Effect of Ammonium and Wastewater Effluent on Riverine Phytoplankton in the Sacramento River, CA.

Final Report

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May 29, 2010

Technical report for the California State Water Resources Board

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Executive Summary

This study was conducted between July 2008 and May 2009 in an effort to understand the effect of wastewater effluent discharge from the Sacramento Regional Wastewater Treatment Plant (SRWTP) on nutrient concentration and phytoplankton abundance and productivity within the Sacramento River between the City of Sacramento and Rio Vista, CA. Five different experimental approaches were used during the study: (1) River characterization of inorganic nutrients and phytoplankton (2) Experimental grow-outs to investigate the time course of potential phytoplankton bloom development in the Sacramento River (3) Sacramento River water aging experiments to investigate the potential for bacterially mediated nitrification (i.e. ammonium (NH_4) conversion to nitrite (NO_2) and nitrate (NO_3) (4) NH_4Cl addition experiments to evaluate the hypothesis that NH_4 inhibits phytoplankton NO_3 uptake and decreases phytoplankton growth rates (5) Wastewater effluent addition experiments to evaluate whether wastewater discharge alters primary production and phytoplankton nitrogen uptake.

River characterizations shows that SRWTP discharge has a profound effect on river nutrient concentrations with an increased concentration of NH_4 and phosphate (PO_4) downstream of the outfall; the peak in NH_4 concentration does not always occur at the outfall location but further downstream. Nutrient concentrations varied substantially on a seasonal basis. The spatial distribution of NH_4, NO_2, and NO_3 suggest nitrification is occurring downstream of the SRWTP. We observed a consistent north to south gradient of declining chlorophyll-a concentrations and fluorescent particle abundance (indicative of phytoplankton). Primary production sometimes declined from north to south consistent with the trends in chlorophyll-a. Phytoplankton NH_4 uptake increased just downstream of the wastewater discharge, presumably in response to higher NH_4 concentration supplied via SRWTP and then declined downstream. Phytoplankton NO_3 uptake was high at stations upstream of the SRWTP discharge, consistent with relatively high primary production found at the upstream stations. NO_3 uptake decreased to near detection limits (0.02 $\mu mol\ L^{-1}\ d^{-1}$) at stations downstream of the SRWTP. The shut down of phytoplankton NO_3 uptake is the result of the well described phenomenon of NH_4 inhibition of phytoplankton NO_3 uptake. The sum of phytoplankton NO_3 and NH_4 uptake declined in the downstream direction.

Results from experimental grow-outs suggest that after removing light limitation phytoplankton bloom magnitude in the Sacramento River at RM-44 (downstream of SRWTP discharge) and GRC (upstream of SRWTP discharge) is likely determined by dissolved inorganic nitrogen (DIN) availability. Grow-out experiments conducted at RM-44 produced more chlorophyll-a than experimental grow-outs conducted at GRC. Phytoplankton appeared to take advantage of additional DIN, whether supplied as NO_3 or NH_4 in experiments conducted with water from GRC, or in the form of NH_4 supplied in the wastewater effluent (at RM-44) to produce greater biomass.

Sacramento River water aging experiments from RM-44 and GRC suggested that nitrification (microbially mediated stepwise conversion of NH_4 to NO_2 and NO_3) may occur in the river but only on time scales of 7 to 14 days. The addition of NH_4 via SRWTP discharge likely increases rates of this process relative to locations upstream of SRWTP (GRC) where NH_4 concentrations were lower. There was little evidence of nitrification occurring in aged river

water on time scales of 7 days or less, even in an experiment in water with elevated NH_4 (RM-44).

During April 2009, experimental additions of NH_4Cl up to +50 μmol L^{-1} resulted in a significant reduction in primary production (by ca. 8%). In contrast, supplements of NH_4Cl to water collected at GRC with additions of 0.25 μmol $L^{-1}$to 100 μmol L^{-1} showed no effect on primary production during short-term (4-hr) experiments during May 2009. Also during May 2009, additions of NH_4Cl enhanced phytoplankton NH_4 uptake up to NH_4 concentrations of ca. 4 μmol N L^{-1} (i.e. half-saturation constant, K_m, for NH_4 in the Sacramento River). In contrast, additions of NH_4Cl reduced phytoplankton NH_4 uptake during the experiment conducted in April 2009. This suggests that NH_4 concentrations greater than 4 μmol N L^{-1} will not further enhance phytoplankton NH_4 uptake but may at times reduce NH_4 uptake. Phytoplankton NO_3 uptake was reduced to near detection limits with small experimental additions of NH_4Cl (<<1 μmol L^{-1}) during both April and May 2009 experiments.

Experimental additions of SRWTP effluent (containing NH_4), from 0.25 μmol NH_4 L^{-1} to 100 μmol NH_4 L^{-1} resulted in decreased primary production rates (up to ca. 36% of controls); the decline in primary production became evident at effluent-NH_4 concentrations greater than 8 μmol N L^{-1}. Similarly, NH_4 supplied by SRWTP effluent decreased phytoplankton NH_4 uptake at concentrations greater than 4 μmol N L^{-1} by as much as 29%. Consistent with results for NH_4Cl addition experiments, phytoplankton NO_3 uptake was also reduced to near detection limits with effluent-NH_4 additions less than 1 μmol NH_4 L^{-1}. The concentration threshold of 8 μmol N L^{-1} represents a wastewater effluent dilution of greater than 200:1 (based on an undiluted effluent NH_4 concentration of 1933 μmol N L^{-1}), significantly higher dilution than required under the current SRWTP permitting 14:1 (C. Foe, pers comm.); NH_4 concentrations >8 μmol L^{-1} were consistently observed at stations downstream of the SRWTP during river characterizations and were attributed to the SRWTP discharge.

These results indicate that SRWTP discharge has an effect on inorganic nutrient concentrations and may have an effect on phytoplankton processes in the Sacramento River downstream of Garcia Bend. Experimental grow-outs reveal that populations at upstream locations (GRC and RM-44) are in a physiologically unimpaired condition. This is in stark contrast with results obtained previously with grow-outs conducted over 5 to 7 days at Rio Vista (Wilkerson, NH_4 Summit). The two experimentally determined effects of effluent additions are: (1) the direct shut down of phytoplankton NO_3 uptake by the NH_4 supplied with SRWTP effluent and (2) the negative effect of sewage effluent on primary productivity and phytoplankton NH_4 uptake. The decline in primary production observed *in situ* downstream of the SRWTP is consistent with the results using experimental additions of SRWTP effluent and NH_4Cl which demonstrate an impact to phytoplankton processes in an experimental setting and provide evidence of a potential negative impact to phytoplankton as a result of SRWTP effluent NH_4 discharge in the Sacramento River.

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1.0 Study Background

Compared to most temperate estuarine systems, primary production and rates of phytoplankton nitrogen uptake are low in Suisun Bay and in the Sacramento River as far upstream as Rio Vista. This has also been documented as a long-term decline in phytoplankton abundance in the San Francisco Bay Estuary (SFE) (Jassby 2008). Jassby et al. (2002) estimated that annual primary productivity in the Delta decreased by 43% between 1975 and 1995 and showed that phytoplankton biomass trends were neutral in Suisun Bay and positive in the Delta between 1996-2005 (Jassby, 2008).

Several physical and biological factors are known to influence phytoplankton biomass and productivity in this system. The SFE is not a low nutrient system; light (controlled by turbidity and seasonal irradiance) and freshwater flow are considered key factors controlling primary production. Filtration or grazing by the invasive clam (*Corbula amurensis)* is considered a major contributor to the decrease in phytoplankton abundance that occurred in Suisun Bay and the western Delta after its arrival in 1986 (Alpine & Cloern, 1992; Jassby et al. 2002). *Corbula* is not present in the freshwater reaches of the San Francisco Estuary Delta (the focus of the present study). However, another bivalve, *Corbicula sp.* is present in the freshwater region of the Delta.

Wilkerson et al. (2006) showed that spring blooms of phytoplankton in the northern SFE occurred when at least two conditions were satisfied: (1) vertical stratification driven by salinity that improved light conditions, and (2) ambient concentrations of NH_4 below a threshold of ca. 4 μmol L^{-1} (1 μmol N L^{-1} = 0.014 mg N L^{-1}; i.e. 4 μmol L^{-1} = 0.056 mg N L^{-1}). Tracer experiments using water from the northern SFE indicated that above this NH_4 threshold, phytoplankton almost exclusively took up NH_4 (leaving the NO_3 pool little changed), but the

NH_4 uptake was not accompanied by significant increases in algal biomass (Dugdale et al. 2007). When NH_4 levels dropped below this threshold (~4 μmol L^{-1}), chlorophyll-a increases were observed. It was not until NH_4 dropped below about 1 μmol L^{-1} that rapid NO_3 uptake commenced and rapid growth of phytoplankton took place. As a consequence of these studies in the SFE, ambient NH_4 levels, and hypothesized suppression of phytoplankton blooms by NH_4, have been added to the list of factors that may be affecting the base of the pelagic food web in the SFE. Preliminary results from ongoing CALFED research suggest that NH_4 inhibition of NO_3 uptake may occur in the freshwater reaches of the estuary (Sacramento River at Rio Vista).

The studies described above suggest that ambient NH_4 levels above ~4 μmol L^{-1} may prevent bloom forming phytoplankton taxa (especially diatoms) from accessing the larger NO_3 pool in the northern estuary. The study described herein is an initial investigation into whether the NH_4 and NO_3 uptake interactions observed in experiments using water from the northern estuary will occur in fresh water from the Sacramento River, where the Sacramento Regional Wastewater Treatment Plant (SRWTP) effluent supplies NH_4. Several terms are used to describe interactions between NH_4 and NO_3 use by phytoplankton, such as "inhibition", "suppression", and "preferential uptake". Generally, an indirect interaction between NH_4 and NO_3 is termed preference, and a direct interaction between NH_4 and NO_3 uptake is termed inhibition. NH_4 preference means that NH_4 is more readily utilized than NO_3 although this may not result in more rapid growth than on NO_3. Inhibition results when the presence of one nitrogen source prevents or reduces the uptake of the other. Enzymatic disruption of NO_3 reductase during NH_4 assimilation is one of the proposed mechanisms for true inhibition (Dortch 1990).

The SRWTP discharges an average of 141 million gallons per day (mgd) (representing nearly 60% of all wastewater discharges in the SFE Delta) of secondarily treated municipal wastewater effluent to the Sacramento River immediately downstream of the Freeport Bridge (NPDES, 2009). The SRWTP serves 1.3 million people in the City of Sacramento and surrounding communities and is currently requesting an increase in permitted average dry weather discharge from 181 mgd to 218 mgd (NPDES, 2009).

MacIsaac et al. (1979) performed experiments to observe the effects of treated wastewater on phytoplankton populations in Southern California. Serial enrichments of seawater with treated wastewater or a sterile NH_4Cl solution (of similar NH_4 concentration to the treated wastewater) as a control were made and uptake of ^{15}N and ^{14}C measured. The authors described suppression of NO_3 uptake and NH_4 uptake. Suppression of carbon uptake (primary productivity) also occurred, but at higher concentrations of NH_4 (both alone and in treated wastewater) than for NO_3 and NH_4 uptake suppression. In the Sacramento River, SRWTP treats mainly residential wastewater with a high-purity oxygen activated sludge secondary treatment and chlorination / dechlorination disinfection before discharging a highly diluted final effluent (2% of river flows) into a freshwater receiving environment.

The study described here was conceived to investigate whether the NH_4 effects on primary production that have been observed in the northern SFE also occur in the Sacramento River as a result of wastewater effluent discharge by SRWTP, a secondary level (i.e. discharges primarily NH_4) discharger. Specifically, the study was designed to answer the following research questions:

1. Is the SRWTP a source of NH_4 to the Sacramento River?
2. Does elevated NH_4 concentrations in the Sacramento River inhibit phytoplankton N uptake *in situ*? Does SRWTP effluent inhibit phytoplankton N uptake in an experimental setting?
3. Do elevated NH_4 concentrations reduce primary production or the potential for phytoplankton blooms *in situ* in the Sacramento River? Does SRWTP effluent inhibit primary production in an experimental setting?
4. Is there any indication that phytoplankton species composition changes occur *in situ* as a result of SRWTP effluent (including NH_4) discharge?

2.0 Study Components / Experimental Design

The scope of work for this research project was developed under agreement 06-447-300-0 between the California State Water Quality Control Board and the Romberg Tiburon Center – San Francisco State University (RTC-SFSU); this agreement contained eight TASKS that were developed out of convenience for funding rather than as the experiments were conceived or conducted (referred to here under Section 2, Experimental Design). The results presented in this document (in Section 3, Results) reflect how the experiments were conducted rather than how they were organized by TASKS. Table 1 is provided to aid the reader in determining how each of the sections described below (Section 4: Results) reflects each TASK as outlined in the agreement.

2.1 River Characterization

This study required characterization of nutrients and phytoplankton in the Sacramento River near the Sacramento Regional Wastewater Treatment Plant (SRWTP). This was accomplished twice during 2008 (21 July and 12 November) and three times (9 March, 6 April, 8 May) in 2009 (Table 2) by sampling an eight (8) station transect (Table 3) with three stations upstream of the SRWTP discharge near station RM-44 and five stations downstream of the SRWTP. Surface water samples were collected at each station along with additional vertical profiles of *in vivo* chlorophyll-a using a Wetlabs fluorometer and photosynthetically active radiation (PAR) using a LiCor submersible PAR sensor.

Detailed explanations of nutrient and phytoplankton sample collection, handling and analysis are provided below (Section 3). Briefly, at each station Niskin bottle water samples were collected for nutrients and placed on ice and returned to the lab to determine NO_3, NO_2, silicate ($Si(OH)_4$), PO_4, NH_4, and urea. Separate samples were collected for determination of dissolved inorganic carbon (DIC) concentrations and to estimate number of fluorescing particles, *in vitro* chlorophyll-a concentration in all phytoplankton cells (using GF/F filters with nominal pore size of 0.7 μm) and in larger phytoplankton cells using Nucleopore filters with pore size of 5 μm. In addition, during November 2008 and all of 2009 primary production and phytoplankton nitrogen uptake were assessed using ^{13}C and ^{15}N (NH_4 and NO_3) tracer techniques in 24-hr incubations. These transects were designed to understand the potential role that the SRWTP effluent discharge had on Sacramento River nutrients, turbidity and phytoplankton biomass.

2.2 Aging Experiments

On 21 July 2008, 20-L (LDPE cubitainers) water samples from two locations in the Sacramento River, upstream of SRWTP at Garcia Bend (GRC) and just downstream of the SRWTP (at RM-44), were collected during the River Characterization (Section 3). These samples were returned to RTC, held in the dark at ambient temperature (21ºC) and "aged" for eight weeks. Each of the carboys was sampled weekly for the eight week period for analysis of NO_3 and NH_4 concentrations as well as PO_4 and $Si(OH)_4$. The objective of this experiment was to determine whether nitrification (i.e. sequential oxidation of NH_4 to NO_2 and NO_3) was likely to occur in the Sacramento River.

2.3 River Sample Grow-out Experiments ("Grow-Outs")

Twice during 2008 (21 July and 12 November) and twice during 2009 (9 March and 8 May) water was collected from two locations in the Sacramento River - upstream of SRWTP (GRC) and just downstream of the SRWTP (RM-44) and used to fill triplicate 20-L LDPE cubitainers. These were the same upstream reference and downstream stations that were used for aging experiments (Section 2.2). The cubitainers were transported back to RTC in coolers and incubated in bay water-cooled tubs under natural ambient solar irradiance. Due to the natural suspended sediments in the bay, light was attenuated to ca. ~40% of surface irradiance within the incubation tubs. Grow-outs were sampled daily for the first 96-hr and then after 144-hr and 216-hr for nutrients (NO_3+ NO_2, NH_4, PO_4, $Si(OH)_4$), DIC, chlorophyll-a, fluorescent particles and temperature. After 96-hr, nutrient limitation was observed in grow-out experiments and so analysis of results was limited to the first 96-hr. During 2009 additional 20-L cubitainers were collected at GRC and amended with NO_3 and NH_4 to concentrations comparable to the dissolved inorganic nitrogen concentrations found at RM-44. In addition, during 2009, primary production and phytoplankton nitrogen uptake were assessed daily in the grow-outs using ^{13}C and ^{15}N (NH_4 and NO_3) tracer techniques. The goal of these experiments was to determine the potential for phytoplankton biomass accumulation at stations upstream (GRC) and downstream (RM-44) of the SRWTP under non-light limited conditions simulating a phytoplankton bloom.

2.4 Clean NH_4Cl Addition Experiments

On 6 April 2009 water was collected at GRC for short term (4-hr) primary productivity experiments with 3 experimental additions of NH_4 (added as NH_4Cl). NH_4 was added at concentrations of +1 μmol L^{-1}, +4 μmol L^{-1}, and +50 μmol L^{-1} NH_4. Incubations were performed in triplicate 160-ml incubation bottles using ^{14}C-$NaHCO_3$ under natural light that had been attenuated to 50% of surface PAR using window screening. The goal of this experiment was to evaluate the potential for acute NH_4 inhibition of primary production in the Sacramento River as had been previously shown for other regions of the northern San Francisco Estuary (see study background).

2.5 Diluted Effluent Experiments

This experimental protocol follows closely that used in experiments on Los Angeles effluents (MacIsaac et al. 1979). A flow-weighted 24 hour composite of dechlorinated final effluent from SRWTP was provided by C. Foe on 28 April 2009 and transported to the Romberg

Tiburon Center by the Central Valley Regional Water Quality Control Board (CVRWQB). Nutrient concentrations (NH_4, NO_3, and PO_4) in the effluent were analyzed so that nutrient additions based upon the effluent concentrations of NH_4 and NO_3 could be made.

The following day, four sets of 20 280-ml incubation bottles were filled with water collected at GRC on 28 April 2009 by C. Foe. To one set of incubation bottles serial additions of SRWTP effluent were added to make an addition series of 10 concentrations of NH_4 from 0 to 100 µmol N L^{-1}. To the second set of incubation bottles serial additions of NH_4Cl were added to make a parallel set of additions from 0 to 100 µmol N L^{-1}. Once the additions were made, isotopic tracers, $^{15}NO_3$, $^{15}NH_4$ and ^{13}C were added at approximately 10% of ambient NO_3, NH_4, and DIC concentrations. The third and fourth sets of bottles were used for ^{14}C-based primary production estimates. Samples were then incubated for 6-hr in flowing bay-water tables under natural light attenuated to 50% of surface irradiance using window screening. The goal of this experiment was to evaluate whether elevated concentrations of effluent-NH_4 or NH_4 alone influenced primary production or phytoplankton NH_4 and NO_3 uptake and if an effect was observed, to determine the lowest concentration at which it occurred.

3.0 Detailed Sampling and Analytical Methods

3.1 Water Column Sampling

At each station, a Seabird Electronics SBE-32 rosette mounted with three 6-L Niskin bottles and fitted with a Seabird SBE-19 plus CTD was lowered over the side. Salinity, temperature and *in vivo* fluorescence (using a Wetlabs fluorometer) were measured continuously from surface to approximately 0.5 m off of the bottom. In addition, light attenuation was assessed using a LiCor PAR sensor mounted on the rosette. A separate measure of light attenuation was made using a 0.3-m diameter Secchi disk. The light attenuation coefficient, k, was determined by either plotting the natural log of PAR versus depth and using the slope of least-squares regression or, in the case of the Secchi disc, using the equation k = 0.4 + 1.09/Secchi depth (Cloern 1991). Water was collected at the surface (<0.5m) using the Niskin bottle samplers. Each Niskin bottle was numbered (1 to 3) with bottles closed sequentially starting at bottle 1. Samples for chemical and biological analyses were collected from assigned bottles using the following sampling order:

Bottle 3: Sample for dissolved inorganic carbon was collected into a 22-ml glass scintillation vial and preserved with 200-µl of 5%w/v $HgCl_2$.

Bottles 1, 2 and 3: Using syringe fitted with a 25-mm Whatman GF/F filter, one sample was collected in a 25ml HDPE scintillation vial for NO_3+NO_2, PO_4 and $Si(OH)_4$ analyses.

Bottles 1, 2 and 3: Using syringe fitted with a 25-mm Whatman GF/F filter, one 25-ml sample was collected in a 60-ml centrifuge tube for NH_4 analysis.

Bottles 1, 2 and 3: Using syringe fitted with a 25-mm Whatman GF/F filter, one 25-ml sample was collected in a 60ml centrifuge tube for urea analysis.

Bottles 1, 2 and 3: 500-ml sample was collected into an opaque plastic bottle for *in vitro* chlorophyll-a and particulate organic carbon and nitrogen (POC and PON, respectively) analyses.

Bottle 1: 20-ml sample was collected into a 25-ml plastic scintillation vial for flow cytometry.

Bottle 1: 200-ml sample was collected in a 250-ml glass amber bottle and preserved with acid-Lugols solution for phytoplankton enumeration.

Bottle 2: Two 160-ml clear polycarbonate bottles filled for $^{13}C/^{15}N$ tracer uptake incubations (during 2009 transects only).

Bottle 3: Sample collection by SRWTP (Michael Cook).

3.2 Nutrient Analysis

Samples for NO_3, NO_2, PO_4, and $Si(OH)_4$ were first filtered through 25-mm GF/F filters using a hand syringe into 20-ml HDPE scintillation vials and frozen until analysis. Before analysis samples were thawed at room temperature for 24-hr to minimize $Si(OH)_4$ polymerization effects (MacDonald et al. 1986) and then analyzed using a Bran and Luebbe AutoAnalyzer II (Whitledge et al. 1981 for NO_3, NO_2 and PO_4; Bran and Luebbe, 1999 for $Si(OH)_4$). Water samples for NH_4 analysis, filtered through GF/F filters into 60-ml sterile Falcon tubes were frozen until analysis. Ammonium concentrations were manually determined by the phenol-hypochlorite colorimetric method of Solorzano (1969) using a Hewlett Packard diode array spectrophotometer and 10-cm path length cell. Urea was measured according to Revilla et al. (2005) on 25-ml surface samples, filtered through GF/F filters.

3.3 Dissolved Inorganic Carbon

Dissolved inorganic carbon (DIC) was measured according to Friederich et al. (2002) using a Monterey Bay Research Institute-clone DIC analyzer with acid-sparging and a LiCor nondispersive infra-red detector (Model 6252). DIC concentrations were calculated using the certified reference material prepared at the Scripps Institution of Oceanography (A. Dickson Laboratory).

3.4 *In Vitro* Chlorophyll-a, Phaeophytin and Flow Cytometry Analysis

Extracted chlorophyll-a and phaeophytin was determined on GF/F filters (for all cells >0.7-µm) and 5-µm pore-size Nucleopore filters (>5.0-µm cell sized phytoplankton). Filtration volumes were 50 - 100-ml and chosen to ensure sufficient material collection but also to minimize filtration times. Filtration was carried out under a gentle (<250-mm Hg) vacuum on a manifold with a Cole-Palmer vacuum pump. Filters were collected and stored frozen in glass culture tubes until analysis within one week of collection. Analysis was performed fluorometrically on acetone extracts according to Arar and Collins (1992) using a Turner Designs Model 10 AU fluorometer calibrated with commercially available chlorophyll-a (Sigma

Chemical). Flow cytometry was assessed using a CytoBuoy flow cytometer (Dubelaar & Gerritzen, 2000).

3.5 Primary Production, Phytoplankton Nitrogen Uptake and Particulate Organic Carbon and Nitrogen.

Carbon and nitrogen uptake were estimated using dual-labeled stable isotope tracer techniques (Legendre and Gosselin, 1996). Surface water was dispensed into two 160-ml clear polycarbonate bottles. One bottle was inoculated with $^{15}NH_4Cl$ and the other with $K^{15}NO_3$ (99 atom % ^{15}N) at approximately 10% of anticipated ambient NH_4 or NO_3 concentration. $Na_2{}^{13}CO_3$ was added to each bottle at approximately 10% of ambient DIC concentration. After inoculation, bottles were placed in bay water-cooled incubator tables at 50% surface irradiance (under window screening). Incubations were carried out for 24-hr (river transect samples) or 4-hours (for grow-out/enclosure samples) and were terminated by gentle vacuum filtration onto pre-combusted (450°C for 4 hours) 25 mm GF/F filters. Phytoplankton ^{13}C and ^{15}N enrichment were measured on a Europa 20/20 gas chromatograph – mass spectrometer. Transport rates (ρ, in μmoles L^{-1} h^{-1} or μmol L^{-1} d^{-1}) and specific uptake (V, in h^{-1}) were calculated according to Dugdale and Wilkerson (1986) and Legendre and Gosselin (1996).

During the NH_4Cl and effluent addition experiments ^{14}C-based primary production estimates were made using the JGOFS (1996) method. Samples were dispensed into 160-ml incubation bottles and 6.4 μCi additions of ^{14}C-$NaHCO_3$ were added. Incubation bottles were then placed in baywater cooled incubation tables under ambient light attenuated to 50% of surface PAR for 4-hr. Incubations were terminated under gentle vacuum filtration with particulate collection onto 25-mm GF/F filters. Samples were then acid fumed (with addition of 250-μl 10% HCl) for 24-hr, dried and placed in 7-ml scintillation vials. Scintillation cocktail was then added (OptiPhase 'HighSafe' 3, Perkin Elmer) and samples were dark acclimated for 24-hr prior to be counted on a Wallac Scintillation counter.

We observed a systematic bias in primary production estimates determined from ^{13}C and ^{14}C approaches. It is unclear what the reason is for the observed bias which resulted in substantially higher ^{13}C-based primary production compared to ^{14}C-based primary production. Some of the bias may be the result of nonliving carbonate in the POC (C. Kendall, pers comm.) which would explain the observations as ^{13}C-based primary production relies on POC determinations whereas ^{14}C primary production is dependent on DIC concentrations and does not rely on estimates of POC. We present results of both ^{13}C and ^{14}C here focusing analysis on trends and caution against comparisons of absolute values reported for 13C-primary production between this dataset and those previously reported for the Sacramento River.

4.0 Results

4.1 River Characterization

July 2008 (WBD08-1)

Temperature varied little (± 1.5º C) between I-80 (21.6º C) and Isleton (22.5 ºC) and EC remained constant between all stations (Table 4). Nitrate, nitrite (NO_2), NH_4, urea, PO_4 and silicate ($Si(OH)_4$) showed small fluctuations between stations from I-80 to GRC (Table 4, Fig. 2,

Fig. 3) but then constituents increased downstream of RM-44. Nitrate increased downstream of HOD, along with PO_4; maximum values for NO_3 and PO_4 were observed at ISL (Table 4). Silicate remained consistently high (> 290 µmol L^{-1}) along the sampling transect. Ammonium concentrations increased at RM-44, reaching a maximum at HOD of 25.07 µmol L^{-1} (Table 4, Fig. 3B). The SRWTP discharge is located between GRC and RM-44.

Extracted chlorophyll-a in cells >0.7-µm diameter was 4.6 µg L^{-1} at I80, and declined downstream from OAK. At HOD, chlorophyll-a concentrations (1.9 µg L^{-1}) were less than 50% of values observed at I80 (Table 5). The chlorophyll-a concentrations for each of the two size fractions (>0.7 and >5.0-µm) suggested that there was a larger chlorophyll-a contribution (60 to 79%, mean of 70%) by cells >5-µm than smaller cells. These results were consistent with those measured by flow cytometry (Fig. 4A) which showed that most fluorescent particles ranged in size between 5 and 50-µm (fluorescent particle abundance was >2-fold higher than the abundance in the 1 to 5-µm size category, average for 1-5µm 7 x 10^2 cells ml^{-1} versus 2.2 x 10^3 cells ml^{-1} for 5 to 50-µm). Both size classes showed the same trend, with declining particle number between OAK and HOD consistent with the trends observed for chlorophyll-a (Table 5). Samples for particulate organic carbon (POC) and nitrogen (PON) were not measured during this transect (Table 6). Secchi depth decreased moving downstream between OAK and ISL from 1.4 to 1-m (Fig. 2A, Table 6).

November 2008 (WBD08-2)

Changes in temperature between I80 (13.9º C most northern) and ISL (14.7ºC most southern) were relatively small. Electrical conductivity increased downstream ranging from 181.9 (at GRC) to 233.0 µS (at RM-44) (Table 7). Nitrate was relatively constant (12 µmol L^{-1} to 13 µmol L^{-1}) along the transect from I80 to CRS, but increased ca. 2-fold at ISL (23.4 µmol L^{-1}) (Fig. 3A, Table 7). Phosphate increased at RM-44 and remained elevated at ISL. Similar to July 2008, $Si(OH)_4$ remained consistently high (> 300 µmol L^{-1}) along the transect (Fig. 2B, Table 7). Ammonium was elevated at I80 (7.73 µmol NH_4 L^{-1}) and decreased to GRC, but then sharply increased to a transect maximum concentration at RM-44 (70.82 µmol NH_4 L^{-1}). Ammonium declined throughout the rest of the transect but remained elevated (>35 µM) compared to the upstream stations (I80, OAK, and GRC) southward to ISL (Fig. 3B, Table 7). Extracted >0.7-µm chlorophyll-a ranged from 3.3 µg L^{-1} at I80 and decreased regularly southward toward ISL (0.6 µg L^{-1}) (Fig. 4B, Table 8). Chlorophyll-a contained in cells >5-µm in diameter also decreased from north to south (Fig. 4B, Table 8). Overall, cells >5-µm in diameter contributed between 76 and 99% of total chlorophyll-a, (averaging 85% over the whole transect). Flow cytometry results showed a pattern similar to chlorophyll-a with a declining trend from north to south (Fig. 4B); the highest fluorescent particle counts were observed at I80 (1.58 x 10^6 cells L^{-1}) and minimum values were measured at ISL (Table 8).

Particulate organic carbon (POC) ranged between 36 µmol L^{-1} and 97 µmol L^{-1} across all stations, and generally mirrored the decreasing chlorophyll-a trends. POC did increase from 38 µmol L^{-1} at GRC to 97 µmol L^{-1} at RM-44. This increase was inconsistent with chlorophyll-a suggesting that POC other than phytoplankton may have contributed to the POC pool at that station. Particulate organic nitrogen (PON) was low throughout the transect, ranging between 1 and 4 µmol L^{-1}. Daily primary production and phytoplankton nitrogen uptake rates were low (<0.3 µmol L^{-1} for C) throughout the transect (Fig. 6, Table 9). Secchi depth increased from I80

to ISL in November (from 0.9 to 1.7-m) indicating an increase in photic zone depth (Fig. 2A, Table 9)

March 2009 (WBD09-1)

Electrical conductivity and temperature did not vary appreciably between I80 and ISL (ca. 100 µS and ca. 11.4ºC for EC and temperature, respectively); EC did increase at RIO to 144 µS (Table 10). Nitrate, NO_2, $Si(OH)_4$, and urea showed little fluctuation between I80 and ISL (Table 10); while NH_4, which was low at I80 (1.0 µM) increased at RM-44 (10.09 µmol L^{-1}) and remained elevated (>10 µmol L^{-1}) to ISL before decreasing to 6.4 µmol L^{-1} at RIO (Fig. 3B, Table 10). Nitrate and NO_2 increased between ISL and RIO (Fig. 3A, Fig. 3D). Phosphate concentrations increased at RM-44 to 1.94 µmol L^{-1} (Fig. 2C, Table 10). Total chlorophyll-a was 1.9 µg L^{-1} at I80 and reached a maximum value of 3.8 µg L^{-1} immediately downstream at OAK and then decreased downstream to RIO (Table 11, Fig. 5). Chlorophyll-a in cells >5-µm in diameter ranged between 1.0 µg L^{-1} at RIO and 2.2 µg L^{-1} at OAK and GRC (Table 11) and chlorophyll-a in cells >5-µm contributed on average 62% to the total chlorophyll-a (Table 11). Flow cytometry data generally showed decreasing trends from I80 to RIO for fluorescent particle abundance with the highest numbers at I80 (1.1 x 10^6 particles mL^{-1}) and minimum values (0.7 x 10^6 particles mL^{-1}) measured at RIO (Table 10). Fluorescent particles between 5 to 50-µm in size were roughly 2-fold more abundant than smaller particles (average for size range 1 to 5-µm was 3.2 x 10^2 particles mL^{-1} and 6.6 x 10^2 particles mL^{-1} for 5 to 50-µm) (Fig. 5A).

POC values varied between 108 and 152 µmol C L^{-1} and PON values ranged between 6 and 12 µmol N L^{-1} (Table 11). Secchi depth remained relatively constant ranging between 0.4 and 0.5-m (Fig. 2A, Table 12). Primary production rates varied between 6.48 and 11.52 µmol L^{-1} d^{-1} across all stations (Fig. 6B, Table 12), and phytoplankton NH_4 uptake rates ranged from 0.53 to 0.98 µmol L^{-1} d^{-1} with the peak value observed at RM-44. Phytoplankton NO_3 uptake rates were greatest at the 3 upstream stations, I80, OAK, GRC, ranging from 0.34 to 0.41 µmol L^{-1} d^{-1} above SRWTP and then dropped to ≤0.07 µmol L^{-1} d^{-1} at all southward stations to RIO (Fig. 6B, Table 12) reflecting the increasing NH_4 concentrations moving in the downstream direction.

April 2009 (WBD09-2)

Temperature varied between 14.4 and 15.7ºC and EC varied between 104 and 140 µS between I80 and RIO; EC values did increase substantially at RIO (192.7 µS) (Table 13). Nitrate, NO_2, $Si(OH)_4$ and urea showed small fluctuations between I80 and ISL (Fig. 2C, Fig. 3A,C,D,Table 13); NO_3 increased from ISL to RIO from 8.50 to 24.60 µmol L^{-1} (ca. 3-fold increase). At HOD NH_4 and PO_4 increased (Fig. 2C, Fig. 3B, Table 13); NH_4 increased from <1 µmol L^{-1} up to 21 µmol L^{-1} while PO_4 increased from 0.6 µmol L^{-1} to 2.4 µmol L^{-1}, and remained elevated until RIO (Table 13). Total chlorophyll-a was relatively high between I80 and GRC (averaging 5.72 µg L^{-1}) and then declined to 4.7 µg L^{-1}. Downstream of RM-44 the values were in the same range as in March 2009. Chlorophyll-a in cells >5-µm contributed on average 76% to the total chlorophyll-a over the entire transect. Higher chlorophyll-a concentrations on the April 2009 cruise matched the higher observed concentrations of fluorescent particles (Table 14) (average for 1 to 5µm was 3.4 x 10^3 cells mL^{-1} and 1.1 x 10^3 cells mL^{-1} for 5 to 50µm) (Fig. 5B). In contrast to the other transects the smaller fluorescent particle size class was more abundant than the large size class.

POC values varied from 45 µmol L^{-1} to 87 µmol L^{-1} while PON ranged between 3 and 9 µmol L^{-1} (Table 14). Both POC and PON generally trended downward from northern to southern station following the pattern observed for chlorophyll-a. Secchi depth increased moving downstream from I80 to ISL (0.8-m to 1.8-m) and then decreased to 0.6-m at RIO (Table 15). Primary production ranged between 3.1 and 12.0 µmol L^{-1} d^{-1} across all stations, decreasing from I80 to ISL (Fig. 6C, Table 15). Phytoplankton NH_4 uptake rates ranged between 0.47 and 1.19 µmol L^{-1} d^{-1} along the transect, with the maximum value at RM-44; Phytoplankton NO_3 rates were highest at the 3 northernmost stations (I80, OAK, and GRC) ranging between 1.70 and 2.26 µmol L^{-1} d^{-1}. Below RM-44 NO_3 uptake decreased to <0.03 µmol L^{-1} d^{-1} to RIO (Fig. 6C, Table 15).

May 2009 (WBD09-3)

Temperature (16.2ºC ± 0.7) varied little between I80 and ISL while EC varied by ca. 25% (104 ± 25 µS) between these stations, increasing (to 125.7 µS) at RIO (Table 16). Nitrate, NO_2, $Si(OH)_4$ and urea showed little change between I80 and ISL (Fig. 2B, Fig. 3A,C,D, Table 16); with small increases in concentrations observed at RIO (particularly for NO_3, NO_2, and urea) (Table 16). At RM-44 NH_4 and PO_4 increased, in the case of NH_4 the increase was roughly 8-fold (from ca. 1 µmol L^{-1} up to 11.1 µmol L^{-1}). Phosphate increased from 0.9 µmol L^{-1} to 1.4 µmol L^{-1}, and remained elevated to ISL with another increase in concentrations at RIO (Table 16).

Total and >5-µm chlorophyll-a showed minor changes in concentration between I80 and ISL (4.5 to 6.2 µg L^{-1}), and then decreased significantly at RIO (2.26 µg L^{-1} and 0.79 µg L^{-1} for total and >5-µm fractions, respectively) (Fig. 5C, Table 17). The >5-µm size fraction made up on average, 69% of the total chlorophyll-a in the water column, with maxima at OAK (89%) and a gradual decrease towards RIO (35%). Both size classes of fluorescent particles showed a decreasing trend that was not reflected in the extracted chlorophyll-a analysis. Fluorescent particles counts were in the range of 4.1 to 9.4 x 10^2 mL^{-1} (average 6.75 x 10^2 mL^{-1}) for particles 1 to 5-µm and 8.4 to 15.5 x 10^2 particles mL^{-1} (average 11.6 x 10^2 particles mL^{-1}) for particles 5 to 50-µm. The highest fluorescent particle counts were observed at OAK (2.2 x 10^6 particles L^{-1}) while the minimum values (1.3 x 10^6 particles L^{-1}) were measured downstream at RIO (Fig. 5C, Table 17).

POC and PON values showed relatively large differences between stations (POC ranged between 56 and 180 µmol L^{-1}); PON values varied between 6 and 17 µmol L^{-1} across the eight stations (Table 17). There were no discernable trends in either parameter except for ISL where maximal values for both POC (180 µmol L^{-1}) and PON (17 µmol L^{-1}) were observed and were associated with an increase in chlorophyll-a (Table 17). Secchi depth remained relatively unchanged between I80 and ISL (0.4-m to 0.6-m) and then increased to 0.7-m at RIO (Table 18). Primary production ranged between 5.7 and 12.0 µmol L^{-1} d^{-1} along the transect, with no obvious trends along from north to south. Phytoplankton NH_4 uptake was lowest at I80, OAK and GRC and increased at RM-44 and remained high downstream (Table 18). Phytoplankton NO_3 uptake rates were greatest at the 3 northernmost stations above RM-44 (I80, OAK, GRC) ranging between 0.48 and 1.13 µmol L^{-1} d^{-1}, corresponding to where NH_4 concentrations were low (to just above detection limits). Phytoplankton NO_3 uptake rates were <0.06 µmol L^{-1} d^{-1} for the rest of the transect (Fig. 6D, Table 18).

Seasonal Comparisons of River Transects

Comparisons of river parameters between transect dates are shown in Figures 2, 3, 4, 5 and 6. Nitrate concentrations were lowest in July 2008 and April 2009, whereas during November 2008, March 2009 and May 2009, NO_3 concentrations were similar (ca. 10 μmol L^{-1} to 13 μmol L^{-1} at I80 (Fig. 3A). This difference may have been due in part to Sacramento River flow which was relatively low (13,033 and 12,035 cfs for July 2008 and April 2009, respectively). During the other two cruises in 2009, Sacramento River flow was >2-fold higher (Table 2). Sacramento River flow in November 2008 was similar to July 2008 and April 2009 but NO_3 concentrations were more similar to the high flow periods in 2009. NH_4 concentrations were always low ca. ≤1.0 μmol L^{-1} upstream of RM-44 and generally increased at RM-44. However, the maximal NH_4 concentrations were not always found at RM-44. The November 2008 NH_4 concentrations were substantially higher (up to 4-fold) than NH_4 concentrations on any other transect (Fig. 3B). Urea concentrations showed some spatial variation but no consistent spatial patterns across cruises (Fig. 3C). Urea concentrations were lower than either NO_3 or NH_4, ranging between <0.1 μmol L^{-1} and 1.3 μmol L^{-1} (Fig. 3C). NO_2 concentrations were also low throughout all transects but showed an increase in concentrations downstream of RM-44 (Fig. 3D). During March 2009 NO_2 concentrations were also elevated (approaching 0.6 μmol L^{-1}) at I80.

Nitrite concentrations were low along all transects relative to both NO_3 and NH_4 (below 2 μmol L^{-1}) however, the spatial patterns in NO_2 concentration may provide some indication of microbial processes likely to play a role in the magnitude and distribution of DIN in the Sacramento River. Figure 7 (April 2009) shows the typical pattern for NO_3, NH_4 and NO_2 along the eight station transect (note, for all other transect graphs and tables NO_2 is reported along with NO_3 as "NO_3 + NO_2"). Unlike NH_4, which increased rapidly downstream of RM-44, NO_2 concentrations tended to gradually increase downstream of RM-44. This suggests that SRWTP was not a large source of NO_2, however, the oxidation of NH_4 to NO_2 (i.e. the first step in the sequential oxidation of NH_4 to NO_3, nitrification) is likely the underlying mechanism responsible for at least some of the increase in NO_2. In addition, NO_3 concentrations steadily increased downstream of the SRWTP. Again, the gradual increase in NO_3 does not suggest that SRWTP is a major source of NO_3, but rather nitrification within the river may be important in the conversion of NH_4 to NO_3. Stable isotope analysis of NO_3 and NH_4 within the Sacramento River along this region also indicates the potential for substantial rates of nitrification (C. Kendall, pers comm.).

Secchi depth was lower during March 2009 and May 2009 compared to July 2008, November 2008 and April 2008 (Fig. 2A). This difference is likely due to the differences in Sacramento River flow with higher flows (ca. >25,000 cfs) resulting in lower Secchi depths (ca. 0.4-m) (Fig. 4A). Silicate concentrations were all high relative to the other inorganic nutrients measured during these transects, however, July 2008 did show lower $Si(OH)_4$ concentrations at all stations compared to the other transects (Fig. 2B). PO_4 concentrations all increased at RM-44 and generally continued to increase along transects moving southward. The exception to this trend was during November 2008, when PO_4 concentrations were all high relative to the other dates when these stations were visited (Fig. 2C).

Primary production was low in November 2008, compared to primary production estimates made during the three cruises in 2009 (Table 9, Fig. 6). During March and May, when Sacramento River flow was high (ca. 25,000 cfs, Table 2) primary production estimates did not show a discernable trend along the north to south transect (Fig. 6). In contrast, during April 2009 when Sacramento River flow was low (12,000 cfs) primary production estimates revealed a steady decline in rates from north to south, consistent with the patterns observed for chlorophyll-a (Table 15, Fig. 6).

4.2 River Grow-Out Experiments

July 2008

During July 2008 grow-out experiments were carried out for 216-hr using un-amended water collected at GRC and RM-44. Initial conditions of both stations (Table 19) were similar for inorganic nutrients, NO_3, PO_4, $Si(OH)_4$, and for chlorophyll-a. Initial NH_4 concentrations at GRC were 0.4 μmol L^{-1} and 9.06 μmol L^{-1} at RM-44. By 96-hr, NO_3 was exhausted in both enclosures and NH_4 and PO_4 were <1 μmol L^{-1}. Final chlorophyll-a concentrations were 14.7 μg L^{-1} at RM-44 versus 1.2 μg L^{-1} at GRC. The low initial concentrations of all inorganic nutrients in the GRC water resulted in nutrient limitation early in the grow-out experiments at GRC, resulting in lower overall chlorophyll-a GRC enclosures compared to RM-44. The larger pool of DIN, due to more available NH_4 at RM-44, was able to support a substantial gain in chlorophyll-a.

November 2008

Similar to July 2008, 96-hr grow-out experiments were carried out on un-amended natural assemblages of river phytoplankton from GRC and RM-44. Initial inorganic nutrients at both stations (Table 20) were similar for NO_3 and $Si(OH)_4$, but PO_4 and NH_4 were elevated at RM-44 (71.87 μmol L^{-1} compared to GRC 3.4 μmol L^{-1}). Initial chlorophyll-a concentrations were low, ca. 1μg L^{-1}. After 96-hrs of incubation RM-44 chlorophyll-a concentrations increased to 11.4 μg L^{-1} based mainly on NH_4 consumption of 12.5 μmol L^{-1}. The GRC grow-out chlorophyll-a increased to 16.7 μg L^{-1} based mainly on NO_3 drawdown of 9.2 μmol L^{-1}.

March 2009

Grow-out experiments during 2009 were different than those performed in 2008 in that additions of inorganic nitrogen were made (both as NO_3 and NH_4) to GRC enclosures so that both GRC and RM-44 grow-outs started with roughly the same ambient DIN (Table 21). Grow-outs were monitored daily for 96-hr. Nutrient amended samples were designated either as GRC + NO_3 or GRC + NH_4 for GRC grow-outs with additions of NO_3 or NH_4, respectively. We have reported nutrient and chlorophyll-a after the 96-hr incubation period under ambient temperature and 50% surface solar irradiance.

Nutrient concentrations in un-amended (i.e. no DIN additions) GRC and RM-44 grow-outs were similar (Table 21) for NO_3, $Si(OH)_4$, chlorophyll-a and PO_4. In contrast, NH_4 concentrations were 12-fold higher at RM-44 compared to GRC (12.47 versus 1.02 μmol L^{-1}). By 96-hr, NO_3 and PO_4 were exhausted in the un-amended GRC grow-outs, resulting in a total

chlorophyll–a yield of 12.6 μg L^{-1}. Un-amended grow-outs from RM-44 showed roughly 33% more chlorophyll-a (18.83 μg L^{-1}) compared to the un-amended GRC grow-outs. Larger phytoplankton cells (> 5-μm) made up about 70% of the chlorophyll-a biomass in GRC and 60% in RM-44. RM-44 phytoplankton growth appeared to be driven more by NH_4 drawdown; while NO_3 drawdown was more significant in un-amended GRC grow-outs (Table 21).

In the amended grow-outs, the GRC+NH_4 treatment behaved similarly to un-amended RM-44, accumulating similar concentrations of chlorophyll-a over the 96-hr period, (17.0 μg L^{-1}) and utilizing NH_4 rather than NO_3 in support of that phytoplankton growth. The GRC + NO_3 treatment accumulated the lowest chlorophyll-a concentrations (10.9 μg L^{-1}) during the 96-hr growth period and behaved mostly similarly to the un-amended GRC river water.

May 2009

Both un-amended and DIN amended grow-out experiments were also carried out in May 2009 (Table 22). Un-amended grow-outs from GRC and RM-44 were similar in initial concentrations of NO_3, $Si(OH)_4$, and chlorophyll-a (Table 22) while initial NH_4 was 7-fold higher at RM-44 compared to GRC (12.47 vs. 1.02 μmol L^{-1}) and PO_4 was 2-fold higher at RM-44 compared to GRC (1.24 versus 0.77 μmol L^{-1}). By 96-hr, NO_3 and PO_4 were exhausted in the un-amended GRC grow-out resulting in chlorophyll-a concentrations of 9.0 μg L^{-1}. Chlorophyll-a concentrations in un-amended RM-44 grow-outs were roughly 25% higher than those at GRC (13.8 μg L^{-1}). By 96-hr RM-44 grow-outs showed both NO_3 and NH_4 drawdown, with both constituents approaching 0 (Table 22). Interestingly, unlike previous grow-out experiments there was significant silicate drawdown observed in the RM-44 grow-out.

Amended grow-out treatments behaved similarly to each other, with accumulations of chlorophyll-a of 13 μg L^{-1} over 96-hr. Both treatments used both NO_3 and NH_4 to support growth. Silicate drawdown was noted more significantly in these experiments than in the previous months.

4.3 River Water Aging Experiment

20-L cubitainers were collected on July 2008 at GRC and RM-44 and incubated in the dark at 21ºC for 8 weeks (Table 23). Initial NO_3 + NO_2 and $Si(OH)_4$ concentrations were similar between the two water samples (0.64 μmol N L^{-1} and 0.47 μmol N L^{-1} and 311 μmol $Si(OH)_4$ L^{-1} and 295 μmol $Si(OH)_4$ L^{-1} respectively). Initial PO_4 concentration in RM-44 water was much higher (3.8-fold higher) than PO_4 in GRC water, as was the initial NH_4 concentration that was >20-fold higher in RM-44 water (17.5 μmol L^{-1}) compared to GRC (0.8 μmol L^{-1}). Inorganic nutrient concentrations measured weekly in the two cubitainers showed a decrease in NH_4 and an increase in NO_3+NO_2 over time. This was well demonstrated for RM-44 where initial NH_4 was higher (17.5 μmol L^{-1}) and all converted to NO_3 (NO_3 = 17.6 μmol L^{-1}) after 2 weeks. The overall change in NO_3+NO_2 and NH_4 in the GRC cubitainer was less clear. After one week there was little change in any of the inorganic nutrients monitored in either cubitainer (GRC or RM-44). The initiation of the decrease in NH_4 and conversion to NO_3+NO_2 occurred after two weeks and had reached completion (i.e. near complete conversion of all available NH_4 to NO_3 + NO_2) after 3 weeks. Phosphate concentrations did not vary substantially from the initial concentrations suggesting that PO_4 was not required or produced (e.g. via DOM degradation) as a result of this

nitrogen transformation. The same was observed for $Si(OH)_4$ that remained high throughout the 8 weeks.

4 .4 NH_4Cl Addition Experiment

During April 2009 (WBD09-2) NH_4 was added to river water from GRC (upstream of the SRWTP with low NH_4 concentrations) and the effect on primary production and phytoplankton nitrogen uptake was compared to un-amended water from GRC and RM-44 (RM-44 is immediately downstream of SRWTP discharge) (Table 24). Interestingly both GRC and RM-44 had low initial NH_4 concentrations; 0.35 µmol L^{-1} at GRC and 0.87 µmol L^{-1} in RM-44 water, atypical for RM-44. Three concentration additions of NH_4Cl were made to the GRC water: +1 µmol NH_4 L^{-1}, +4 NH_4 µmol L^{-1}, and +50 NH_4 µmol L^{-1}. Primary production was lowest at RM-44 as was chlorophyll-a (Table 14) (0.9 µmol C L^{-1} h^{-1}, Table 24). In experiments using GRC water there was little effect on primary production with NH_4Cl additions (Table 24) up to 4 µmol L^{-1}. The small decline in primary production (0.1 µmol C L^{-1} h^{-1}, ca. 8% of primary production at GRC) between un-amended GRC and GRC +50 µmol NH_4 L^{-1} observed in ^{13}C-based primary production was significant (t-test, P = 0.02) but similar declines were not evident in the ^{14}C-based estimates. Phytoplankton NH_4 uptake showed a small decrease with additions of NH_4 (0.013 µmol L^{-1} h^{-1}). The strongest effect of added NH_4Cl was observed in estimates of phytoplankton NO_3 uptake, which was reduced in all the NH_4 addition treatments (from 0.028 µmol L^{-1} h^{-1} to 0 µmol L^{-1} h^{-1} with the +50 µmol NH_4 L^{-1} addition. The total N uptake declined steadily with NH_4 additions from 0.088 µmol L^{-1} h^{-1} to 0.050 µmol L^{-1} h^{-1} at the +50 µmol L^{-1} addition, a decrease of 43% over GRC +1 µmol L^{-1}. This experiment unfortunately started with water that had uncharacteristic initial nutrient conditions; lower NO_3 at GRC and lower NH_4 at RM-44 than typically measured.

4.5 Effluent Addition Experiments

An experiment similar to that described by MacIsaac et al. (1979) was carried out in May 2009 to evaluate the impact of SRWTP effluent on primary production and phytoplankton N uptake. Primary productivity and phytoplankton nitrogen uptake were estimated in bottles filled with water from GRC and serial additions of SRWTP effluent-NH_4 or equivalent concentrations of NH_4Cl. Ambient NH_4 concentrations were 1.65 µmol L^{-1} and 31.71 µmol L^{-1} at GRC and RM-44, respectively. NH_4 concentrations at GRC were well below the 4 µmol L^{-1} total NO_3 uptake inhibition threshold described by Dugdale et al (2007); but ca. 1 µmol L^{-1} ambient NH_4 may have had some inhibitory effect on measured phytoplankton NO_3 uptake at GRC during the experiment. The initial NO_3 concentration at GRC was 2.16 µmol L^{-1} (versus 2.71 µmol L^{-1} at RM-44).

The NH_4 concentration in the SRWTP composite sample was 1933 µmol L^{-1}. This was added to GRC water as a dilution series along with a comparable set of bottles using a dilution series of NH_4Cl. The NH_4 addition series ranged from 0.40 µmol N L^{-1} to 100 µmol N L^{-1}, representing effluent dilutions of 1:20 to 1:5000 (Table 25, Table 27, Table 28). The SRWTP must maintain a dilution of 1:14; based on the effluent composite NH_4 concentration, the 1:14 dilution would result in river concentrations of ca. 143 µmol L^{-1} in the immediate discharge receiving waters. It should be noted that the river concentrations that were observed during the transects completed in 2008 and 2009 never reached more than 50% of this theoretical value (the highest recoded NH_4 value during all transects was ca. 70 µmol L^{-1} at RM-44, recorded during

November 2008). As with the NH_4Cl addition experiment described in Section 4.4, the relationship between primary production and NH_4 concentration was investigated using both ^{14}C-HCO_3 and ^{13}C-HCO_3 tracers.

^{14}C-Primary production under ambient NH_4 concentrations at RM-44 was also measured for comparison with rates for un-amended GRC water and was lower- by almost a factor of 2. ^{14}C-primary production at GRC varied between 1.00 to 1.33 μmol L^{-1} h^{-1} across the NH_4 addition series with the highest primary production rates reported at NH_4Cl additions of +0.50 and +16 μmol NH_4 L^{-1} (Table 26). ^{14}C-primary production rates in bottles with additions of SRWTP effluent varied between 0.61 and 1.20 μmol C L^{-1} h^{-1} with the highest primary production values measured at +4 and +16 μmol effluent-NH_4 L^{-1}. The lowest ^{14}C-primary production estimates were found at +32 and +100 μmol effluent-NH_4 L^{-1} h^{-1} and the difference between "GRC + 0" effluent-NH_4 versus "GRC+100" effluent NH_4 was 0.35 μmol C L^{-1} h^{-1}, equivalent to a 36% decline in primary production.

Primary production estimates using ^{13}C were higher than those reported using ^{14}C (see Section 3.5) but showed similar trends. Treatments with additions of NH_4Cl resulted in ^{13}C-primary production values that ranged between 1.49 and 1.74 μmol L^{-1} h^{-1} with no discernable trends with increasing additions of NH_4Cl (Table 27). ^{13}C-Primary production in treatments with wastewater effluent-NH_4 additions showed declining trends at wastewater effluent-NH_4 >8 μmol NH_4 L^{-1}. At effluent concentrations <8 μmol NH_4 L^{-1} ^{13}C-primary production ranged between 1.64 and 1.83 μmol L^{-1} h^{-1}. At effluent-NH_4 additions >8 μmol L^{-1} h^{-1}, ^{13}C primary production ranged between 1.28 and 1.65 μmol L^{-1} h^{-1}. The difference in ^{13}C-primary production estimates between "GRC+0" effluent-NH_4 and "GRC+100" effluent-NH_4 addition represented a 22-27% decline in primary production.

The impact of NH_4 additions on phytoplankton NH_4 uptake also differed between NH_4Cl and effluent-NH_4 treatments. The relationship between phytoplankton NH_4 uptake versus NH_4 concentrations (as effluent NH_4 or NH_4Cl) was investigated using specific NH_4 uptake, V (NH_4 uptake normalized to particulate organic nitrogen in the sample, analogous to a specific growth rate, at "balanced" growth) in order to evaluate nutrient kinetic relationships (i.e. Michaelis-Menten formulation). The data fit the Michaelis-Menten equation reasonably well at low concentrations but data at higher NH_4 concentrations deviated from the model expectation. A theoretical maximum NH_4 uptake rate was calculated (conservatively, dotted line, Fig 9) but confidence in this value is low and the experiment should be repeated. Phytoplankton NH_4 uptake responded positively to NH_4Cl additions (Table 28, Fig. 8) and could be modeled as a classical Michaelis-Menten response (Fig. 9). In contrast, phytoplankton NH_4 uptake initially showed a positive response to additions of effluent-NH_4 to an effluent-NH_4 addition of 8 μmol L^{-1} but then declined by 29% at 100 μmol L^{-1} compared to maximum phytoplankton N uptake. This decline in phytoplankton NH_4 uptake as well as the minimum effluent-NH_4 addition at which the decline begins to occur is consistent with the decline in primary production described above (Table 26 and 27, Fig. 8).

Increasing concentrations of either effluent or NH_4Cl caused a decrease in NO_3 uptake by GRC phytoplankton (Table 27). With no NH_4 additions, NO_3 uptake was 0.036 to 0.038 μmol N L^{-1} h^{-1}. Phytoplankton NO_3 uptake was reduced to 0.006 – 0.007 with 100 μmol N L^{-1} h^{-1} (Table 27). The decrease in NO_3 uptake was evident even at the some of the lowest additions (0.5 μmol L^{-1} NH_4); at the highest NH_4 concentration addition (100 μmol NH_4 L^{-1}) phytoplankton NO_3 was

reduced by 80% compared to control samples with no addition (Table 27). The total N uptake declined from a peak of 0.165 μmol L^{-1} h^{-1} (at +0.25 μmol NH4 L^{-1}, ρNH_4 = 0.132 μmol L^{-1} h^{-1}, ρNO_3 = 0.033 μmol L^{-1} h^{-1}) to 0.078 μmol L^{-1} h^{-1} (at 100 μmol L^{-1} effluent-NH_4, ρNH_4 = 0.071 μmol L-1 h-1, ρNO_3 = 0.007 μmol L^{-1} h^{-1}). A reduction of total N uptake at high NH_4 concentrations is consistent with the April 2009 NH_4Cl addition experiment results.

5.0 Discussion

Analysis of long-term trends in environmental parameters, including nutrients and nitrogen speciation provide insight into potential bottom-up controls on phytoplankton biomass in the San Francisco Estuary (SFE) Delta (e.g. Jassby, 2008; Glibert, 2010). These studies result in an integrated view of ecosystem-level changes and help to elucidate the primary drivers of the observed declines in phytoplankton that have been tied to collapse of the pelagic organism decline (POD) species (Sommer et al., 2007). One limitation of such integrative analysis of long term monitoring data is the potential that more subtle, but nonetheless important, changes in bottom-up drivers of phytoplankton growth will be masked by the complex interaction of several drivers operating together to impact the POD (Sommer et al., 2007). The goal of the present study was to investigate the potential for anthropogenic NH_4 loading in the SFE to act as a bottom-up control on phytoplankton blooms (Wilkerson, et al 2006; Dugdale et al 2007; Glibert, 2010). The study was designed to look at three time and spatial scales (i.e. in situ measurements during river transects, 96-hr "grow-out" experiments, and 4-hr bottle incubations with manipulated NH_4) and relies, in part, on phytoplankton physiology (i.e. primary production rates and phytoplankton N uptake) to provide a more sensitive indicator of environmental stress. The strength of using phytoplankton physiology as an indicator of environmental stress is that individual environmental stressor effects can potentially be detected on time scales of minutes to hours. The reliance on changes in phytoplankton biomass (or chlorophyll-a) or shifts in phytoplankton community composition as indicators of environmental stress require time scales of days to weeks – over these time scales interaction of multiple environmental stressors likely mask the contribution that an individual stressor makes to overall declines in phytoplankton. The results presented here are an attempt to constrain the role that anthropogenic NH_4 loading and subsequent elevated NH_4 concentrations play as a bottom-up control on phytoplankton in the Sacramento River.

In situ measurements made during the five Sacramento River transect surveys, and those completed by Foe et al. (2010) help to elucidate in a broad sense the environmental setting in the Sacramento River (i.e. nutrient concentrations, phytoplankton biomass and in situ primary production and phytoplankton N uptake). 96-hr "grow-out" experiments provide results on time scales of days whereas the 4-hr incubation experiments provide results on time scales of minutes to hours. Because of the differences in temporal and spatial scales of each of these approaches it is not surprising that interpretation of results from each approach leads to different and sometimes conflicting conclusions. Insight into the potential effect of NH_4 on phytoplankton biomass, primary production and phytoplankton N uptake can be gained from considering each of the approaches used here.

5.1 SRWTP Effect on Inorganic Nutrient Distributions in the Sacramento River

River characterizations show that while nutrient concentrations varied substantially in the Sacramento River on a seasonal basis, discharge from the SRWTP has a large effect on river nutrient concentrations, particularly for NH_4 and PO_4. Secondarily, NH_4 from the SRWTP outfall appears to influence the spatial distribution of elevated NH_4, NO_2, and NO_3 at locations downstream of the SRWTP, likely as the result of nitrification (Fig. 7). We cannot further assess nitrification rates with the current dataset available but additional studies will be initiated to better constrain this biogeochemical pathway for inorganic nitrogen in the Sacramento River.

Because of the limited number of data points for NH_4 concentrations collected at each station during this study (n = 5), and the large range in values encountered during transect surveys, mean NH_4 concentrations were not calculated for the Sacramento River immediately downstream of the SRWTP outfall (i.e. at RM-44 and HOD). However, estimates of mean river NH_4 concentrations are necessary to determine "environmental relevance" for results obtained during short duration bottle incubations (i.e. "clean" NH_4Cl and effluent-NH_4 addition experiments). Specifically, are the concentrations used in the short duration bottle incubations similar to NH_4 concentration found in the Sacramento River downstream of the SRWTP outfall? The Central Valley Regional Water Quality Control Board (CVRWQCB) surveyed NH_4 concentrations in the river downstream of SRWTP over 16 months from March 2009 to February 2010 (Foe, 2010). The CVRWQCB found NH_4 concentrations at HOD averaged 32.99 ± 7.40 μmol L^{-1} (95% CI, n = 16) (Foe 2010, Table 1A). Similar to the results presented here, variability in NH_4 concentrations collected by CVRQWCB at HOD was large; ranging between 2.86 to 50.71 μmol L^{-1} (more than a factor of 17-fold) with no pronounced seasonal pattern. The SRWTP also collected samples weekly between July 2008 and June 2009 at HOD for NH_4 analysis (Fig 10). The SRTWP dataset also shows large annual variation in NH_4 (20.71 to 92.86 μmol L^{-1}) with a mean NH_4 of 47.86 ± 5.70 μmol L^{-1} (95% CI, n = 49). In most months NH_4 exceeded 50 μmol L^{-1} at HOD (the exceptions were August 2008 (maximum NH_4 = 41.43 μmol L^{-1}) and May 2009 (maximum NH_4 = 29.29 μmol L^{-1})). Finally, Larry Walker and Associates calculated NH_4 concentrations downstream of the SRWTP discharge from records of NH_4 discharge and Sacramento River Flow and suggested mean NH_4 of 39.01 ± 1.38 (95%CI, n = 365) and a range of 10.93 to 77.36 μmol L^{-1} during the period between July 2008 and June 2009 (SRCSD, 2010). The same modeling study suggests increasing NH_4 concentrations at HOD over the POD years (2002-2009) of approximately 2 μmol L^{-1} yr^{-1} and a median value of 25.7 μmol L^{-1} over the entire period. Each of these approaches clearly show that the NH_4 concentration range used in the short duration NH_4 incubation experiments (1 to 50 μmol L^{-1} and 1 to 100 μmol L^{-1}) are "environmentally relevant" representing NH_4 concentrations regularly experienced by phytoplankton in the Sacramento river between the SRWTP outfall and HOD, approximately 10 km downstream of the discharge.

While samples for total dissolved nitrogen (TDN) and dissolved organic nitrogen (DON) were collected and analyzed during this study by the SRTWP, these results were not available at the time of the report. Foe et al. (2010) presented TND and DON from their 16 month long survey of river nutrient chemistry at HOD. NH_4 represented 60% ± 12 (±95% CI, n = 16) of the TDN (range 4 to 92%). In contrast DON contributed between 5 and 9% of the N to TDN (removing on sampling data, March 16) (Foe et al., 2010, Table 1A). In >2/3 of the sampling events, DON increased between GRC and HOD presumably as a result of SRWTP discharge. In 10 of 16 sampling events, DON decreased between HOD and Walnut Creek. The DON decrease

averaged 4.85 μmol L^{-1} or roughly 35% of the DON concentration at HOD. The decrease in DON may have been through ammonification (DON conversion to NH_4), contributing a relatively small amount of NH_4 (ca. 5μmol l-1) to the NH_4 pool.

5.2 SRWTP Effect on Phytoplankton N Uptake Rates

Compared to the three stations (I80, OAK, GRC) upstream of the SRWTP, phytoplankton NH_4 uptake increased immediately downstream of the wastewater discharge (at RM-44), presumably in response to higher NH_4 concentration supplied via SRWTP. Upstream of SRWTP NH_4 concentrations were typically low (<1 μmol L^{-1}) and so phytoplankton NH_4 uptake may have been NH_4-limited. Experimental additions of NH_4Cl up to +50 μmol L^{-1} resulted in a decrease in phytoplankton NH_4 uptake during April 2009 whereas experimental additions of NH_4Cl made in May 2009 enhanced phytoplankton NH_4 uptake up to ca. 4 μmol NH_4 L^{-1} (i.e. apparent saturation for NH_4 in the May 2009 Sacramento River N uptake kinetics experiment). This would imply that NH_4 concentrations greater than 4 μmol N L^{-1} should not lead to further enhancement of phytoplankton NH_4 uptake. SRWTP effluent (tracked as "effluent-NH_4") led to a decrease in phytoplankton NH_4 uptake at concentrations greater than 4 μmol N L^{-1}; in the one experiment conducted using effluent-NH_4 additions, phytoplankton NH_4 uptake decreased by 29% at 100 μmol "effluent NH_4" L^{-1} when compared to the maximum value observed. The concentration threshold of 8 μmol N L^{-1} represents a wastewater effluent dilution of greater than 200:1 (based on an undiluted effluent NH_4 concentration of 1933 μmol N L^{-1}), significantly higher dilution than required under the current SRWTP permitting (http://www.waterboards.ca.gov/centralvalley/board_decisions/adopted_orders/sacramento/5-00-188_npdes.pdf). NH_4 concentrations supplied to the Sacramento River by SRWTP as wastewater effluent resulted in NH_4 consistently >4 μmol L^{-1} observed at stations downstream of the SRWTP during river characterizations.

Phytoplankton NO_3 uptake was high at stations upstream of the SRWTP but decreased abruptly to near detection limits (0.02 μmol L^{-1} d^{-1}) at stations downstream of the SRWTP. The shut down of phytoplankton NO_3 uptake appears to be the result of the well described phenomenon of NH_4 inhibition of phytoplankton NO_3 uptake. During all manipulation experiments where NH_4 was added to river water (either as NH_4Cl or SRWTP effluent-NH_4; i.e. manipulated grow-outs, clean NH_4Cl addition and SRWTP effluent addition experiments) phytoplankton NO_3 uptake was always reduced to near detection limits with NH_4 additions as low as <1 μmol NH_4 L^{-1} indicating that the phytoplankton community was sensitive to NH_4 concentrations, consistent with previous findings for the northern SFE (Wilkerson et al., 2006; Dugdale et al., 2007); this condition was observed during all sampling dates.

Clearly SRTWP discharge reduces phytoplankton access to NO_3. There remains some uncertainty as to the impact of the reduction of phytoplankton NO_3 uptake on primary production and nitrogen processing in the Sacramento River. Dugdale et al (2007) hypothesized that fast growing coastal diatoms were more sensitive to NH_4 inhibition of NO_3 uptake in the northern SFE and suggested that anthropogenic NH_4 loading to the estuary likely reduces the relative importance of diatoms during spring bloom events. We cannot fully evaluate potential shifts in the phytoplankton community with the present dataset (see below) but phytoplankton samples were collected and preserved for later analysis.

Table 28 summarizes the observed impacts of effluent and NH_4Cl additions on phytoplankton nitrogen uptake during experiments conducted using NH_4Cl and a composite sample of effluent from SRWTP. Primary production and phytoplankton NH_4 uptake appeared to be negatively impacted by SRWTP effluent at additions >8 μmol NH_4. In the one experiment with SRWTP conducted primary production was by up to ca. 30% with additions of 100 μmol L^{-1} effluent-NH_4 and phytoplankton NH_4 uptake was reduced by nearly 1/3. The impact of additions of NH_4 (either as NH_4Cl or effluent NH_4) on phytoplankton NO_3 uptake was consistent with a reduction of 80% of phytoplankton NO_3 uptake compared to the controls (with no NH_4Cl added). Additions of NH_4 as effluent or NH_4Cl result in reduced uptake of DIN at concentrations >4 to 8 μmol L^{-1}. The reductions in phytoplankton N uptake shown during the one experiment with SRWTP effluent provide evidence for a potential effect of SRWTP additions. However, this experiment should be repeated with the impacts established here validated.

5.3 SRWTP Effect on Primary Production and Phytoplankton Blooms

Primary production estimates obtained during the river transects show a decline in productivity from the City of Sacramento south to Rio Vista. The decline in primary production was consistent with the decrease in chlorophyll-a, which raises the question of whether the change in primary production is a function of a change in biomass or of photosynthetic activity. Chlorophyll-a normalized primary production (P/B, Tables 9, 12, 15, 18) was calculated for stations during each of the transects where primary production estimates were made. Patterns in P/B were not consistent spatially across cruises. During March and April 2009 P/B values were depressed slightly compared to upstream stations. During November and May the opposite trend or no trend was apparent. The patterns observed in March and April are consistent with the experimental results obtained with effluent addition experiments (May 2009, Section 4.5) where primary production rates decreased at elevated SRWTP effluent NH_4 additions. However, the validity of this type of analysis is very sensitive to low chlorophyll-a concentrations; at these lower chlorophyll-a concentrations sampling artifacts and associated error may undermine interpretation of *in situ* phytoplankton physiology.

Results from experimental grow-outs suggest that after removing light limitation phytoplankton bloom magnitude in the Sacramento River at RM-44 and GRC is likely determined by dissolved inorganic nitrogen (DIN) availability. Grow-out experiments conducted downstream of the SRWTP (RM-44) produced more chlorophyll-a than experimental grow-outs conducted upstream of the SRWTP (GRC). Phytoplankton used additional DIN, either as NO_3 or NH_4 in experiments conducted with water from GRC, or in the form of NH_4 supplied in the wastewater effluent (at RM-44) to produce greater biomass. Water collected at RM-44 and used in "grow-out" experiments may not be completely representative of the Sacramento River after SRWTP discharge has been introduced. Routinely, the highest NH_4 concentrations were not found at RM-44 but further downstream at HOD suggesting that wastewater effluent was not completely mixed at RM-44 (D. Engle, pers. comm.). During separate Sacramento River surveys conducted in spring 2009, Parker, et al (in review) found a depression in primary production and phytoplankton NH_4 uptake with increasing NH_4 concentrations at 6 stations between RM-44 and Isleton also suggesting that using water collected at RM-44 for :grow-out" enclosures may not have fully captured the influence of SRWTP in the Sacramento River.

Additions of NH_4Cl to water collected at GRC, at additions of 0.25 μmol L^{-1} to 100 μmol L^{-1} during May 2009 showed no effect on primary production during 4-hr experiments suggesting that phytoplankton uptake potential was not N-limited in the Sacramento River above the SRWTP discharge location. If phytoplankton were N-limited, primary production and phytoplankton N uptake would have increased in response to additions of NH_4. The apparent N-limitation noted in July 2008 grow-out experiments conducted using water from GRC, occurred around 48-hr and under saturating irradiance (50% of surface PAR). The irradiance values and biomass accumulation in grow-out experiments are not encountered *in situ* in the Sacramento River. *In situ* conditions, including low irradiance conditions and phytoplankton biomass losses (e.g. zooplankton grazing or sinking phytoplankton cells (Foe et al., 2010) are eliminated during grow-out experiments. In contrast to the results from May 2009, during April 2009 additions of NH_4Cl (up to +50 μmol L^{-1}) did result in decreased primary production. During May 2009, experimental additions of SRWTP effluent-NH_4 resulted in decreased primary production rates (in the one experiment the decrease was up to ca. 36% compared to the control) (Table 28); Similar to the results for phytoplankton NH_4 uptake, the decline in primary production became evident at effluent-NH_4 concentrations greater than 8 μmol N L^{-1}. The NH_4 concentrations that were measured during the five transect surveys made during this study appear to be low compared to the more comprehensive sampling programs for NH_4 by Foe et al (2010) and the SRWTP. Based on the more comprehensive measurements of NH_4, the NH_4 concentrations that were tested during the effluent NH_4 addition experiments and NH_4Cl appear to reflect NH_4 concentrations *in situ* in the Sacramento River. Because of the April 2009 results, we cannot rule out an effect of NH_4Cl on primary production but the decrease in primary production that was observed with additions of effluent-NH_4 may be the result of NH_4 or some unidentified component of the SRWTP discharge; if that was the case, NH_4 in this experiment was a tracer of increasing concentrations of this toxicant.

It is unlikely that the decline in chlorophyll-a and fluoroprobe pigments that were observed along transects from the upper river to RM44 is the result of phytoplankton light adaption (i.e. changes in chlorophyll-a cell quota due to changes in the light field experienced by phytoplankton *in situ*) as flow cytometer particle counts declined along with chlorophyll-a. In addition, photic zone depths did not show a consistent trend of either increase or decrease moving downstream from I80 to RM-44 providing evidence of changes in light availability.

The degradation product of chlorophyll-a, phaeophytin, was measured during transects and provides insight into the declining patterns observed for chlorophyll-a in the Sacramento River (Fig. 11). Phaeophytin concentrations were relatively low upstream of the wastewater treatment plant (1 to ~3μg L^{-1}), equivalent to 30-40% of the chlorophyll-a concentration. Phaeophytin concentrations generally increased towards RM44 or HOD (while chlorophyll-a concentration consistently decreased) both in absolute and relative concentrations, representing 90-100% of the chlorophyll-a concentrations at RM-44 during July 2008 and April 2009. Downstream of RM44, phaeophytin and chlorophyll-a decreased at roughly the same rate and maintaining similar proportions. The patterns in chlorophyll-a and phaeophytin are less clear during March and May 2009 when river flows were high and the phaeophytin signal may have been diluted.

Phaeophytin is produced through the degradation of active chlorophyll-a; elevated phaeophytin is typically ascribed to zooplankton grazing on phytoplankton but phaeophytin can also be produced through the senescence of phytoplankton cells. Zooplankton grazing was not

assessed during the study and we are not aware of zooplankton studies for the Sacramento River so there is no means to test this potential mechanism to explain the phaeophytin pattern but increased zooplankton activity downstream of Sacramento could explain the patterns observed. The grazing hypothesis is puzzling as there is no clear reason to expect zooplankton grazing to be low at upstream stations with increased grazing toward the SRWTP, and relatively constant grazing pressure maintained at downstream stations. An alternative hypothesis is that chlorophyll-a was produced mainly at upstream locations with little additional *in situ* production of chlorophyll-a moving south of GRC. The increase in absolute phaeophytin and the relative increase in phaeophytin compared to chlorophyll-a may reflect the ongoing senescence of active chlorophyll-a with little new production of chlorophyll-a.

Foe et al. (2010) examined the relationship between the decline in chlorophyll-a and Sacramento River mean daily flow and 15-minute river velocity. The authors found a relationship between 15-minute instantaneous flow and the loss of chlorophyll-a, suggesting that the loss in chlorophyll-a may be the result of settling of phytoplankton cells and inability for phytoplankton cells to be resuspended. This plausible hypothesis still supports the contention that chlorophyll-a production occurs primarily upstream of the SRWTP with little in situ chlorophyll-a production at locations downstream of the SRWTP. Had there been sufficient chlorophyll-a production at downstream locations there would be a resupply of surface chlorophyll-a.

5.4 Phytoplankton Species Composition Changes as a Result of SRWTP Effluent (Including NH_4) Discharge

Flow cytometer data and size-fractionated chlorophyll-a concentrations provide a proxy for assessing changes in the phytoplankton community along the longitudinal extent of the Sacramento River covered during these transects. Based solely on size fractionated chlorophyll-a there does not appear to be consistent spatial patterns in the partitioning of chlorophyll-a into two size classes (>5-µm and <5-µm diameter cells). In general, the highest proportion of chlorophyll-a in >5-µm cells appears to be in stations upstream of SRWTP (i.e. November 2008, March, April and May 2009) but this is not always the case (i.e. July 2008) and in general the percent differences are not large between stations. If these general trends were validated with additional sampling events it would be consistent with the hypothesis put forth by Dugdale et al (2007) in which larger cells (assumed to by diatoms) would be favored in the NO_3-driven upper Sacramento River and smaller cells (i.e. flagellates, cyanobacteria) would be favored in the NH_4-driven SRWTP influenced region of the River. Analysis of the archived phytoplankton samples would more fully elucidate any changes in phytoplankton community composition.

6.0 Recommendations for Future Studies

6.1 Determination of the Underlying Causes for Declining Chlorophyll-a Concentrations From the City of Sacramento to Rio Vista, CA.

A critical feature, previously undescribed until this study, was the apparent decline in chlorophyll-a along the full extent of the north-south transect to Rio Vista. Based on the present results it is not clear whether the decline in chlorophyll-a is due in part to SRWTP and there may

be additional factors also responsible. More detailed characterization of this phenomenon, including full spatial extent (i.e. chlorophyll-a and phytoplankton uptake rate measurements beginning north of I80 in the Sacramento River to RIO) and phytoplankton counts (see below) are needed.

Hypotheses:

1) Phytoplankton are starved for nitrogen due to the shut-down of phytoplankton NO_3 uptake and the impairment of phytoplankton NH_4 uptake (suggested by the effluent addition experiment).
2) There is only localized production of chlorophyll-a at upstream stations (I80 to GRC). Downstream in proximity to the SRWTP phytoplankton are unable to maintain cell numbers.
3) Increased grazing rates of chlorophyll-a from upstream to downstream results in a loss of chlorophyll-a.
4) Changes in phytoplankton species composition brought about by the change from NO_3 to NH_4 supported production results in inherent growth rates that are too low to offset grazing and other loss rates.
5) Some unknown toxin is synthesized *in situ* or supplied to the Sacramento River, and gains potency downstream reaching a maximum near Rio Vista.

Detailed analysis of phytoplankton community composition and phytoplankton C and N uptake rates along the north to south transect would help to more fully characterize the potential effect of SRWTP effluent on phytoplankton responses in the Sacramento River. It must be noted that chlorophyll-a is not strictly biomass (mass in carbon) and variation in C : chlorophyll-a ratio can "mask" more subtle patterns in phytoplankton biomass and production within the Sacramento River. Using a Fast- Repetition Rate Fluorometer (FRRF) would reveal impairment to the photosystems as a result of changing nutrient or the presence of toxicants.

Further, results from the present study cannot address the potential for SRWTP effluent to act to negatively shape the phytoplankton community in the Sacramento River (i.e. reduce the abundance of nutritious taxa and increase the abundance of less desirable species). Again, samples for phytoplankton identification were collected and preserved from each of the river characterization stations and should be analyzed.

6.2 Determination of the Underlying Causes for Variability in NH_4 Concentrations Downstream of the SRWTP Discharge at RM-44.

The present results show that SRWTP discharge is a significant source of NH_4 and PO_4 to the Sacramento River. However, the highest concentrations of NH_4 were not always observed immediately downstream of the effluent source (i.e. RM-44).

Hypotheses:

1) Inappropriate sampling of hydrographic features, i.e. a non-homogeneous field or incomplete mixing.
2) Ammonification of dissolved organic nitrogen in the effluent, i.e. incomplete secondary treatment.
3) Unknown source (e.g. sediment flux).

Future studies are needed to close the nutrient budget for downstream flow of NH_4, NO_2, NO_3, and DON. Measurements of these variables at cross sections (e.g. at RM-44 and HOD) along with bottom tracking ADCP (Acoustic Doppler Current Profiler) measurements should reveal the possibility of unaccounted for sources of nitrogen in addition to SRWTP. It should then be possible to link river flow/modeling, discharge records and nutrient, phytoplankton and bacterial processes in an effort to determine the relative importance of SRWTP discharge versus other nutrient sources and microbial processes in determining the distribution and concentration of inorganic nutrients (particularly nitrogen) in the Sacramento River. This type of study will be crucial in developing a comprehensive model of the fate of inorganic nutrients supplied to the Delta from SRWTP.

6.3 Characterization of Phytoplankton Community Composition in Experimental Grow-outs with Varying DIN (i.e. NO_3 and NH_4) Composition.

The experimental grow-out results presented here for GRC and RM-44 are in contrast to those previously found for experiments conducted in Suisun Bay and Rio Vista (re: Wilkerson presentation at NH_4 Summit). Specifically, results from RM-44 grow-outs showed substantial phytoplankton growth by phytoplankton supplied with NH_4 as their primary N source whereas earlier results from these other locations show delayed or no phytoplankton growth when NH_4 was their primary N source. Experimental phytoplankton blooms from water collected at GRC (where NO_3 was the primary N source for phytoplankton) provides a contrast to results obtained at RM-44 and the potential for detailed examination of how phytoplankton community composition is shaped by the form of inorganic nitrogen available (i.e. either NH_4 or NO_3).

Hypothesis:

1) Experimental phytoplankton blooms driven by NH_4 result in nutritionally inferior phytoplankton species. Experimental phytoplankton blooms driven by NO_3 result in nutritionally superior phytoplankton species (e.g. diatoms).
2) Additional sources of water, carrying phytoplankton inhibitors enters the Sacramento River near Rio Vista.

Samples for phytoplankton identification were collected and preserved from each of experimental grow-outs and should be analyzed.

6.4 Determination of Underlying Causes of SRTWP Effluent Toxicity on Primary Production and Phytoplankton Nitrogen Uptake.

While it unclear at this time whether NH_4 (as NH_4Cl) has a direct negative effect on the phytoplankton parameters that were assessed here (but see above, Section 6.1) it does appear that some other constituent(s) may be present in the SRWTP effluent that do result in negative effects on the phytoplankton parameters. These negative effects were observed at concentrations of NH_4 that are almost certainly maintained within the Sacramento River downstream of the SRWTP discharge.

Hypotheses:

1) Elevated NH_4 concentrations acts to suppress primary production, phytoplankton NH_4 and NO_3 uptake, leading to reduced phytoplankton biomass in the Sacramento River.

2) Elevated SRWTP discharge (traced with elevated NH_4) acts to suppress primary production, phytoplankton NH_4 and NO_3 uptake, leading to reduced phytoplankton biomass in the Sacramento River.

Experiments need to be made to sequentially address the questions 1) is NH_4 the only factor suppressing primary production in the river downstream of SRWTP (RM-44)? This could be addressed by removing NH_4 to low levels (using phytoplankton or bacterial growth) and inoculating phytoplankton into to this "cleaned" water. 2) Is there trace metal toxicity that increases downstream from RM-44? Amendments of chelators or other techniques to remove potential toxicants (e.g. pesticides) are available and would help answer this question. Additional experiments with additions of NH_4Cl are needed to resolve the inconclusive results of the direct effect of NH_4 on primary production and phytoplankton NH_4 uptake.

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Table 1: Cross listing of agreement tasks and results between SFSU-RTC and the Central Valley Regional Water Quality Control Board..

Task from Contract	Description	Results Section
1	**River Transects** – Characterizing the Sacramento River above and below the SRWTP (I-80 Bridge in Sacramento to Rio Vista) in July and November 2008, with nutrient concentrations, phytoplankton biomass.	Section 3.1
2	**Aging** Sacramento River water in the dark to observe if ammonium oxidation (nitrification) might be possible.	Section 3.3
3	**Experimental Grow-outs** in 2008 (WB-08-1, WB-08-2) with enclosed ambient water from above and near SRWTP and Central SFB (control) to measure nitrate and ammonium draw down and phytoplankton response by monitoring over 10 days for nutrient and chlorophyll-a concentrations.	Section 3.2
4	**Effluent Addition Experiment** in 2009 (WB-09-3 Effluents) in which diluted effluent from SRWTP was added to water collected upstream from SRWTP (Garcia Bend) and the effect on nitrate and ammonium uptake and primary productivity was measured.	Sections 3.5
5	**River Transects** – Characterizing the Sacramento River above and below the SRWTP (I-80 Bridge in Sacramento to Rio Vista) in March, April and May 2009, with nutrient concentrations, phytoplankton biomass and uptake rates.	Section 3.1
6	**Experimental Grow-outs** in 2009 (WB-09-1, WB-09-3) with enclosed ambient water from above (from Garcia Bend) and near SRWTP (from River Mile 44) and Central SFB (control) to measure nitrate and ammonium draw down and nutrient uptake and productivity by monitoring over 10 days for nutrient and chlorophyll-a concentrations. In addition, ammonium was added to Garcia Bend water and nitrate to River Mile 44 water such that the total initial DIN was equal.	Section 3.2
7	**Clean Ammonium Addition Experiment** in 2009 (WB-09-2) in which increasing ammonium concentrations were added to water collected upstream from SRWTP (Garcia Bend) and the effect on nitrate and ammonium uptake and primary productivity was measured.	Section 3.4
8	**Management and Data Report**	

Table 2: Sampling dates and experiments conducted during "Waterboard" Sacramento River sampling program (WBD) All transects were carried out in a north to south progression beginning at I80. (*)transect was initiated from RM-44. (**) Time of high water reported for the Sacramento River at Tower Bridge Gateway

Event Name	Date	High Water at Sacramento** (hh:mm) local time	Transect Start Time (hh:mm) local time	River Flow @Freeport (FPT) (cfs)	Number of Stations sampled	Additional Experiments
WBD08-1	July 21, 2008	09:02	08:39	13,033	7	10-d ambient "grow-out"
WBD08-2	November 12, 2008	06:18	08:25*	10,530	7	10-d ambient "grow-out"
WBD09-1	March 9, 2009	07:02	08:55	30,337	8	10-d manipulated "grow-out"
WBD09-2	April 6, 2009	05:47	08:33	12,035	8	"clean" NH_4 addition experiment
WBD09-3	May 8, 2009	07:08	08:24	25,900	8	10-d manipulated "grow-out"

Table 3: Station locations occupied during "Waterboard" Sacramento River sampling program. SRWTP discharge is located downstream of RM-44.

Station Name	Geographic reference	Latitude	Longitude
I80	I-80 Bridge, Sacramento	38º 35'54.07"N	121º 32'52.83"W
OAK	Oak Hall Bend	38º 31'01.76"N	121º 36'57.42"W
GRC	Garcia Bend	38º 28'43.35"N	121º 32'43.85"W
RM-44	River Mile 44	38º 26'05.49"N	121º 31'41.03"W
HOD	Hood	38º 22'07.28"N	121º 31'17.65"W
CRS	Delta Cross Channel	38º 14'87.99"N	121º 30'65.99"W
ISL	Isleton	38º 09'58.46"N	121º 37'35.47"W
RIO	Rio Vista	38º 08'08.99"N	121º 41'39.99"W

Table 4: WBD08-1 (July 2008) Surface water chemistry. Nutrient concentrations are mean values (± 95% CL) from three replicate samples collected from separate 3-L Niskin bottles. † indicates that only a single sample was used to determine the reported concentration.

Station Name	Temperature (ºC)	EC (µS cm^{-1})	NO_3+NO_2 (µM)	NH_4 (µM)	PO_4 (µM)	$Si(OH)_4$ (µM)
I-80	21.6	147.9	1.15±0.31	1.73	0.66±0.10	374.38
OAK	21.3	123.2	0.80±0.11	0.68	0.35±0.01	298.85
GRC	21.6	124.2	0.46±0.36	0.78	0.32±0.02	309.90
RM-44	22.0	134.4	0.41±0.11	17.49	1.21±0.02	287.99
HOD	22.2	140.5	1.74±0.16	25.07	1.55†	315.12
CRS	22.7	137.8	2.96±0.13	11.83	0.97±0.00	300.59
ISL	22.5	139.3	8.14±0.11	13.21	1.52±0.06	312.57

Table 5: WBD08-1 (July 2008) Surface phytoplankton measurements including chlorophyll-*a* (extracted, 90% acetone) and POC and PON. n.d. (not determined).

Station Name	Chloro-phyll-a (GF/F) ($\mu g\ L^{-1}$)	Chloro-phyll-a (>5µm) ($\mu g\ L^{-1}$)	% >5µm chl-a	POC ($\mu mol\ L^{-1}$)	PON ($\mu mol\ L^{-1}$)	Flow Cyto. Total ($x10^6$ part. L^{-1})
I-80	4.6	3.3	72%	n.d.	n.d.	4.5
OAK	3.7	2.6	70%	n.d.	n.d.	3.8
GRC	3.4	2.3	68%	n.d.	n.d.	3.7
RM-44	2.6	1.6	62%	n.d.	n.d.	2.5
HOD	1.9	1.2	63%	n.d.	n.d.	1.8
CRS	1.7	1.2	71%	n.d.	n.d.	1.6
ISL	1.9	1.5	79%	n.d.	n.d.	n.d.

Table 6: WBD08-1 (July 2008) Primary production and nutrient uptake measurements. OBS and PAR determinations based on near surface water column light attenuation. Zp was calculated to 1% of surface irradiance. ρC and ρN were determined using stable isotope tracer ($^{13}C/^{15}N$) incubations at 50% of surface irradiance over 24-hr. n.d. (not determined).

Station Name	**Secchi depth (m)**	**OBS**	**k (m^{-1})**	**Zp (m)**	**ρC (µmol L^{-1} d^{-1})**	**ρNO_3 (µmol L^{-1} d^{-1})**	**ρNH_4 (µmol L^{-1} d^{-1})**
I-80	1.2	n.d.	1.31	3.5	n.d.	n.d.	n.d.
OAK	1.4	n.d.	1.18	3.9	n.d.	n.d.	n.d.
GRC	1.4	n.d.	1.18	3.9	n.d.	n.d.	n.d.
RM-44	1.4	n.d.	1.18	3.9	n.d.	n.d.	n.d.
HOD	1.4	n.d.	1.18	3.9	n.d.	n.d.	n.d.
CRS	1.0	n.d.	1.49	3.1	n.d.	n.d.	n.d.
ISL	1.0	n.d.	1.49	3.1	n.d.	n.d.	n.d.
RIO	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table 7: WBD08-2 (November 2008) Surface water chemistry. Nutrient concentrations are from a single sample collected using a acid cleaned bucket. * from 0.5m depth

Station Name	Temperature* (ºC)	EC* ($\mu S\ cm^{-1}$)	NO_3+NO_2 ($\mu mol\ L^{-1}$)	NH_4 ($\mu mol\ L^{-1}$)	PO_4 ($\mu mol\ L^{-1}$)	$Si(OH)_4$ ($\mu mol\ L^{-1}$)
I-80	13.9	204.7	13.34	7.73	1.69	370
OAK	14.0	194.1	11.24	2.38	1.65	347
GRC	13.9	181.9	12.62	2.46	1.65	344
RM-44	14.3	233.0	12.06	70.82	5.09	363
HOD	14.1	226.6	12.20	47.37	3.88	357
CRS	n.d.	n.d	n.d	n.d	n.d	n.d
ISL	14.7	226.0	23.40	37.45	3.94	317

Table 8: WBD08-2 (November 2008) Surface phytoplankton measurements including chlorophyll-*a* (extracted, 90% acetone) and POC and PON. n.d. (not determined)

Station Name	Chloro-phyll-a (GF/F) ($\mu g\ L^{-1}$)	Chloro-phyll-a (>5µm) ($\mu g\ L^{-1}$)	% >5µm chl-a	POC ($\mu mol\ L^{-1}$)	PON ($\mu mol\ L^{-1}$)	Flow Cyto. Total ($x10^6$ part. L^{-1})
I-80	3.3	3.3	100%	84	4	1.6
OAK	1.9	1.7	89%	36	1	1.1
GRC	1.8	1.4	78%	38	1	1.0
RM-44	1.4	1.1	79%	97	4	0.7
HOD	0.8	0.7	88%	63	2	0.5
CRS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d
ISL	0.6	n.d.	n.d.	56	2	0.3

Table 9: WBD08-2 (November 2008) Primary production and nutrient uptake measurements. OBS and PAR determinations based on near surface water column light attenuation. Zp was calculated to 1% of surface irradiance. ρC and ρN were determined using stable isotope tracer ($^{13}C/^{15}N$) incubations at 50% of surface irradiance over 24-hr. n.d. – (not determined).

Station Name	Secchi depth (m)	OBS	k (m^{-1})	Zp (m)	ρC (µmol L^{-1} d^{-1})	ρC/chl-a [µmol C (µg chl-a)$^{-1}$]d^{-1}	ρNO_3 (µmol L^{-1} d^{-1})	ρNH_4 (µmol L^{-1} d^{-1})
I-80	0.90	n.d.	1.61	2.9	0.27	0.08	0.02	n.d.
OAK	1.30	n.d.	1.24	3.7	0.08	0.04	0.02	n.d.
GRC	1.10	n.d.	1.39	3.3	0.08	0.04	0.12	n.d.
RM-44	1.30	n.d.	1.24	3.7	0.16	0.11	0.01	0.26
HOD	1.70	n.d.	1.04	4.4	0.07	0.09	0.00	0.19
CRS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ISL	1.70	n.d.	1.04	4.4	0.06	0.10	0.02	0.24

Table 10: WBD09-1 (March 2009) Surface water chemistry. Nutrient concentrations are mean values (± 95% CI) from three replicate samples collected from separate 3-L Niskin bottles. * from 0.5-m depth

Station Name	Temperature* (ºC)	EC* ($\mu S\ cm^{-1}$)	NO_3+NO_2 ($\mu mol\ L^{-1}$)	NH_4 ($\mu mol\ L^{-1}$)	PO_4 ($\mu mol\ L^{-1}$)	$Si(OH)_4$ ($\mu mol\ L^{-1}$)
I-80	11.3	107.2	12.31 ±0.17	1.01 ±0.26	1.35 ±0.11	445.76 ±46.3
OAK	11.4	104.1	12.55 ±0.30	0.72 ±0.17	1.39 ±0.03	346.2 ±2.2
GRC	11.5	100.8	11.47 ±1.13	0.79 ±0.21	1.26 ±0.12	369.07 ±25
RM-44	11.7	107.5	12.45 ±0.08	10.09 ±0.24	1.94 ±0.09	350.92 ±27
HOD	11.7	111.0	13.17 ±0.06	10.06 ±0.15	2.04 ±0.08	345.85 ±13.3
CRS	11.6	107.5	13.93 ±0.17	14.55 ±0.53	2.16 ±0.03	388.6 ±4.8
ISL	11.6	98.9	14.22 ±0.25	12.52 ±0.25	1.94 ±0.04	355.44 ±4.3
RIO	11.81	144.2	21.77 ±0.16	6.44 ±0.23	2.46 ±0.07	307.7 ±5.4

Table 11: WBD09-1 (March 2009) Surface phytoplankton measurements including chlorophyll-*a* (extracted, 90% acetone) and POC and PON.

Station Name	Chloro-phyll-a (GF/F) ($\mu g\ L^{-1}$)	Chloro-phyll-a (>5µm) ($\mu g\ L^{-1}$)	% >5µm chl-a	POC ($\mu mol\ L^{-1}$)	PON ($\mu mol\ L^{-1}$)	Flow Cyto. Total ($x10^6$ part. L^{-1})
I-80	1.9	1.9	100%	128	9	1.0
OAK	3.8	2.2	58%	114	9	1.1
GRC	3.4	2.2	65%	110	8	1.0
RM-44	3.2	1.7	53%	108	8	1.0
HOD	3.1	1.8	58%	152	5	0.8
CRS	2.6	1.3	50%	114	12	0.7
ISL	2.3	1.5	65%	104	7	0.7
RIO	1.8	1.0	56%	147	6	0.7

Table 12: WBD09-1 (March 2009) Primary production and nutrient uptake measurements. OBS and PAR determinations based on near surface water column light attenuation. Zp was calculated to 1% of surface irradiance. ρC and ρN were determined using stable isotope tracer ($^{13}C/^{15}N$) incubations at 50% of surface irradiance over 24-hr. n.d. – not determined.

Station Name	**Secchi depth (m)**	**OBS**	**k (m^{-1})**	**Zp (m)**	**ρC (µmol L^{-1} d^{-1})**	**ρC/chl-a [µmol C (µg chl-a)$^{-1}$]d^{-1}**	**ρNO_3 (µmol L^{-1} d^{-1})**	**ρNH_4 (µmol L^{-1} d^{-1})**
I-80	0.4	n.d.	3.13	1.5	8.88	4.67	0.34	0.67
OAK	0.4	n.d.	3.13	1.5	9.60	2.53	0.41	0.72
GRC	0.4	n.d.	3.13	1.5	10.08	2.96	0.38	0.79
RM-44	0.5	n.d.	2.58	1.8	7.92	2.48	0.05	0.98
HOD	0.5	n.d.	2.58	1.8	8.16	2.63	0.02	0.82
CRS	0.5	n.d.	2.58	1.8	11.52	4.43	0.07	0.86
ISL	0.4	n.d.	3.13	1.5	6.96	3.03	0.05	0.67
RIO	0.4	n.d.	3.13	1.5	6.48	3.60	0.05	0.53

Table 13: WBD09-2 (April 2009) Surface water chemistry. Nutrient concentrations are mean values (± 95% CL) from three replicate samples collected from separate 3-L Niskin bottles. *indicates mean based on 2 replicates. * from 0.5-m depth

Station Name	Temperature* (ºC)	EC* (µS cm^{-1})	NO_3+NO_2 (µmol L^{-1})	NH_4 (µmol L^{-1})	PO_4 (µmol L^{-1})	$Si(OH)_4$ (µmol L^{-1})
I-80	14.9	115.3	3.9 ±1.6	0.29 ±0.20	0.6 ±0.02	273 ±10.7
OAK	14.5	103.9	3.43 ±0.3*	0.41 ±0.08	0.6 ±0.03	264.9 ±10.2
GRC	14.4	104.2	8.3 ±0.3*	0.35 ±0.01	0.7 ±0.02	271.3 ±6.2
RM-44	14.7	108.1	3.4 ±0.2	0.89 ±0.23	0.6 ±0.05	270.5 ±19.9
HOD	15.1	121.5	4.7 ±0.8	21.01 ±1.04	2.4 ±0.30	280.3 ±25
CRS	15.2	130.8	6.4 ±0.4	20.83 ±2.00	2.1 ±0.10	263.3 ±45.3
ISL	15.7	137.5	8.5 ±0.5	20.46 ±2.80	2.1 ±0.10	309 ±20.5
RIO	15.5	192.7	24.6 ±0.5	19.59 ±1.20	3 ±0.03	295.1 ±1.4

Table 14: WBD09-2 (April 2009) Surface phytoplankton measurements. Chlorophyll-*a* (extracted, 90% acetone) are mean values from 3 replicate samples collected from separate 3-L Niskin bottles. POC, PON and flow cytometer samples are based on analysis of a single sample. n.d. – not determined.

Station Name	Chloro-phyll-a (GF/F) ($\mu g\ L^{-1}$)	Chloro-phyll-a (>5µm) ($\mu g\ L^{-1}$)	% >5µm chl-a	POC ($\mu mol\ L^{-1}$)	PON ($\mu mol\ L^{-1}$)	Flow Cyto. Total ($x10^6$ part. L^{-1})
I-80	7.1 (±2.0)	5.6 (±0.7)	79%	87	9	7.1
OAK	5.0 (±1.5)	4.6 (±0.2)	92%	74	8	6.8
GRC	5.1 (±0.2)	n.d.	n.d.	63	6	5.9
RM-44	4.7 (±0.2)	3.5 (±0.4)	74%	59	7	5.6
HOD	3.5 (±0.1)	2.8 (±0.2)	80%	66	7	4.1
CRS	2.5 (±0.2)	1.8 (±0.1)	72%	61	6	2.3
ISL	1.2 (±0.0)	1.0 (±0.1)	83%	45	3	1.5
RIO	1.7 (±0.1)	1.2 (±0.1)	71%	64	5	1.2

Table 15: WBD09-2 (April 2009) Primary production and nutrient uptake measurements. OBS and PAR determinations based on near surface water column light attenuation. Zp was calculated to 1% of surface irradiance. ρC and ρN were determined using stable isotope tracer ($^{13}C/^{15}N$) incubations at 50% of surface irradiance over 24-hr

Station Name	Secchi depth (m)	OBS	k (m^{-1})	Zp (m)	ρC (µmol L^{-1} d^{-1})	ρC/chl-a [µmol C (µg chl-a)$^{-1}$]d^{-1}	ρNO_3 (µmol L^{-1} d^{-1})	ρNH_4 (µmol L^{-1} d^{-1})
I-80	0.8	21.54	2.12	2.17	12.00	1.69	2.19	0.52
OAK	1	19.01	1.64	2.8	9.84	1.97	2.27	0.57
GRC	1	18.06	1.58	2.92	9.12	1.79	1.77	0.65
RM-44	1.1	21.00	1.66	2.77	7.20	1.53	0.57	1.19
HOD	1.2	19.35	1.46	3.16	5.52	1.58	0.03	0.60
CRS	1.5	17.71	1.3	3.54	4.56	1.82	0.03	0.65
ISL	1.8	14.54	0.87	5.28	3.12	2.60	0.02	0.48
RIO	0.6	24.81	1.82	2.54	3.60	2.12	0.03	0.51

Table 16: WBD09-3 (May 2009) Surface water chemistry. Nutrient concentrations are mean values (± 95% CL) from three replicate samples collected from separate 3-L Niskin bottles. * from 0.5-m depth

Station Name	Temperature* (ºC)	EC* (µS cm^{-1})	NO_3+NO_2 (µmol L^{-1})	NH_4 (µmol L^{-1})	PO_4 (µmol L^{-1})	$Si(OH)_4$ (µmol L^{-1})
I-80	16.2	104.7	10.10 ±0.14	0.87 ±0.3	0.94 ±0.01	275.2 ±5.5
OAK	16.1	95.3	9.78 ±0.08	1.26 ±0.2	0.91 ±0.01	245.7 ±11.02
GRC	16.2	96.0	9.87 ±0.18	1.37 ±0.2	0.89 ±0.04	223.2 ±26.4
RM-44	16.4	101.8	9.86 ±0.12	11.10 ±0.83	1.40±0.04	241.94 ±14.7
HOD	16.5	100.1	10.08 ±0.12	9.60 ±0.42	1.30 ±0.05	146.5 ±28.6
CRS	16.2	95.6	10.34 ±0.05	14.70 ±2.1	1.54 ±0.03	239.4 ±29.2
ISL	16.4	97.3	11.56 ±0.14	10.70 ±0.83	1.34 ±0.08	218.1 ±48.8
RIO	16.8	125.7	16.36 ±0.13	10.10 ±1.07	1.89 ±0.01	235.2 ±7

Table 17: WBD09-3 (May 2009) Surface phytoplankton measurements. Chlorophyll-*a* (extracted, 90% acetone) are mean values (± 95% CL) from three replicate samples collected from separate 3-L Niskin bottles. POC, PON, and flow cytometer samples are based on analysis of a single sample.

Station Name	**Chloro-phyll-a (GF/F) (µg L^{-1})**	**Chloro-phyll-a (>5µm) (µg L^{-1})**	**% >5µm chl-a**	**POC (µmol L^{-1})**	**PON (µmol L^{-1})**	**Flow Cyto. Total (x10^6 part. L^{-1})**
I-80	6.8 (±0.6)	4.7 (±1.9)	69%	107	10	1.8
OAK	5.7 (±0.3)	5.0 (±0.7)	88%	65	6	2.2
GRC	5.4 (±0.8)	4.1 (±0.1)	76%	81	8	1.8
RM-44	5.4 (±0.1)	4.3 (±0.3)	80%	77	9	1.6
HOD	5.3 (±0.8)	3.6 (±0.9)	68%	107	10	1.8
CRS	4.5 (±0.7)	3.4 (±0.6)	76%	95	9	1.5
ISL	6.2 (±0.7)	3.5 (±0.8)	56%	180	17	1.7
RIO	2.3 (±0.2)	0.8 (±0.1)	35%	56	6	1.3

Table 18: WBD09-3 (May 2009) Primary production and nutrient uptake measurements. OBS and PAR determinations based on near surface water column light attenuation. Zp was calculated to 1% of surface irradiance. ρC and ρN were determined using stable isotope tracer ($^{13}C/^{15}N$) incubations at 50% of surface irradiance over 24-hr. "*" indicates that measurement taken from 0.5m water depth

Station Name	Secchi depth (m)	OBS*	k (m^{-1})	Zp (m)	ρC (µmol L^{-1} d^{-1})	ρC/chl-a [µmol C (µg chl-a)$^{-1}$]d^{-1}	ρNO_3 (µmol L^{-1} d^{-1})	ρNH_4 (µmol L^{-1} d^{-1})
I-80	0.4	54.9	4.2	1.1	10.80	1.59	1.13	0.68
OAK	0.5	32.2	2.43	1.9	5.76	1.01	0.23	0.90
GRC	0.5	35.0	2.59	1.8	8.40	1.56	0.48	0.81
RM-44	0.5	35.9	2.62	1.8	8.40	1.56	0.06	1.32
HOD	0.6	34	2.62	1.8	9.12	1.72	0.06	1.23
CRS	0.5	40.1	2.96	1.6	7.92	1.76	0.05	1.13
ISL	0.4	62.5	4.85	1.0	12.00	1.94	0.05	1.55
RIO	0.7	24.2	1.9	2.4	5.76	2.50	0.04	0.88

Table 19: "Grow-out" experiments conducted during WBD08-1 (July 2008). Nutrient and chlorophyll-a conditions in samples collected from experimental "grow-outs" prior to (t=0hr) and after 5-d (96-hr) incubation periods under ambient temperature and 50% of surface solar irradiance.

	RM-44	GRC
Initial Conditions (T=0hr)		
NO_3+NO_2 (µmol L^{-1})	1.50 ± 0.03	1.09 ± 0.05
NH_4 (µmol L^{-1})	9.06 ± 0.79	0.4 ± 0.13
PO_4 (µmol L^{-1})	1.67 ± 0.77	1.05 ± 0.01
$Si(OH)_4$ (µmol L^{-1})	309.83 ± 7.56	303.81 ± 4.55
Chl-*a* (GF/F) (µg L^{-1})	2.94 ± 0.48	2.65 ± 0.48
Chl-*a* (>5 µm) (µg L^{-1})	1.74 ± 0.09	1.31 ± 0.10
Final Conditions (T= 96-hr)		
NO_3+NO_2 (µmol L^{-1})	0.01 ± 0.01	0
NH_4 (µmol L^{-1})	0.36 ± 0.06	0.66 ± 0.25
PO_4 (µmol L^{-1})	0.43 ± 0.09	0.69 ± 0.05
$Si(OH)_4$ (µmol L^{-1})	258.59 ± 10.19	296.77 ± 2.14
Chl-*a* (GF/F) (µg L^{-1})	14.7 ± 1.5	1.2 ± 0.1
Chl-*a* (>5 µm) (µg L^{-1})	10.9 ± 2.0	0.8± 0.1
Net Constituent Changes (T0 – T96)		
NO_3+NO_2 (µmol L^{-1})	1.49	1.09
NH_4 (µmol L^{-1})	8.70	-0.26
PO_4 (µmol L^{-1})	1.64	0.36
$Si(OH)_4$ (µmol L^{-1})	51.24	7.05
Chl-*a* (GF/F) (µg L^{-1})	-11.8 increase)	1.4
Chl-*a* (>5 µm) (µg L^{-1})	-9.2 (increase)	0.6

Table 20: "Grow-out" experiments conducted during WBD08-2 (November 2008). Nutrient and chlorophyll-a conditions in samples collected from experimental "grow-outs" prior to (t=0hr) and after 5-d (96-hr) incubation periods under ambient temperature and 50% of surface solar irradiance.

	RM-44	GRC
Initial Conditions (T=0hr)		
NO_3+NO_2 (μmol L^{-1})	12.58	12.81
NH_4 (μmol L^{-1})	71.87	3.35
PO_4 (μmol L^{-1})	5.18	1.69
$Si(OH)_4$ (μmol L^{-1})	378.5	351.26
Chl-*a* (GF/F) (μg L^{-1})	0.6	1.2
Chl-*a* (>5 μm) (μg L^{-1})	0.4	0.8
Final Conditions (T= 96-hr)		
NO_3+NO_2 (μmol L^{-1})	11.93	3.61
NH_4 (μmol L^{-1})	59.34	1.47
PO_4 (μmol L^{-1})	4.2	0.98
$Si(OH)_4$ (μmol L^{-1})	361.85	298.1
Chl-*a* (GF/F) (μg L^{-1})	12.01	16.72
Chl-*a* (>5 μm) (μg L^{-1})	13.6	17.35
Net Constituent Changes (T0 – T96)		
NO_3+NO_2 (μmol L^{-1})	0.65	9.2
NH_4 (μmol L^{-1})	12.53	1.88
PO_4 (μmol L^{-1})	0.98	0.71
$Si(OH)_4$ (μmol L^{-1})	16.65	53.16
Chl-*a* (GF/F) (μg L^{-1})	-11.4 (increase)	-15.5 (increase)
Chl-*a* (>5 μm) (μg L^{-1})	-13.2 (increase)	-16.6 (increase)

Table 21: "Grow-out" experiments conducted during WBD09-1 (March 2009). Nitrogen additions were made to water collected at station GRC (either as NO_3; i.e. GRC+NO_3, or NH_4, i.e. GRC+NH_4) to match ambient DIN ($NO_3 + NO_2 + NH_4$) concentrations at RM-44). Nutrient and chlorophyll-a conditions in samples collected from experimental "grow-outs" after nitrogen addition but prior placing "grow-outs" in incubation chambers (t=0hr). Nutrient and chlorophyll-a results are also reported after 5-d incubation period (T=96hr) under ambient temperature and 50% of surface solar irradiance.

	RM-44	GRC	GRC+NO_3	GRC+NH_4
Initial Conditions (T=0hr)				
NO_3+NO_2 (µmol L^{-1})	13.34 ±0.13	13.1 ±0.07	24.6 ±0.33	13.14 ±0.03
NH_4 (µmol L^{-1})	12.47 ±1.53	1.02 ±0.36	1.47 ±0.39	12.1 ±1.21
PO_4 (µmol L^{-1})	2.18 ±0.02	1.61 ±0.05	1.65 ±0.01	1.66 ±0.03
$Si(OH)_4$ (µmol L^{-1})	330.7 ±2.24	328.83 ±9.54	324.7 ±13.15	326.28 ±4.26
Chl-*a* (GF/F) (µg L^{-1})	1.9 ±0.4	2.6 ±0.5	2.4 ±0.4	3.9 ±2.5
Chl-*a* (>5 µm) (µg L^{-1})	n.d.	n.d.	n.d.	n.d.
Day 5 Conditions (T=96-hr)				
NO_3+NO_2 (µmol L^{-1})	8.5 ±0.35	0.29 ±0.14	14.08 ±0.5	10.71 ±0.47
NH_4 (µmol L^{-1})	0.61 ±0.31	0.39 ±0.35	0.24 ±0.11	0.43 ±0.2
PO_4 (µmol L^{-1})	0.91 ±0.12	0.7 ±0.1	0.84 ±0.12	0.52 ±0.21
$Si(OH)_4$ (µmol L^{-1})	306.11 ±40.24	288.84 ±215.28*	305.76 ±39.05	275.86 ±49.08
Chl-*a* (GF/F) (µg L^{-1})	18.83 ±3	12.61 ±1.07	10.86 ±2.76	17.02 ±5.5
Chl-*a* (>5 µm) (µg L^{-1})	11.21 ±3.7	8.86 ±0.52	8.08 ±1.22	10.09 ±2.52
Net Constituent Changes (T0 – T96)				
NO_3+NO_2 (µmol L^{-1})	4.83	12.81	10.52	2.43
NH_4 (µmol L^{-1})	11.85	0.63	1.24	11.67
PO_4 (µmol L^{-1})	1.27	0.92	0.82	1.14
$Si(OH)_4$ (µmol L^{-1})	24.59	39.99	18.94	50.42
Chl-*a* (GF/F) (µg L^{-1})	-16.9 (increase)	-10.1 (increase)	-8.5 (increase)	-13.1 (increase)
Chl-*a* (>5 µm) (µg L^{-1})	n.d.	n.d	n.d.	n.d.

Table 22: "Grow-out" experiments conducted during WBD09-3 (May 2009). Nitrogen additions were made to water collected at station GRC (either as NO_3; i.e. GRC+NO_3, or NH_4, i.e. GRC+NH_4) to match ambient DIN (NO_3 + NO_2 + NH_4) concentrations at RM-44). Nutrient and chlorophyll-a conditions in samples collected from experimental "grow-outs" after nitrogen addition but prior placing "grow-outs" in incubation chambers (t=0hr). Nutrient and chlorophyll-a results are also reported after 5-d incubation period (T=96hr) under ambient temperature and 50% of surface solar irradiance.

	RM-44	GRC	GRC+NO_3	GRC+NH_4
Initial Conditions (T=0hr)				
NO_3+NO_2 (µmol L^{-1})	10.35 ±0.19	9.91 ±0.06	16.25 ±0.08	9.9 ±0.2
NH_4 (µmol L^{-1})	9.54 ±0.42	1.44 ±0.61	1.48 ±0.31	6.8 ±0.22
PO_4 (µmol L^{-1})	1.24 ±0.06	0.77 ±0.01	0.78 ±0.01	0.75 ±0.01
$Si(OH)_4$ (µmol L^{-1})	273.43 ±4.73	269.66 ±3.07	268.53 ±3.63	265.77 ±3.58
Chl-*a* (GF/F) (µg L^{-1})	2.2 ±0.1	2.3 ±0.1	2.5 ±0.1	3.0 ±0.2
Chl-*a* (>5 µm) (µg L^{-1})	1.4 ±0.1	1.2 ±0.2	1.4 ±0.1	1.5 ±0.1
Day 5 Conditions (T= 96-hr)				
NO_3+NO_2 (µmol L^{-1})	0.48 ±0.73	0	0.68 ±0.67	0.52 ±0.48
NH_4 (µmol L^{-1})	0.35 ±0.11	0.7 ±0.1	0.65 ±0.37	0.74 ±0.27
PO_4 (µmol L^{-1})	0	0.01 ±0.017	0	0
$Si(OH)_4$ (µmol L^{-1})	103.92 ±84.8	177.82 ±42.12	140.74 ±82.1	70.42 ±73.5
Chl-*a* (GF/F) (µg L^{-1})	13.8 ±0.4	9.0 ±0.6	11.6 ±1.5	11.1 ±1.3
Chl-*a* (>5 µm) (µg L^{-1})	14.7 ±0.6	9.9 ±0.7	13.2 ±1.9	13.0 ±1.4
Net Constituent Changes (ΔT0 – ΔT96)				
NO_3+NO_2 (µmol L^{-1})	9.87	9.91	15.57	9.37
NH_4 (µmol L^{-1})	9.19	0.74	0.83	6.06
PO_4 (µmol L^{-1})	1.24	0.76	0.78	0.75
$Si(OH)_4$ (µmol L^{-1})	169.5	91.84	127.79	195.35
Chl-*a* (GF/F) (µg L^{-1})	-11.6 (increase)	-6.8 (increase)	-9.1 (increase)	-8.2 (increase)
Chl-*a* (>5 µm) (µg L^{-1})	-13.2 (increase)	-8.7 (increase)	-11.8 (increase)	-11.6 (increase)

Table 23: Eight week time series of inorganic nutrient concentrations from water samples collected at RM-44 and GRC and incubated at 21ºC in the dark.

Date	Week	Station	$NO_3 + NO_2$ ($\mu mol\ L^{-1}$)	NH_4 ($\mu mol\ L^{-1}$)	PO_4 ($\mu mol\ L^{-1}$)	Si ($\mu mol\ L^{-1}$)
7/22/2008	0	GRC	0.64	0.78	0.33	311
7/22/2008		RM-44	0.47	17.49	1.26	295
7/28/2008	1	GRC	1.01	0.07	0.99	300
7/28/2008		RM-44	2.25	17.3	2.06	305
8/4/2008	2	GRC	1.19	0.15	1.00	304
8/4/2008		RM-44	17.62	2.94	2.00	313
08/11/08	3	GRC	0.28	0.46	0.91	303
08/11/08		RM-44	20.03	0.65	1.87	307
8/18/2008	4	GRC	0.23	0.14	0.75	306
8/18/2008		RM-44	19.98	0.59	1.74	305
8/25/2008	5	GRC	0.18	0.28	0.73	306
8/25/2008		RM-44	19.78	0.69	1.72	308
9/2/2008	6	GRC	0.00	0.33	0.67	313
9/2/2008		RM-44	19.39	0.7	1.68	311
9/8/2008	7	GRC	0.00	0.11	0.76	316
9/8/2008		RM-44	19.35	1.97	1.66	306
9/15/2008	8	GRC	0.00	0.05	0.67	306
9/15/2008		RM-44	18.91	0.57	1.66	305

Table 24: Primary production and phytoplankton nitrogen uptake (± 95% CL) measured in "Clean NH_4" experiments conducted during WBD09-2 (April 2009). Ammonium additions were made as NH_4Cl to water collected at station GRC at concentrations of +1 µmol L^{-1}, +4 µmol L^{-1}, and 50 µmol L^{-1}. Incubations were performed in triplicate in 160-ml HDPE bottles at ambient temperature and 50% of surface solar irradiance for 4-hr. n.d.(not determined).

Treatment	^{14}C-primary production (µmol C $L^{-1}h^{-1}$)	^{13}C-primary production (µmol C $L^{-1}h^{-1}$)	$^{15}NH_4$ – uptake (µmol N $L^{-1}h^{-1}$)	$^{15}NO_3$ - uptake (µmol N $L^{-1}h^{-1}$)	Total N uptake (µmol N $L^{-1}h^{-1}$)	n
RM-44	n.d.	0.90±0.07	0.041±0.00	0.010±0.00	0.051	3
GRC	0.68	1.20±0.02	0.060±0.00	0.028±0.00	0.088	3
GRC+1 µmol L^{-1} NH_4	0.68	1.25±0.05	0.063±0.00	0.013±0.00	0.076	2
GRC+4 µmol L^{-1} NH_4	0.65	1.13±0.12	0.054±0.00	0.006±0.00	0.060	3
GRC+50 µmol L^{-1} NH_4	0.73	1.10±0.03	0.047±0.00	0.003±0.00	0.050	3

Table 25 : Table of SRWTP effluent dilutions used in the wastewater addition experiments conducted in May 2009. SRWTP permitting requires a 14:1 effluent dilution into the Sacramento River (C. Foe, pers. comm.).

Dilution	**NH_4 (µmol L^{-1})**
14 : 1	142.86
20 : 1	100.00
32: 1	62.50
64 : 1	31.25
100 : 1	20.00
200 : 1	10.00
400 : 1	5.00
800 : 1	2.50
1600: 1	1.25
5000 : 1	0.40

Table 26: ^{14}C-primary production rates in experimental incubations with varying concentrations of NH4Cl or SRWTP effluent-NH4. Experiments were conducted during May 2009.

Treatment	^{14}C-primary production (μmol C $L^{-1}h^{-1}$) As effluent	^{14}C-primary production (μmol C $L^{-1}h^{-1}$) as NH_4Cl
RM-44	0.57	0.58
GRC	0.96	1.00
GRC+0.25μmol L^{-1} NH_4	1.13	1.28
GRC+0.50 μmol L^{-1} NH_4	1.13	1.33
GRC+1 μmol L^{-1} NH_4	1.05	1.19
GRC+2 μmol L^{-1} NH_4	1.11	1.12
GRC+4 μmol L^{-1} NH_4	1.20	1.16
GRC+8 μmol L^{-1} NH_4	1.10	1.31
GRC+16 μmol L^{-1} NH_4	1.16	1.33
GRC+32 μmol L^{-1} NH_4	0.73	1.19
GRC+64 μmol L^{-1} NH_4	1.04	1.30
GRC+100 μmol L^{-1} NH_4	0.61	1.26

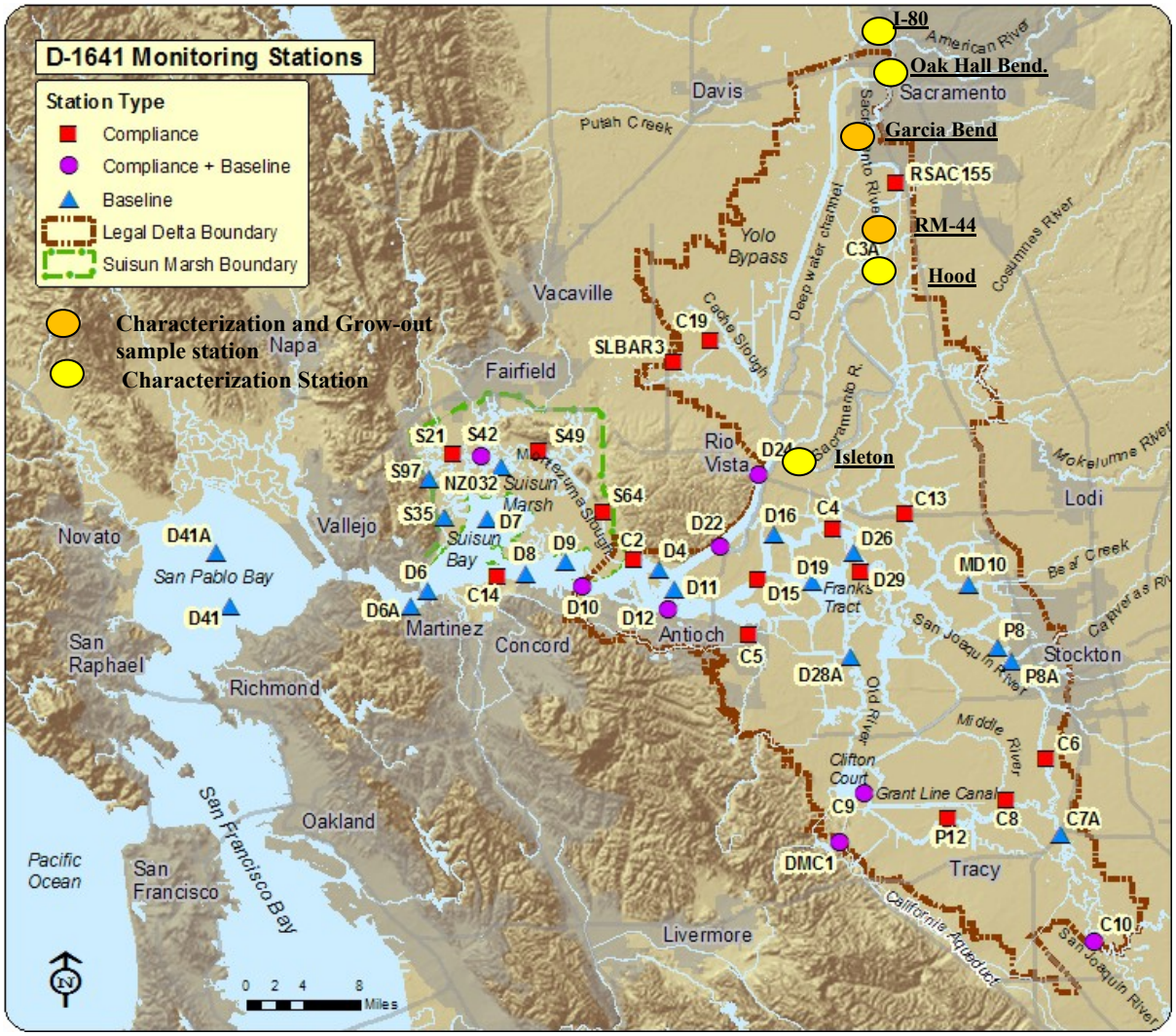
Table 27: ^{13}C-Primary production and ^{15}N-phytoplankton nitrogen uptake rates measured during effluent addition experiment conducted in May 2009.

Treatment	^{13}C-primary production NH_4 (µmol C $L^{-1}h^{-1}$) as wastewater	^{13}C-primary production NO_3 (µmol C $L^{-1}h^{-1}$) as wastewater	^{13}C-primary production NH_4 (µmol C $L^{-1}h^{-1}$) as NH_4Cl	^{13}C-primary production NO_3 (µmol C $L^{-1}h^{-1}$) as NH_4Cl	$^{15}NH_4$ – uptake (µmol C $L^{-1}h^{-1}$) as wastewater	$^{15}NH_4$ – uptake (µmol C $L^{-1}h^{-1}$) as NH_4Cl	$^{15}NO_3$ – uptake (µmol C $L^{-1}h^{-1}$) as wastewater	$^{15}NO_3$ – uptake (µmol C $L^{-1}h^{-1}$) as NH_4Cl
RM-44	0.854	0.865	0.854	0.865	0.027	0.027	0.006	0.006
GRC	n.d	1.749	1.700	1.710	n.d.	0.044	0.036	0.038
GRC+0.25 µmol L^{-1} NH_4	1.716	1.817	1.687	1.687	0.132	0.051	0.033	0.035
GRC+0.50 µmol L^{-1} NH_4	1.737	1.761	1.720	1.698	0.062	0.059	0.028	0.029
GRC+1 µmol L^{-1} NH_4	1.820	1.822	1.475	1.694	0.082	0.059	0.024	0.022
GRC+2 µmol L^{-1} NH_4	1.757	1.815	1.521	1.520	0.076	0.049	0.021	0.019
GRC+4 µmol L^{-1} NH_4	1.675	1.830	1.520	1.494	0.095	0.053	0.019	0.017
GRC+8 µmol L^{-1} NH_4	1.757	1.639	1.507	1.499	0.086	0.062	0.015	0.014
GRC+16 µmol L^{-1} NH_4	1.651	1.570	1.514	1.516	0.076	0.057	0.012	0.011
GRC+32 µmol L^{-1} NH_4	1.473	1.441	1.635	1.627	0.072	0.067	0.011	0.009
GRC+64 µmol L^{-1} NH_4	1.388	n.d.	1.841	1.738	0.073	0.062	n.d.	0.013
GRC+100 µmol L^{-1} NH_4	1.279	1.354	1.787	1.651	0.071	0.080	0.007	0.006

Table 28 : Summary of observed impacts from SRWTP effluent and NH_4Cl additions on primary production and phytoplankton NH_4 and NO_3 uptake during addition experiments conducted in April([1]) and May([2]) 2009. Effective concentration was the concentration at which any impact was first noted. SRWTP dilution equivalent is based on 1933 μmol L^{-1} effluent concentration, as measured on the effluent supplied for the addition experiments.

	Process	Effective Concentration	Effect	SRWTP Dilution Equivalent
Effluent	Primary Production	(>8 μmol L^{-1})[2]	22-36% decrease[2]	333 : 1
	NH_4 Uptake	(>4 μmol L^{-1})[2]	29% decrease[2]	333 : 1
	NO_3 Uptake	(≥ 1 μmol L^{-1})[2]	>80% decrease[2]	2000: 1
NH_4	Primary Production	Unclear[1]	Unclear[1]	None
	NH_4 Uptake	(>1 μmol L^{-1})[1] Unclear[2]	34%[1] Unclear[2]	None
	NO_3 Uptake	(≥ 1 μmol L^{-1})[1] (≥ 1 μmol L^{-1})[2]	>80% decrease[1] >80% decrease[2]	2000 : 1
Current SRWTP Dilution				14 : 1

Figure 1: Station Map for "Waterboards" experiments conducted from July 2008 to May 2009. SRWTP discharge is located immediately upstream of RM-44



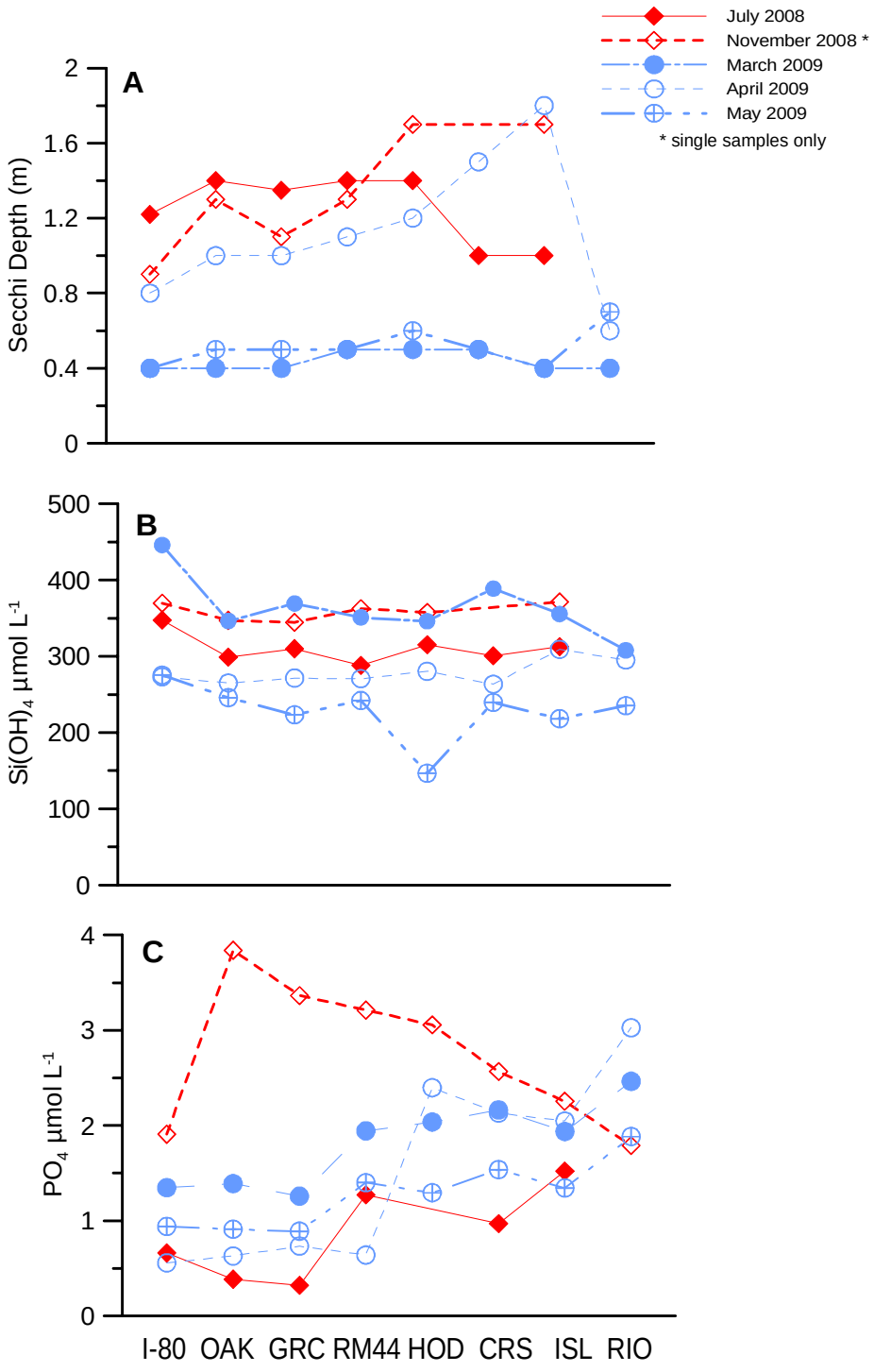


Figure 2: A) Secchi depth, B) silicate and C) phosphate concentrations along the Sacramento River during 5 transects completed between July 2008 and May 2009.

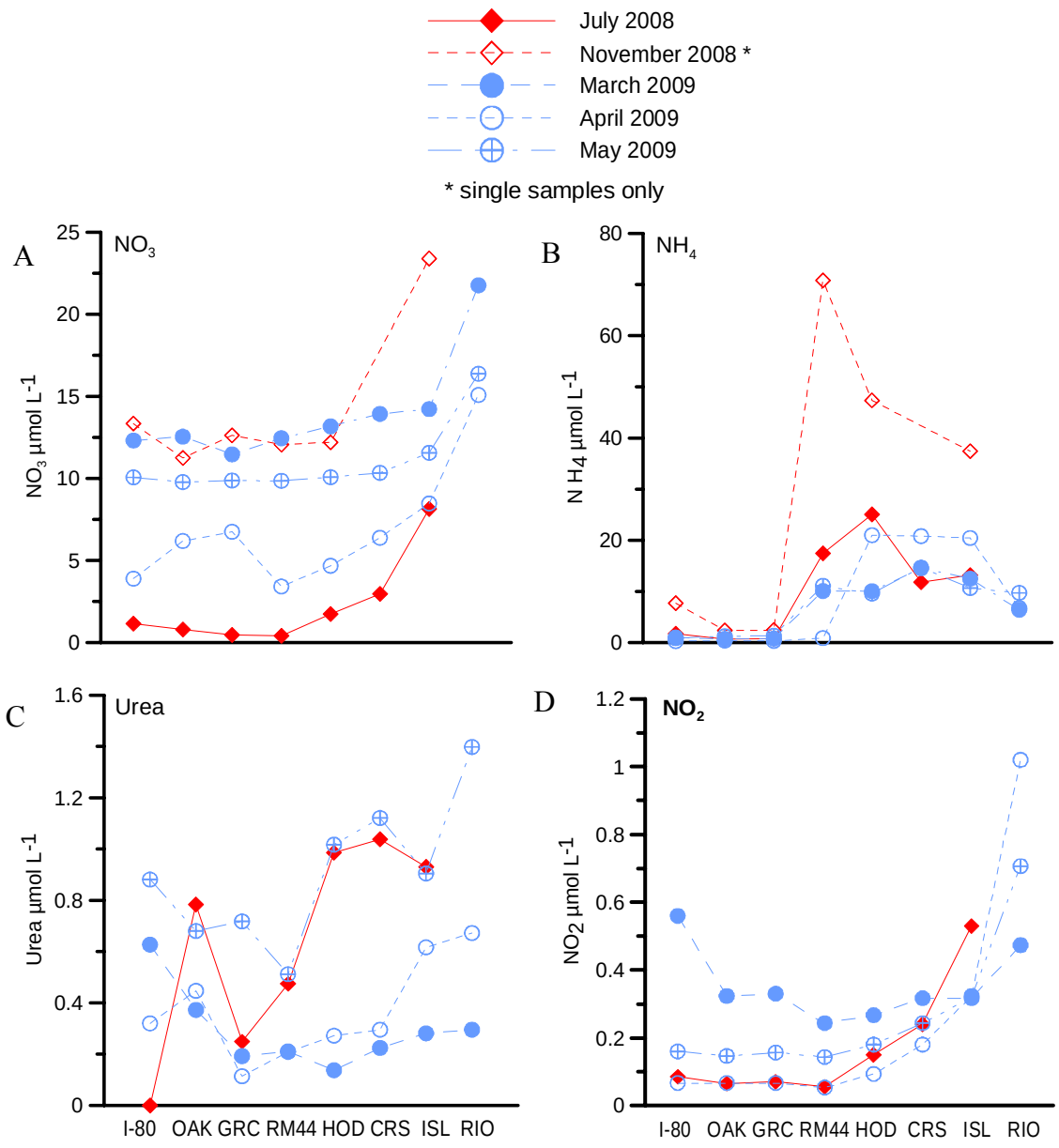


Figure 3: A)Nitrate, B)ammonium, C)urea and D)nitrite concentrations along the Sacramento River completed between July 2008 and May 2009.

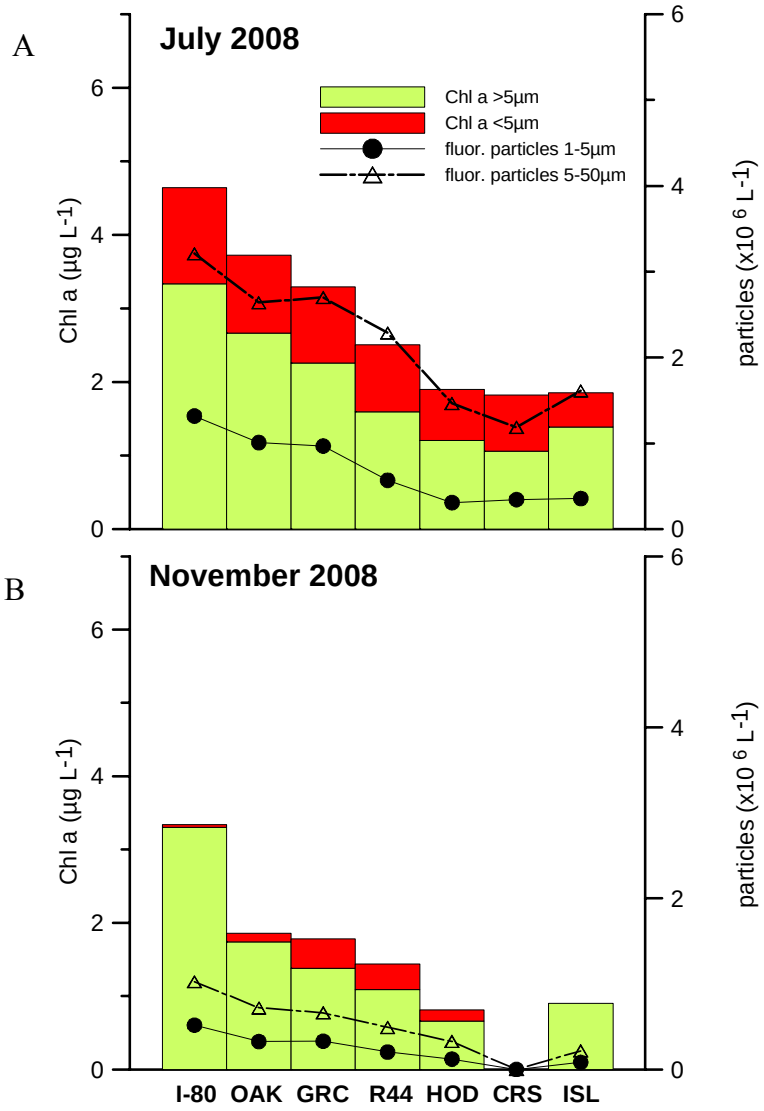


Figure 4: Chlorophyll concentrations for cells >5µm and <5µm in diameter and particle counts (1 to 5-µm and 5 to 50-µm in diameter) determined by flow cytometry. Samples were collected in the Sacramento River in A) July and B)November 2008.

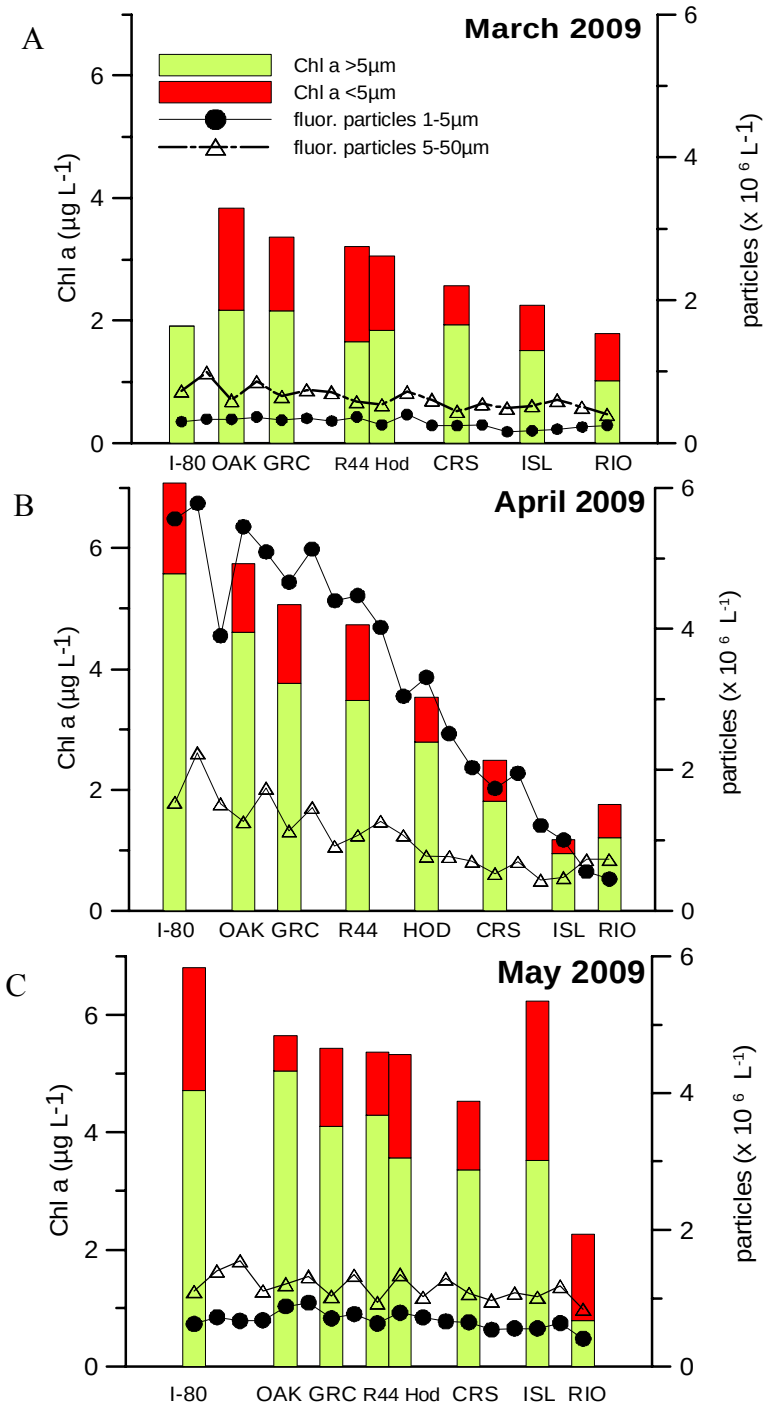


Figure 5: Chlorophyll concentrations for cells >5µm and <5µm in diameter and particle counts (1 to 5-µm and 5 to50-µm in diameter) determined by flow cytometry. Samples collected during transect sampling in the Sacramento River during A)March, B)April, and C)May 2009.

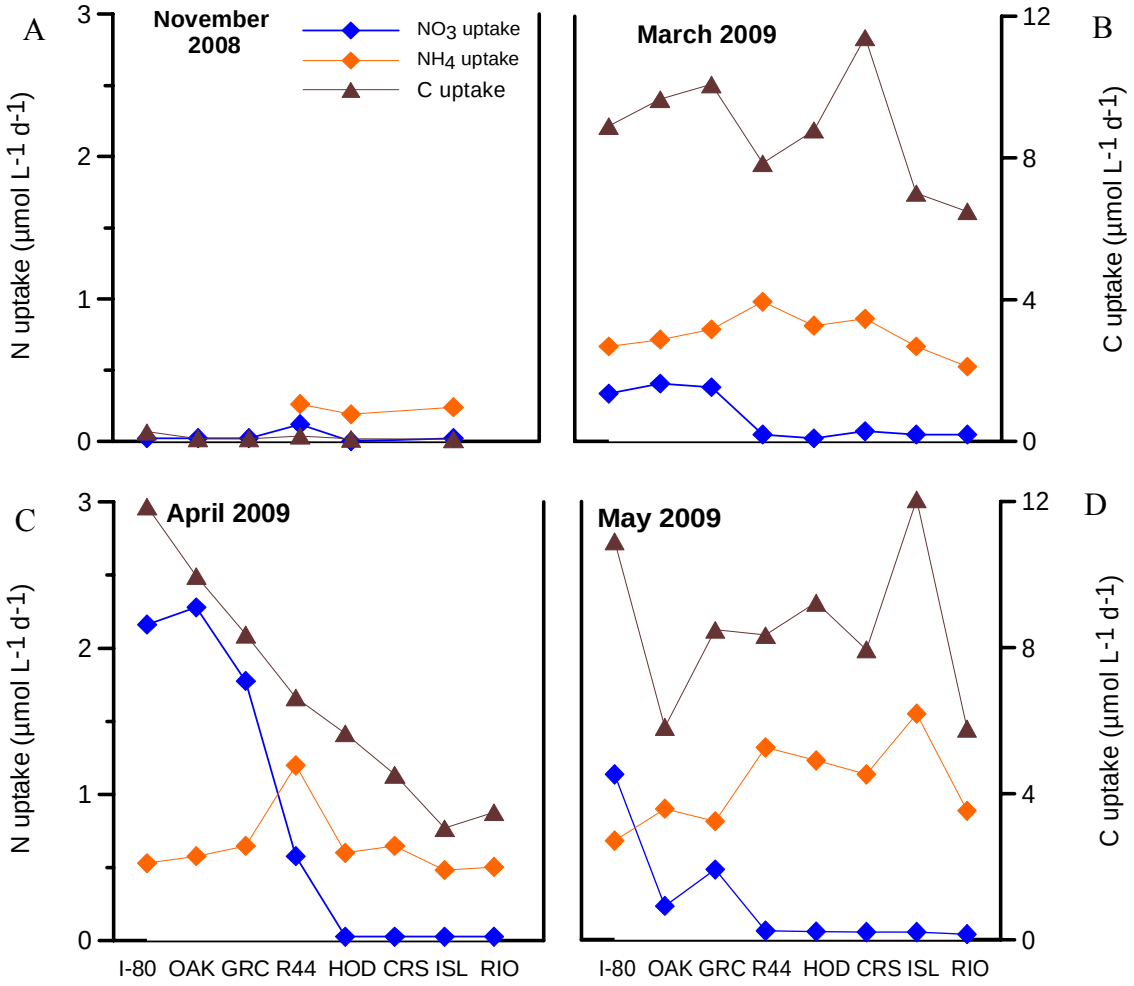


Figure 6: Primary production (C uptake) and phytoplankton nitrogen uptake rates (NH_4 and NO_3) made during 24-hr incubations carried out during eight station transect surveys of the Sacramento River during A)November 2008, B)March 2009, C) April 2009, and D)May 2009.

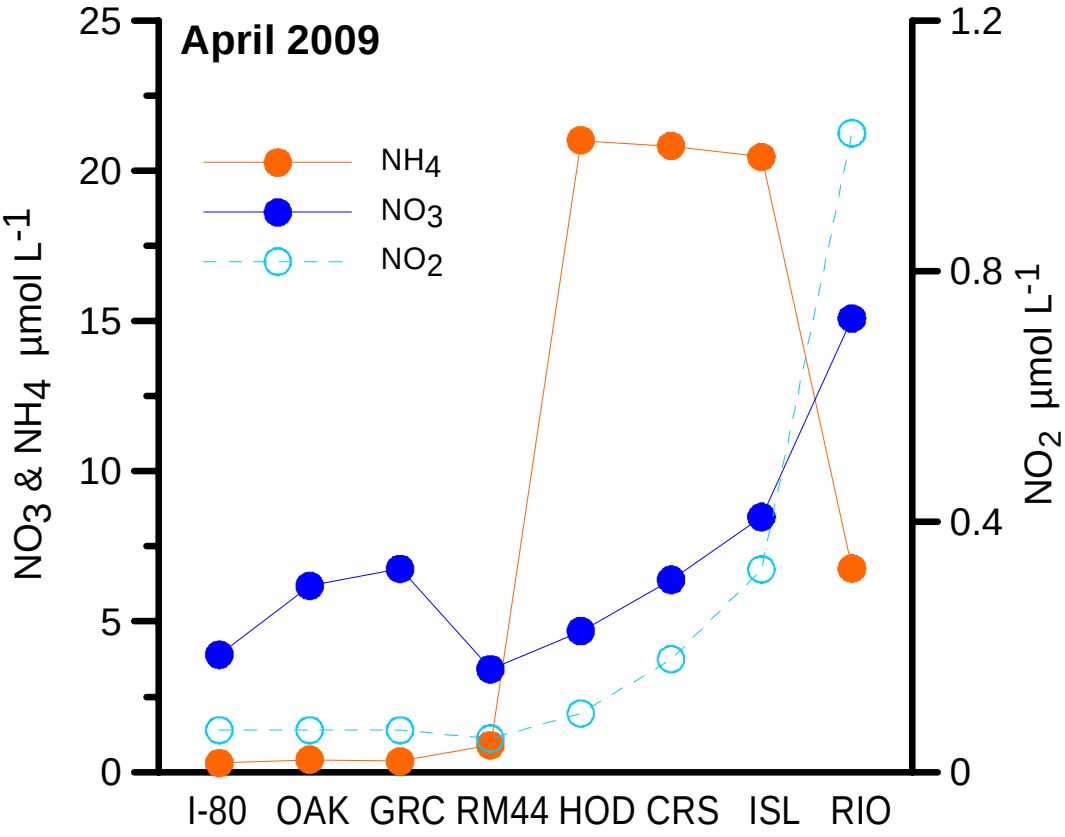


Figure 7: Ammonium (closed red), nitrate (closed blue), and nitrite (open blue) concentrations along the Sacramento River during April 2009.

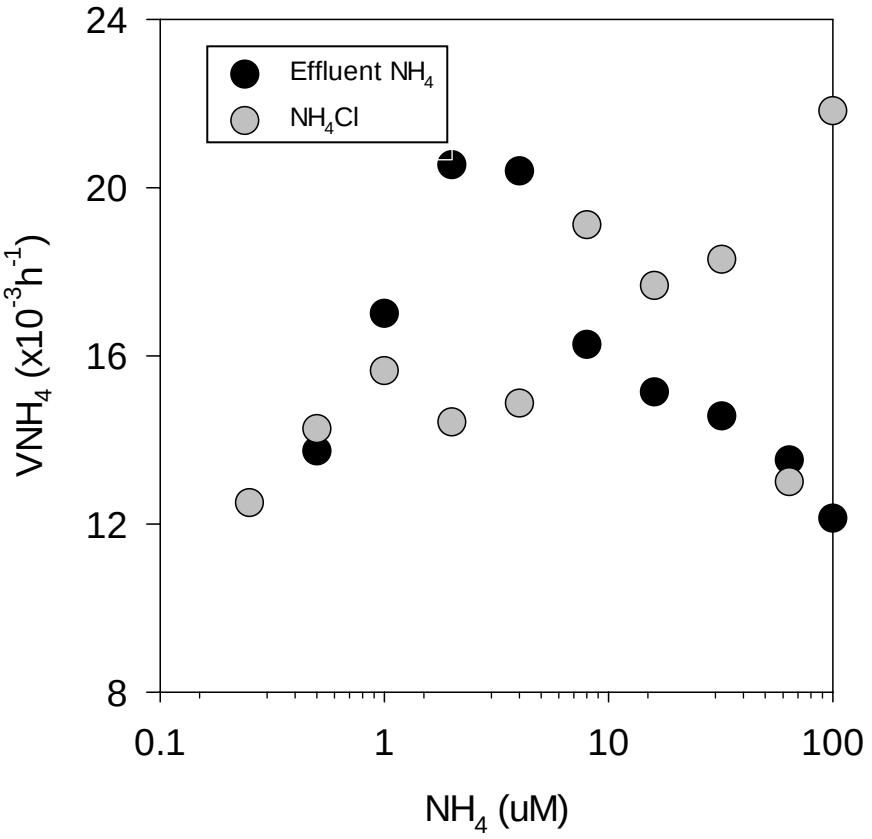


Figure 8: Specific NH_4 (VNH_4, x $10^{-3}h^{-1}$) uptake versus log NH_4 concentration during the May 2009 effluent addition experiment. Closed circles are VNH_4 after exposure to experimental additions of effluent-NH_4, gray circles are VNH_4 after exposure to experimental additions of NH_4Cl.

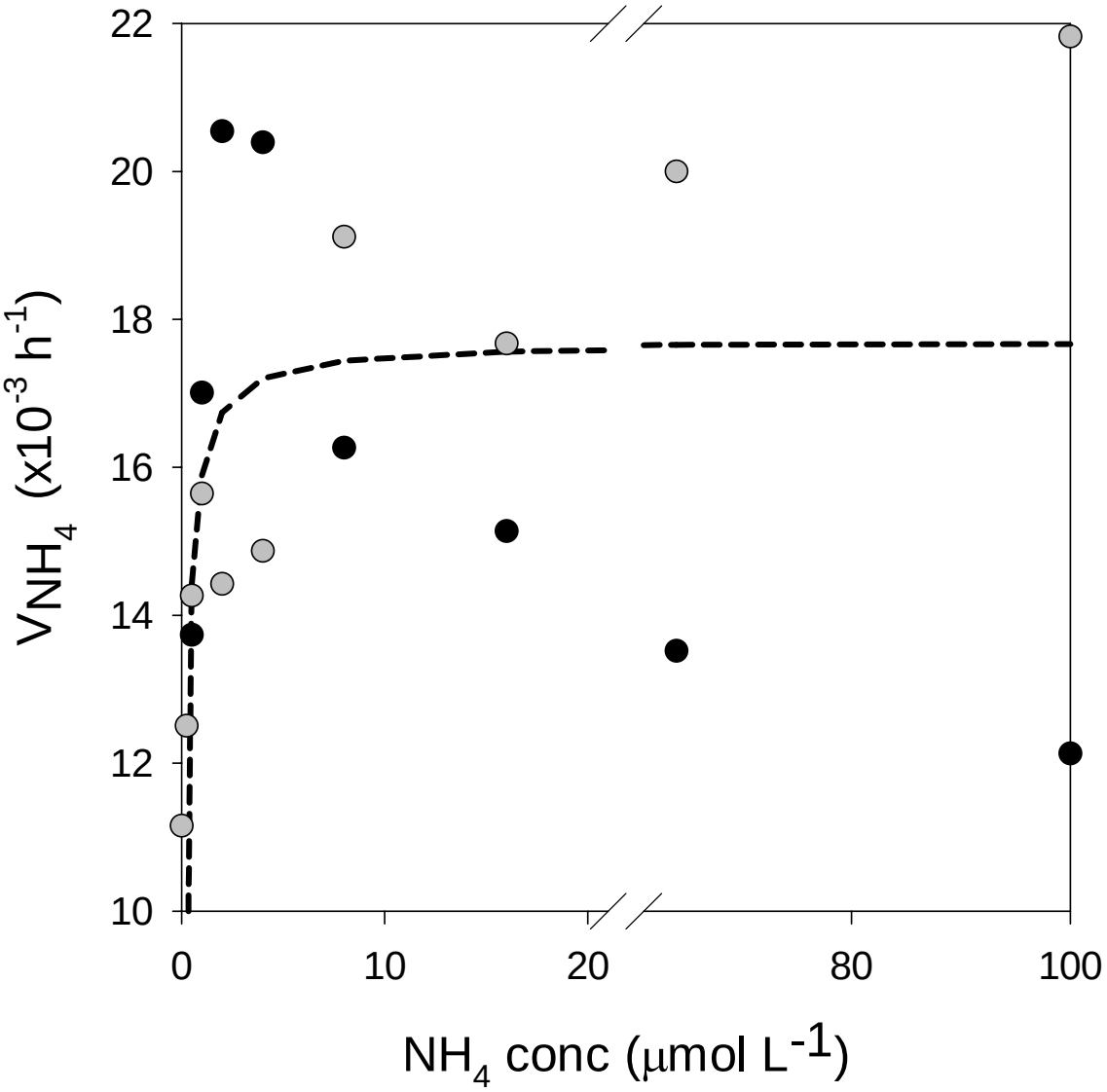


Figure 9: Ammonium uptake vs. NH_4 showing Michaelis-Menten kinetics for the added NH_4 incubations (grey circles).

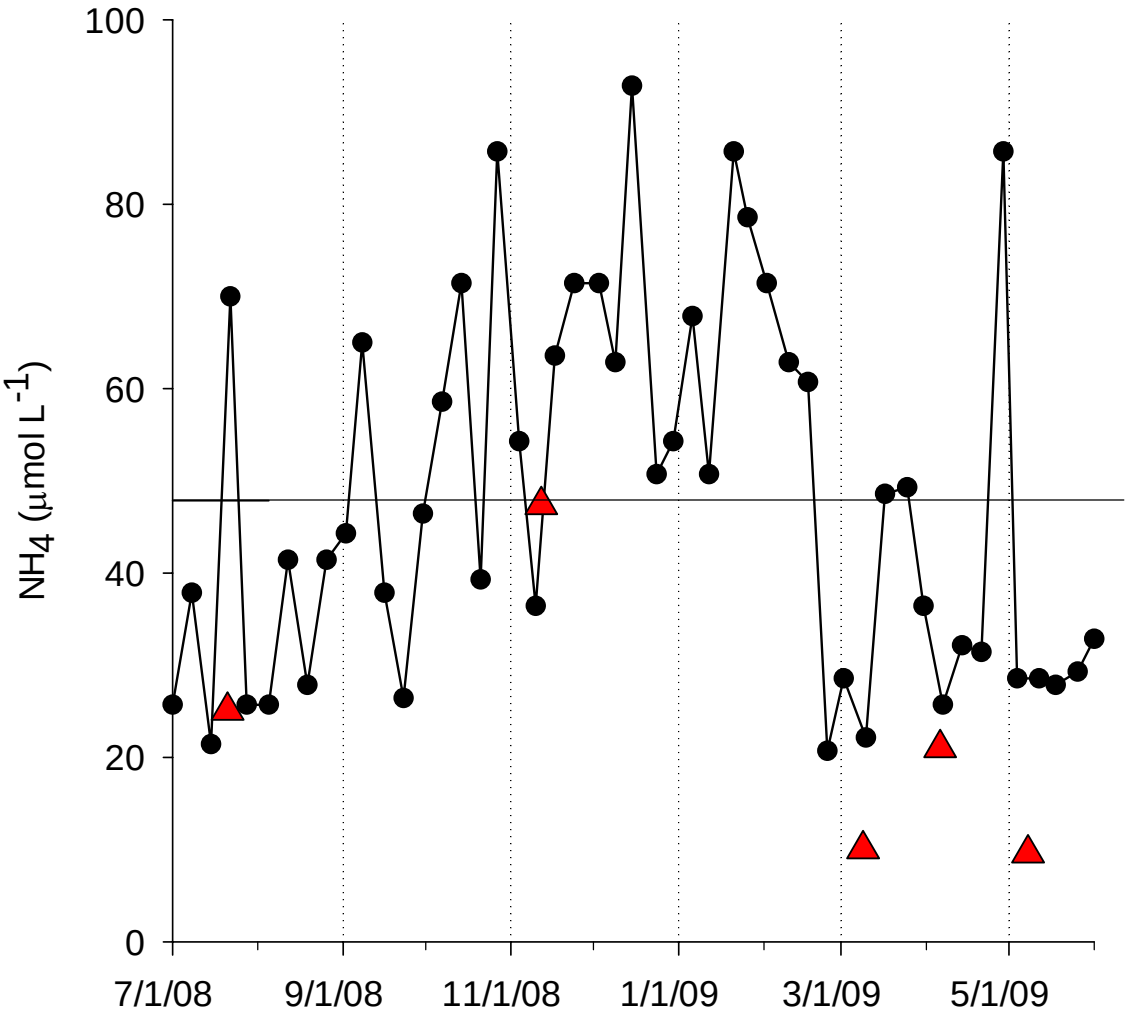


Figure 10: (Closed circles) NH_4 concentration measured at HOD by SRWTP from July 2008 to June 2009. (Red triangles) NH_4 concentrations measured by RTC during the five survey transects made of the Sacramento River in 2008 and 2009.

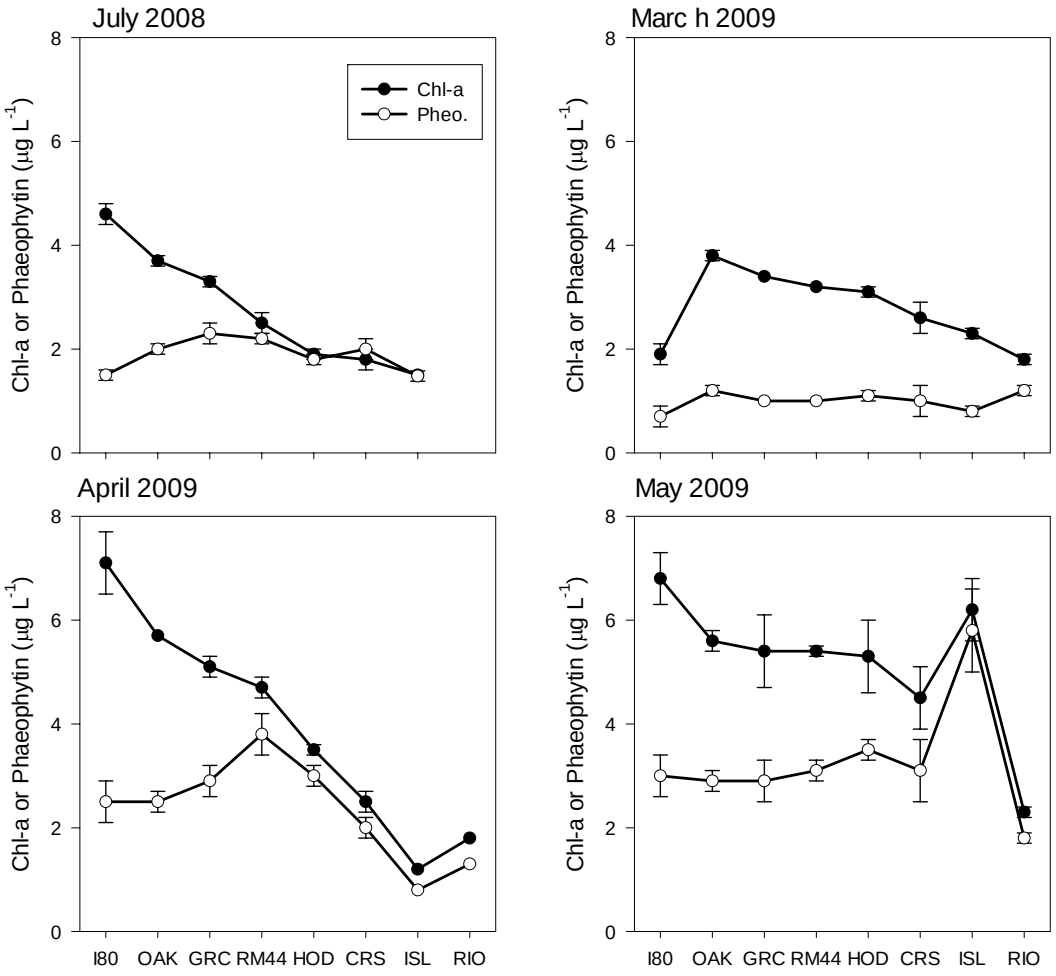


Figure 11: Chlorophyll-a and phaeophytin concentrations (±95% CL) from water samples collected along the Sacramento River on cruises between November 2008 and May 2009.