbesi_co/Eurycercus longirostris_cl
9	28	cyclopoid copepodid_co/nauplii /Acanthocyclops vernalis_co
10	49	Moina sp._cl/nauplii/Bosmina longirostris_cl
11	51	Eurycercus longirostris_cl/veliger

SUPPORTING INFORMATION - FIGURES

Figure S-1. Constituent concentrations in relation to travel time for the October 2013 and June 2014 Lagrangian experiments, showing trends in samples from effluent containing (+EFF) and effluent free (-EFF) parcels, Sacramento River, California: (A) specific conductivity, (B) dissolved organic carbon (DOC), (C) total suspended sediment (TSS), (D) dissolved oxygen (DO) percent saturation, (E) pH, and (F) water temperature. Oct, October; AmR, American River; I80 Br, Interstate-80 Bridge.

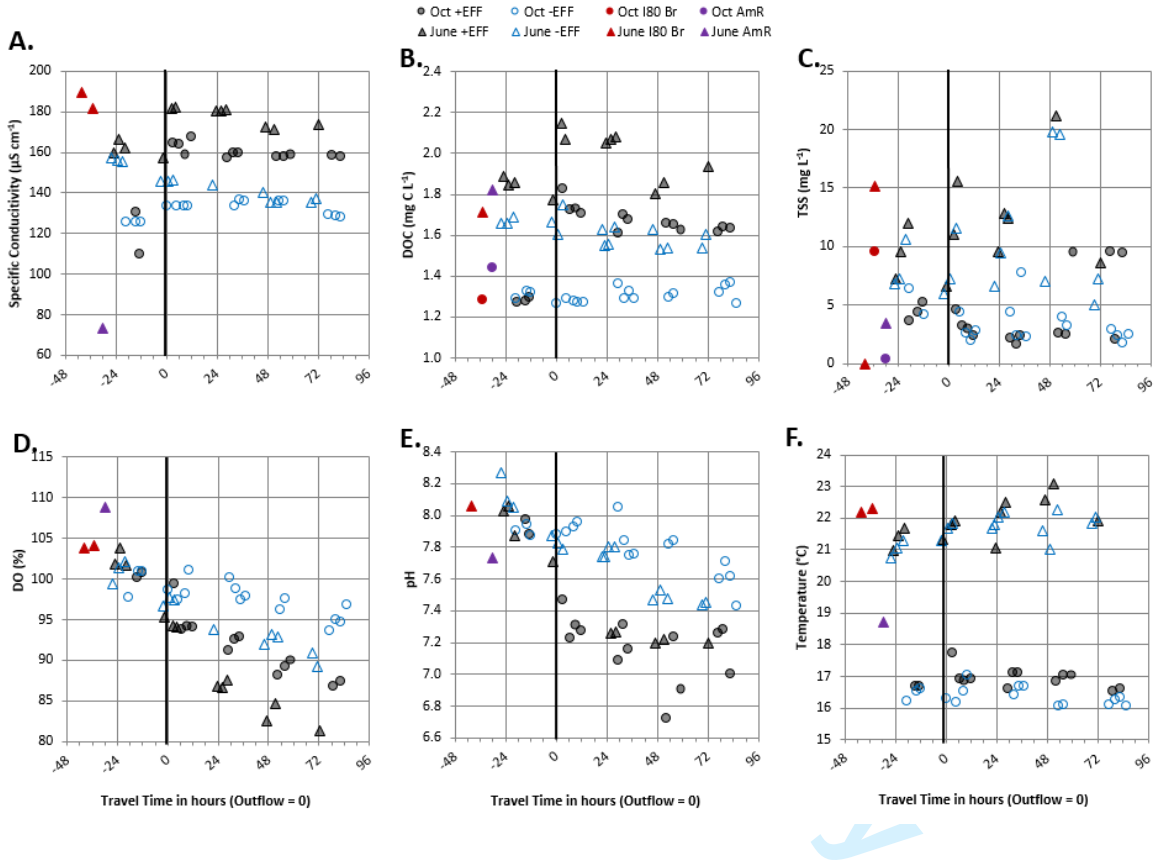
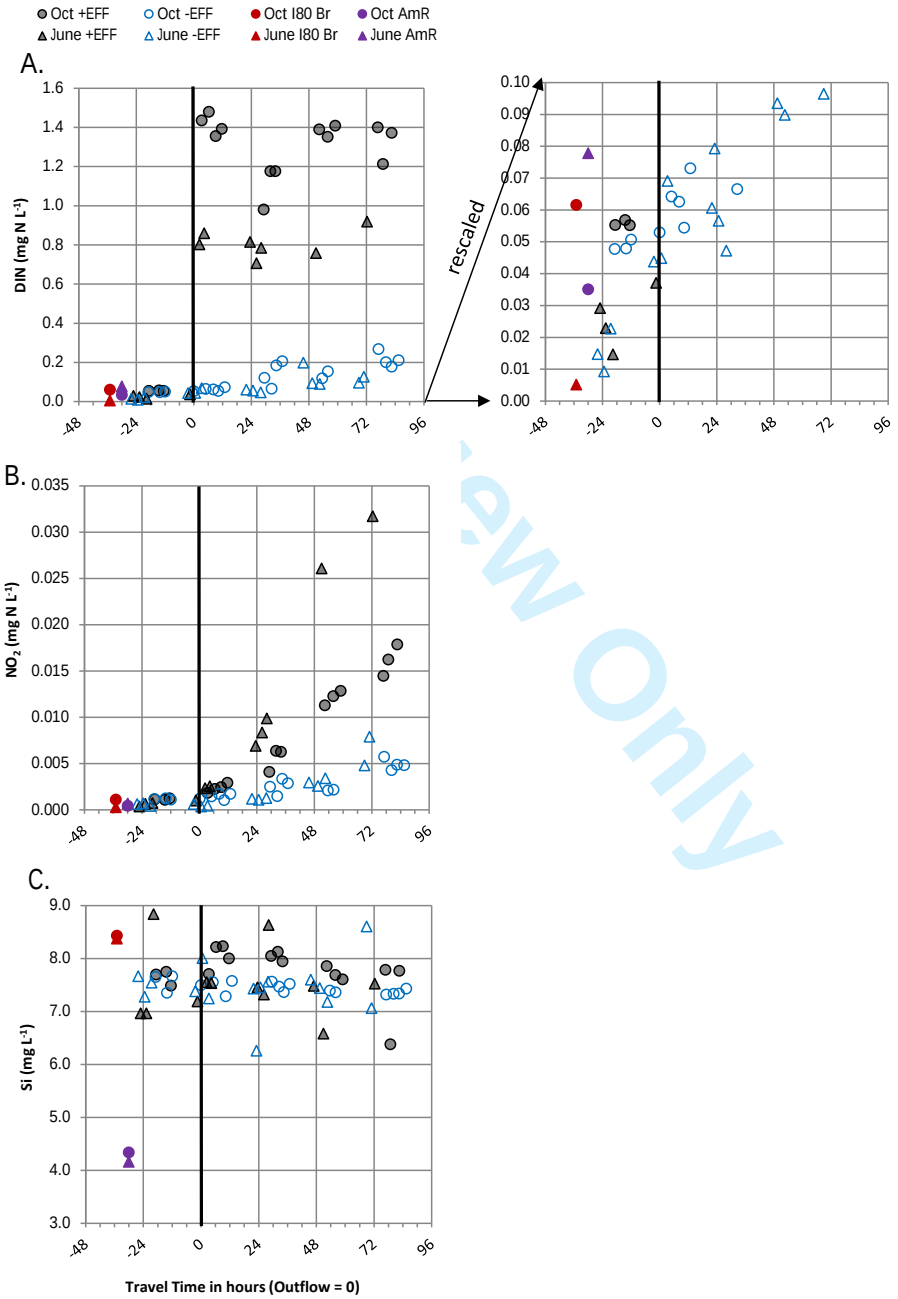


Figure S-2. Constituent concentrations in relation to travel time for the October 2013 and June 2014 Lagrangian experiments, showing trends in samples from effluent containing (+EFF) and effluent free (-EFF) parcels, Sacramento River, California: (A) dissolved inorganic nitrogen [DIN (NH4+NO3+NO2)], all data and rescaled to show lower concentrations, (b) nitrite (NO_2), and (C) silica (Si). Data plotted in relation to travel time where zero time indicates passage past the wastewater treatment plant (WWTP). Oct, October; AmR, American River; I80 Br, Interstate-80 Bridge.



89 **Figure S-3.** Phytoplankton uptake rates in relation to travel time for the October 2013 and June 2014
90 Lagrangian experiments, showing trends in samples from effluent containing (+EFF) and effluent free (-
91 EFF) parcels, Sacramento River, California: (A) ammonium (NH4) uptake, (B) nitrate (NO3) uptake, (C)
92 carbon (C) uptake. Data plotted in relation to travel time where zero time indicates passage past the
93 wastewater treatment plant (WWTP). Oct, October; AmR, American River; I80 Br, Interstate-80 Bridge.
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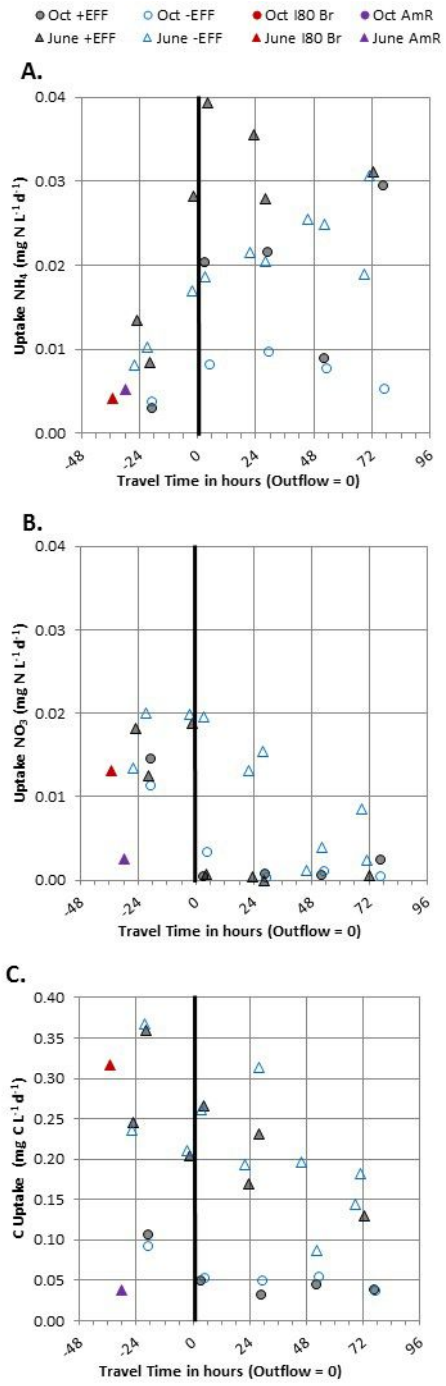

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96 **Figure S-4.** Tidal cycles in the Sacramento River at the U.S. Geological Survey Freeport and Walnut Grove
97 continuous monitoring stations and collection of samples from effluent free (-EFF) and effluent
98 containing (+EFF) parcels, Sacramento River, California, during the Lagrangian experiments in (A)
99 October 2013 and (B) June 2014.
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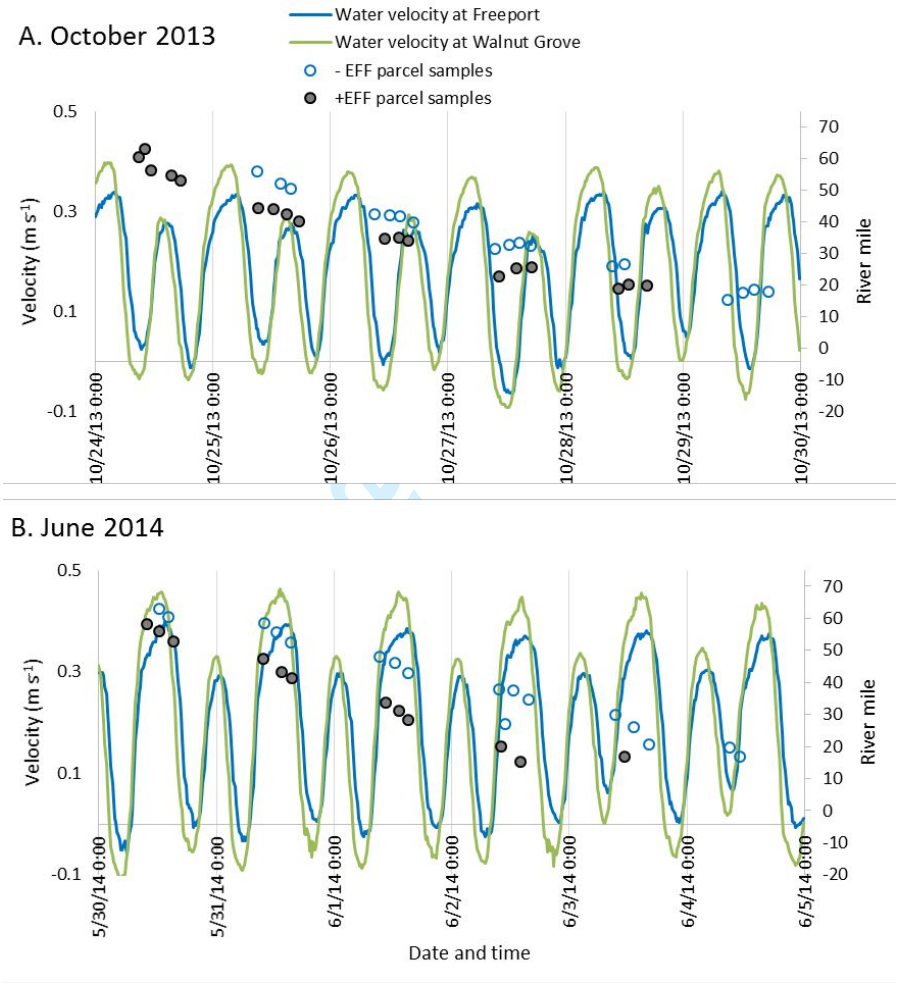

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Figure S-5. Tidal variations in velocity and turbidity in the Sacramento River at Walnut Grove, California, during Lagrangian-based experiments in (A) October 2013 and (B) June 2014. (Data from the U.S. Geological Survey continuous monitoring station near Walnut Grove (http://waterdata.usgs.gov/usa/nwis/uv?11447890).

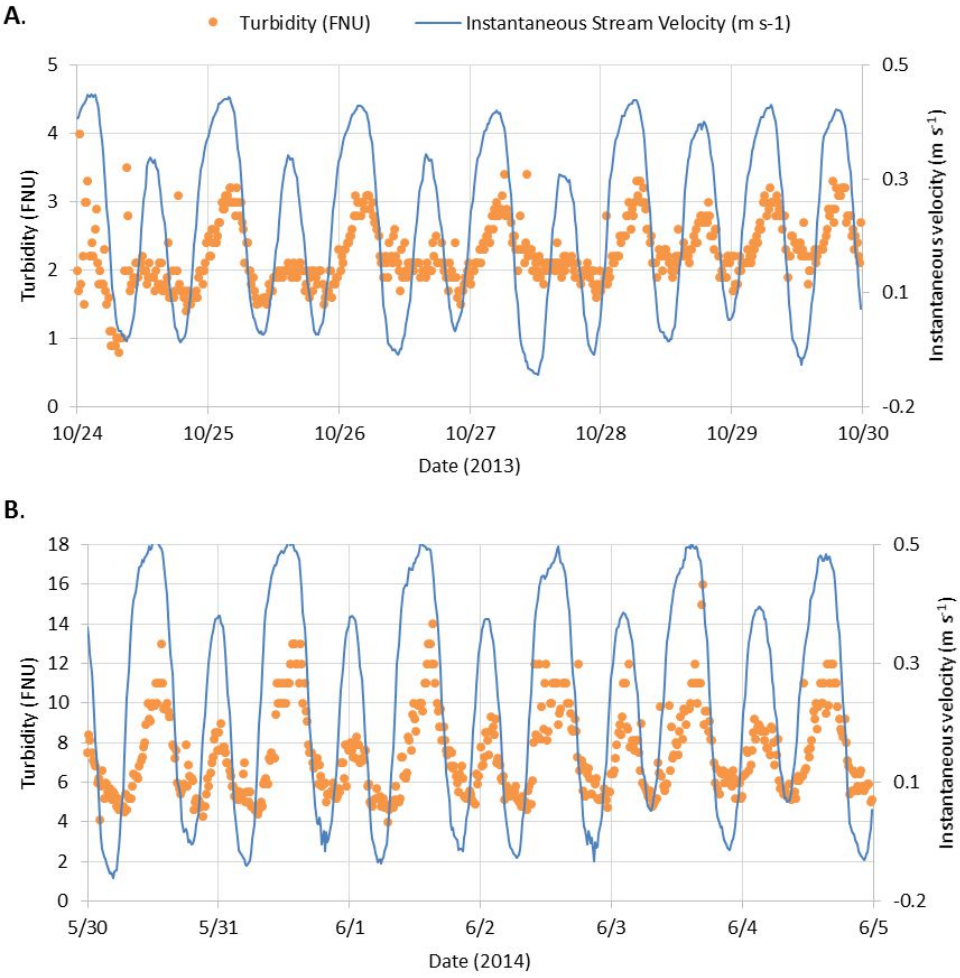


Figure S-6. Chlorophyll-a as a function of tidally averaged discharge at the U.S. Geological Survey Freeport monitoring station (Sacramento River, California), highlighting the dominant algal taxa during small to moderate blooms. Discrete samples collected by Sacramento Regional County Sanitation District.

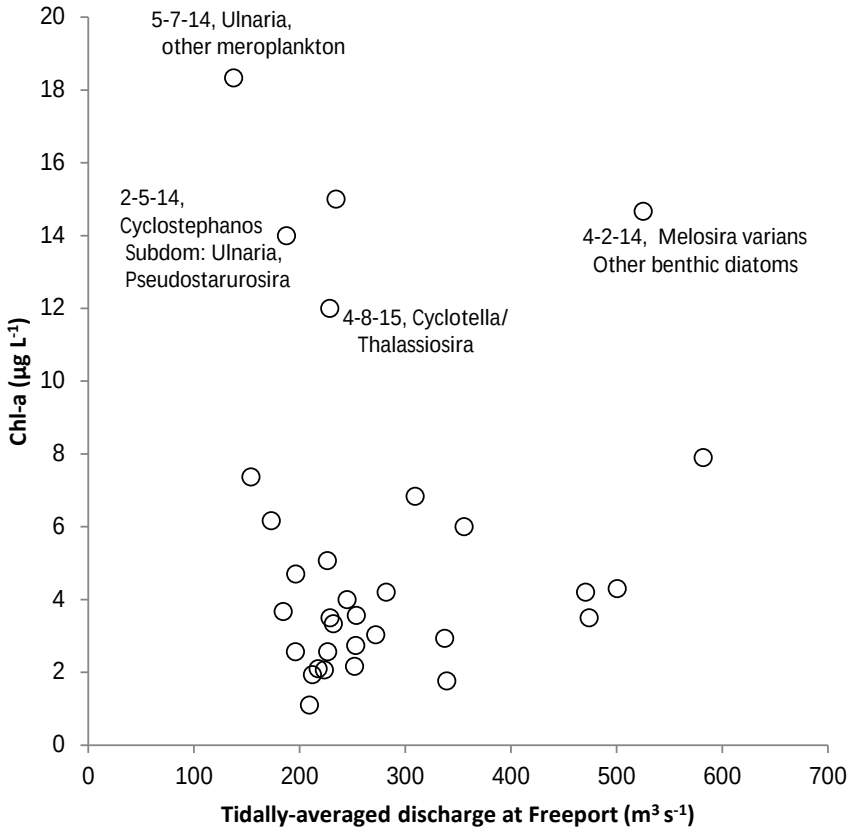
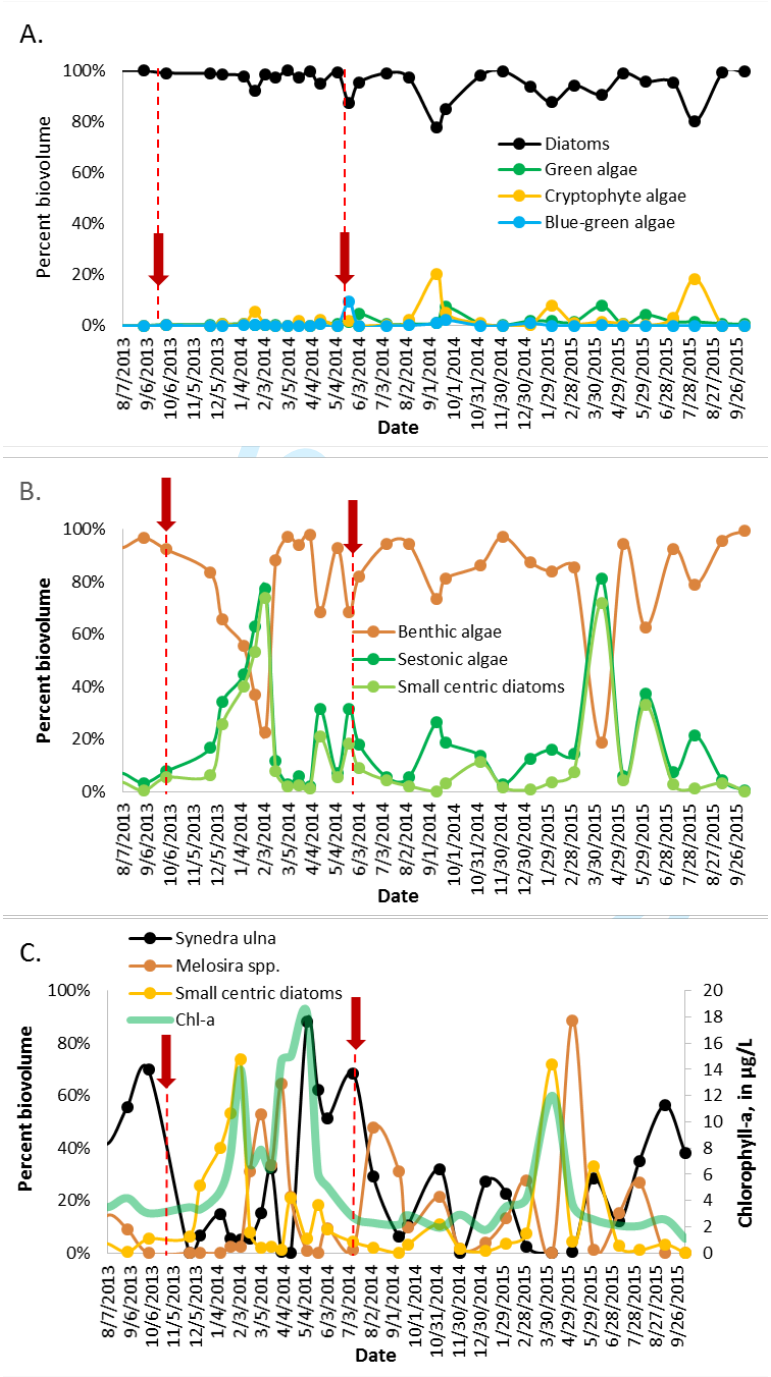


Figure S-7. Seasonal pattern in phytoplankton species composition in the Sacramento River at Freeport, California, 2013-15: (A) major algal groups, (B) selected habitat and species metrics, and (C) dominant species during periods of elevated chlorophyll-*a*. Red arrows and dashed lines indicate October 2013 and June 2014 Lagrangian-based experiments.



125 **Figure S-8.** Photographs of *Ulnaria ulna* showing (A-B) healthy colonies, (C-D) frustules in decay, (E-F)
126 colony of newly hatched resting cells, and (G) *Melosira varians* filament packed with chloroplasts.
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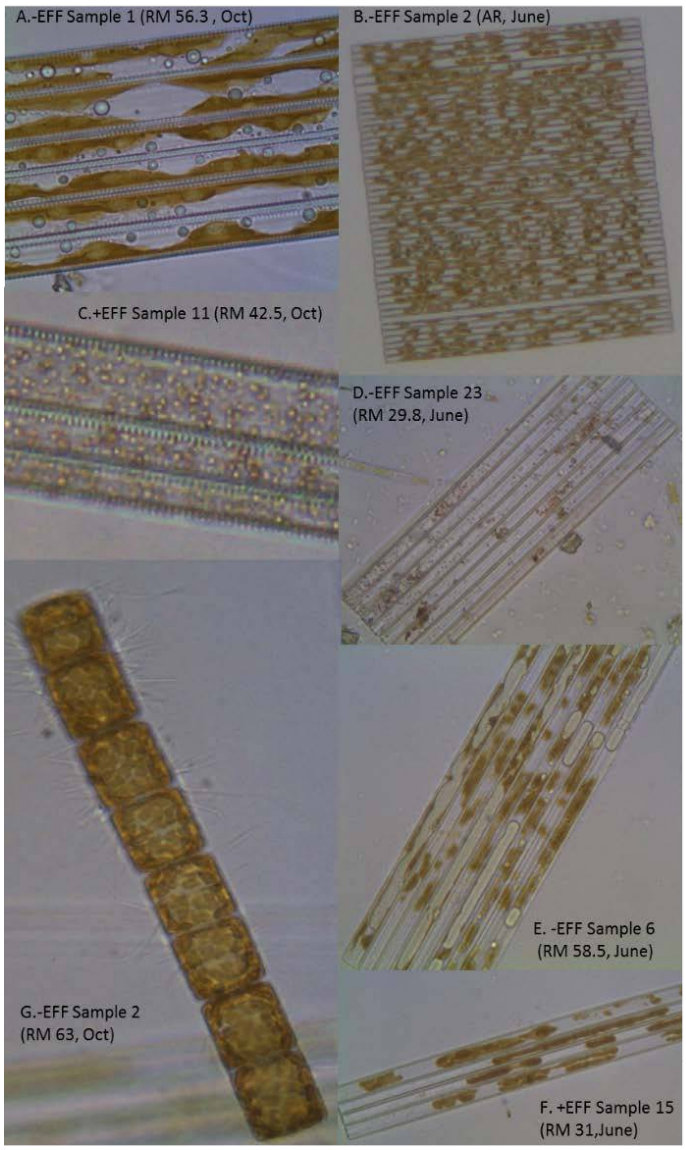

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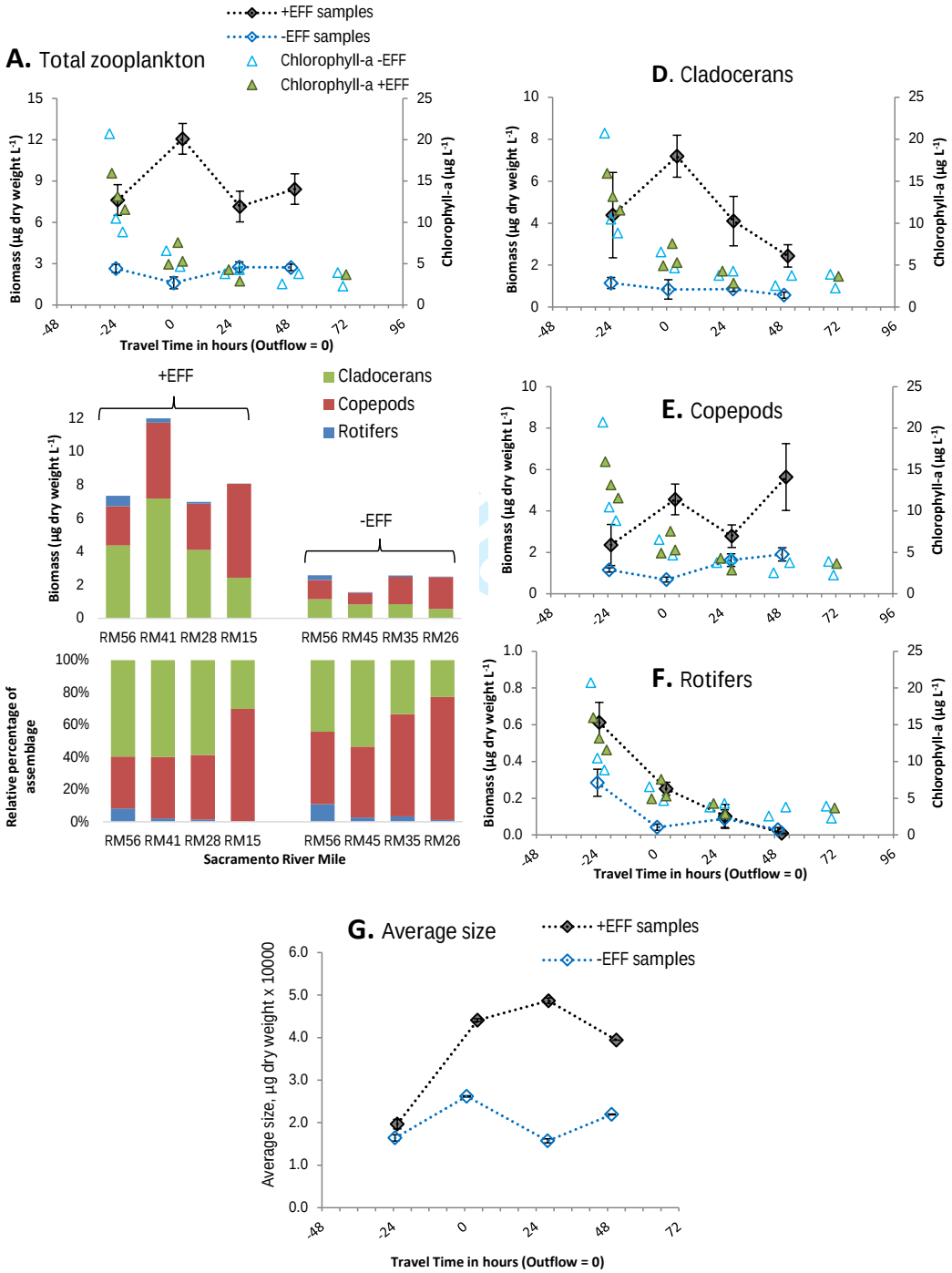
Figure S-9. Photographs of (A-D) Cladocerans and (E-G) copepods in the Sacramento River, California, collected during the October 2013 and June 2014 Lagrangian experiments.

136 **Figure S-10.** Zooplankton and clam (*Corbicula fluminea*) biomass data collected from the Sacramento
137 River, California, during the June 2014 Lagrangian experiment, in relation to (A) travel time, (B-C) river
138 mile, (D-G) travel time. Error bars indicate standard error. Chlorophyll-*a* concentrations in samples from
139 effluent free (-EFF) and effluent containing (+EFF) parcels during the June 2014 Lagrangian experiment
140 are also shown on each graph.



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