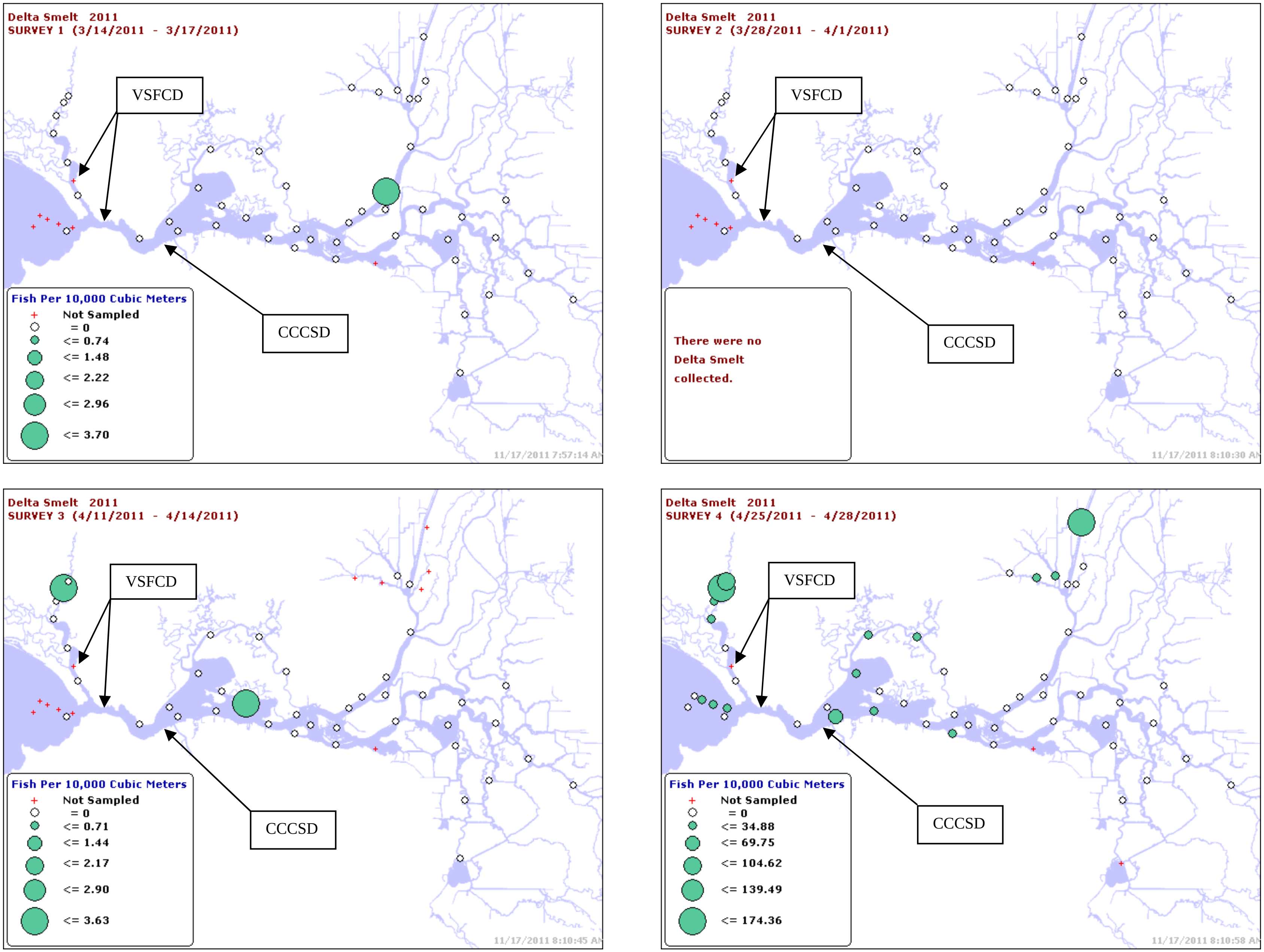
ATTACHMENT 1

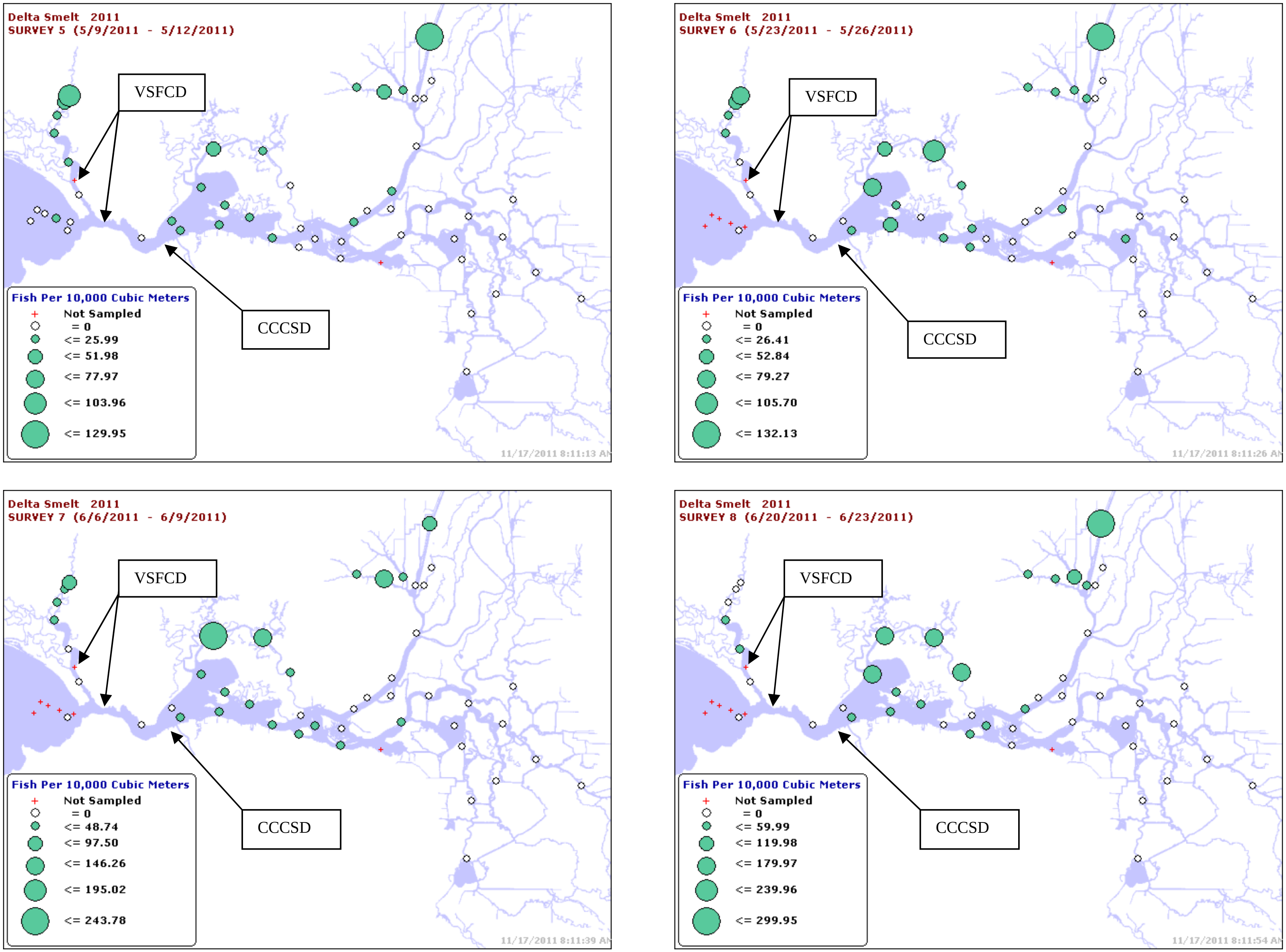
2011 20mm Survey Distribution Maps

Figure 1. Delta Smelt 2011 20 mm Surveys 1-4.



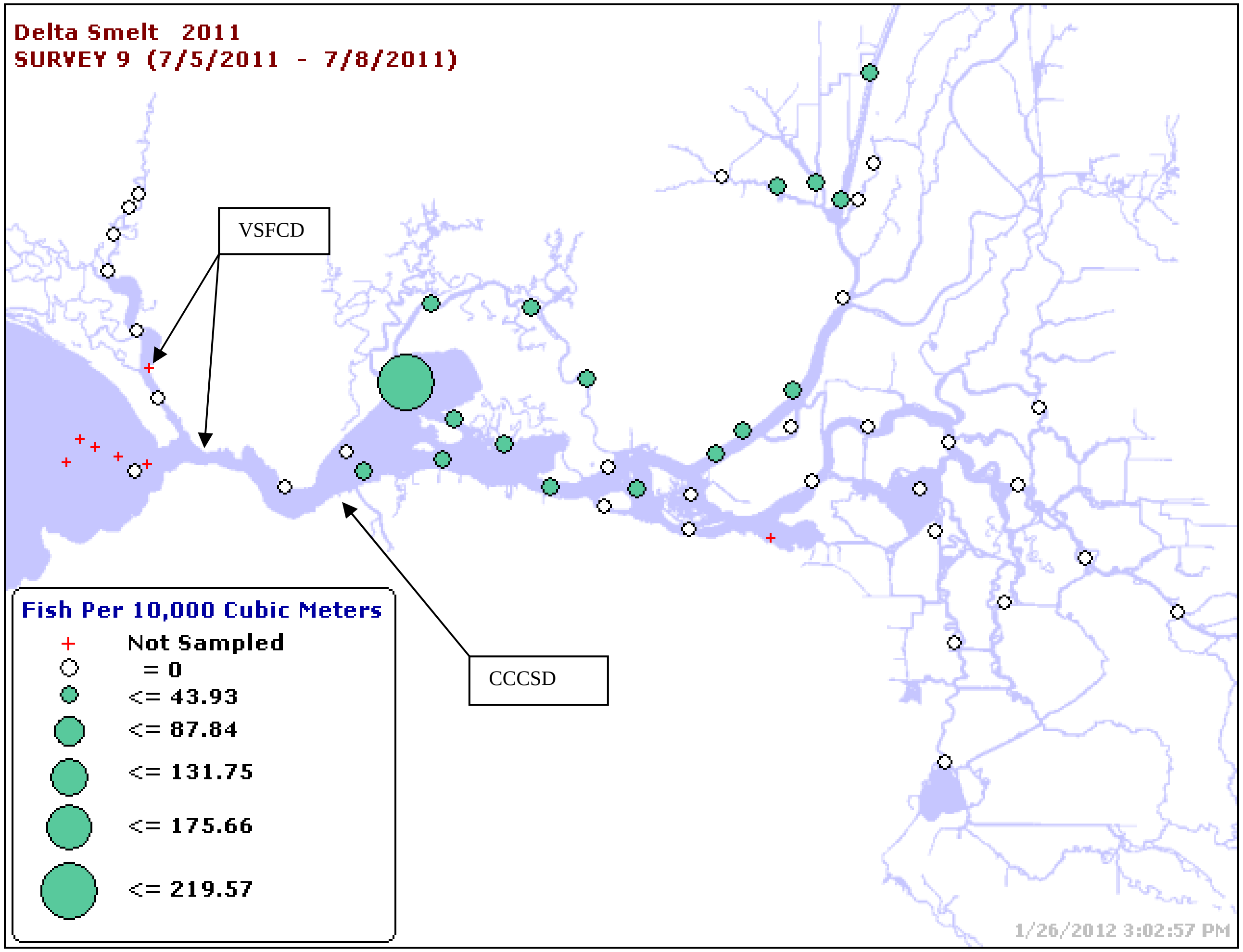
Survey maps and underlying data from Cal. Dept. of Fish & Game, available at http://www.dfg.ca.gov/delta/projects.asp?ProjectID=20mm.

Figure 2. Delta Smelt 2011 20 mm Surveys 5-8.



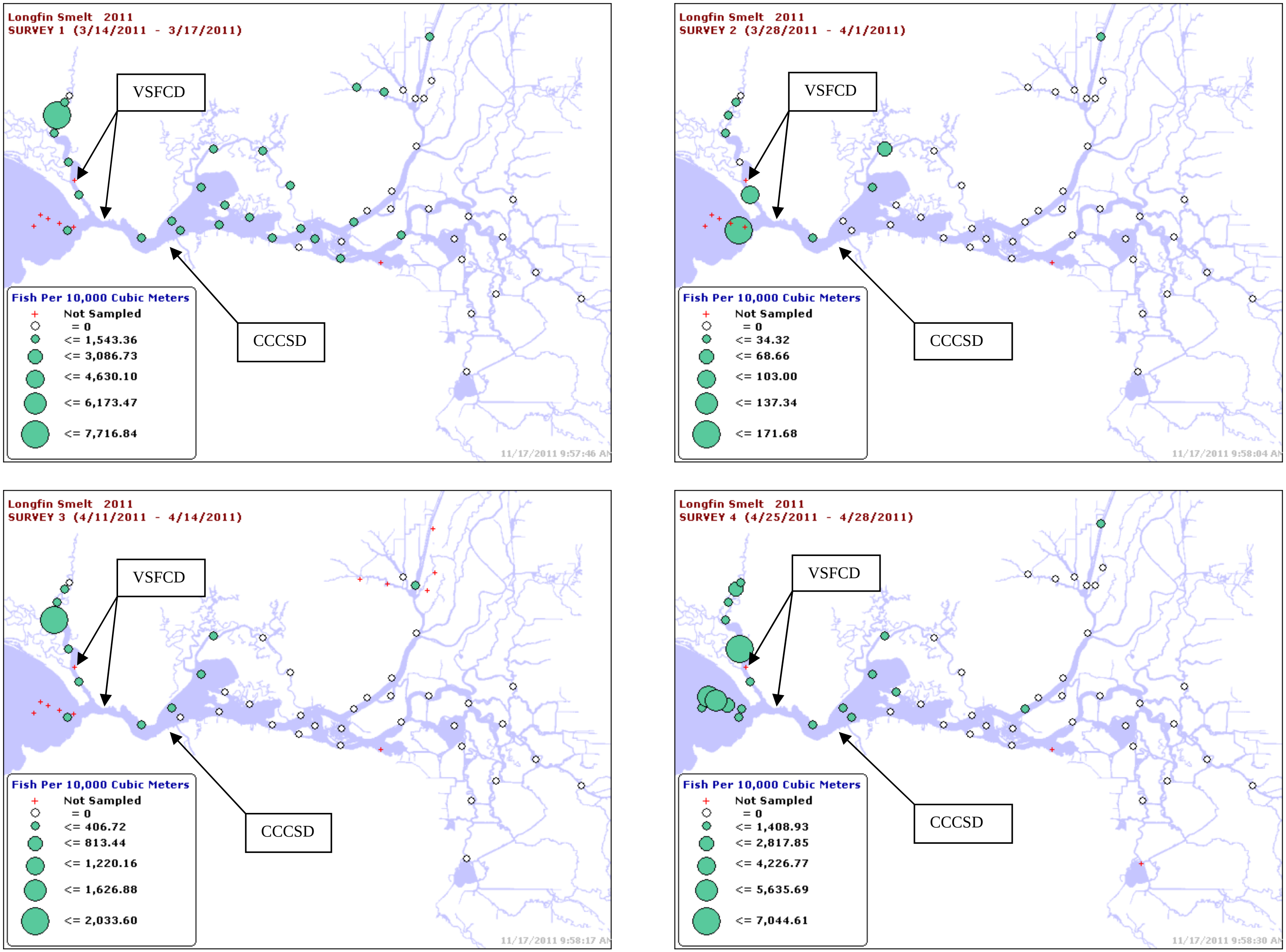
Survey maps and underlying data from Cal. Dept. of Fish & Game, available at http://www.dfg.ca.gov/delta/projects.asp?ProjectID=20mm.

Figure 2B. Delta Smelt 2011 20 mm Survey 9.



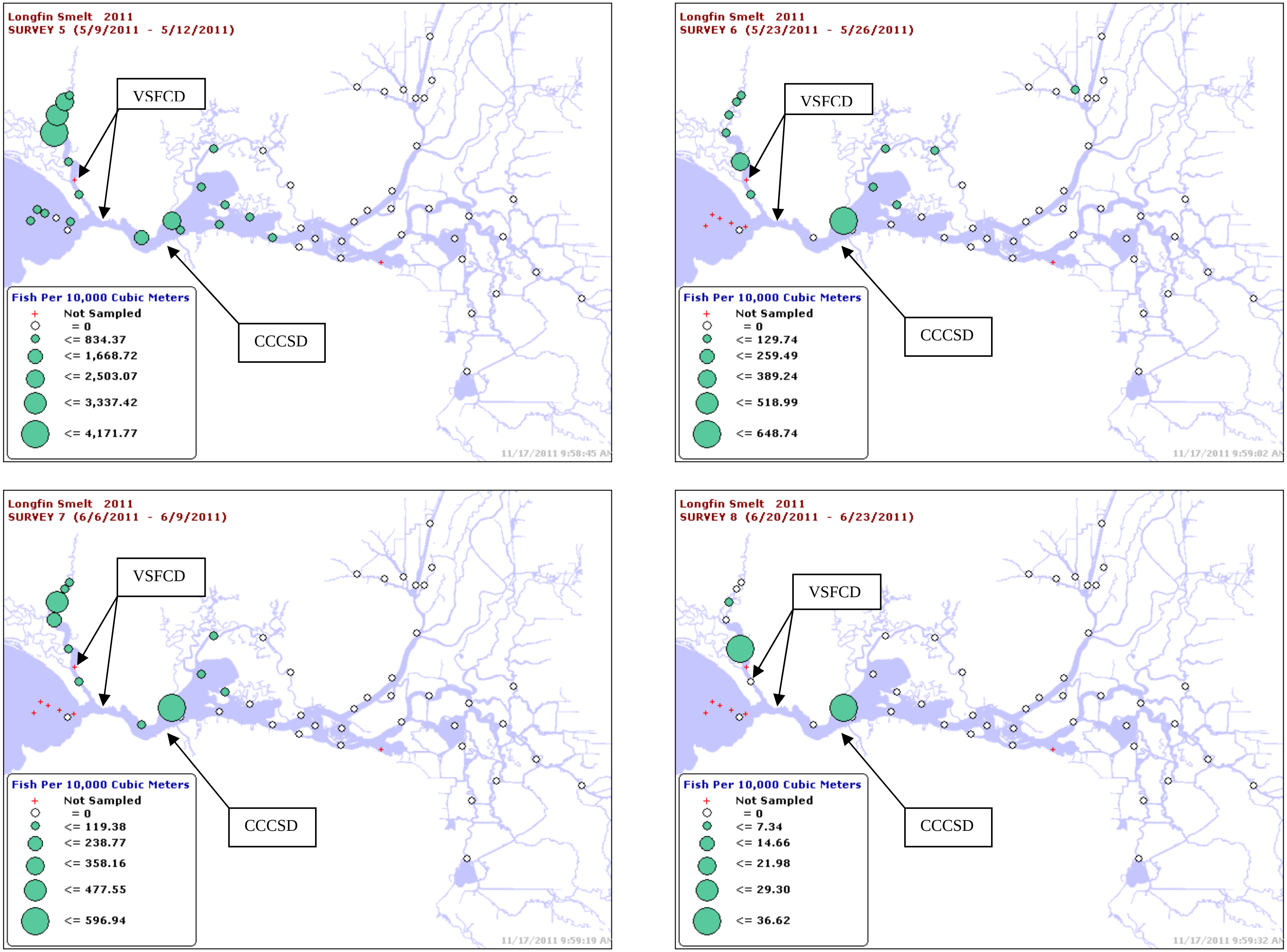
Survey maps and underlying data from Cal. Dept. of Fish & Game, available at http://www.dfg.ca.gov/delta/projects.asp?ProjectID=20mm.

Figure 3. Longfin Smelt 2011 20 mm Surveys 1-4.



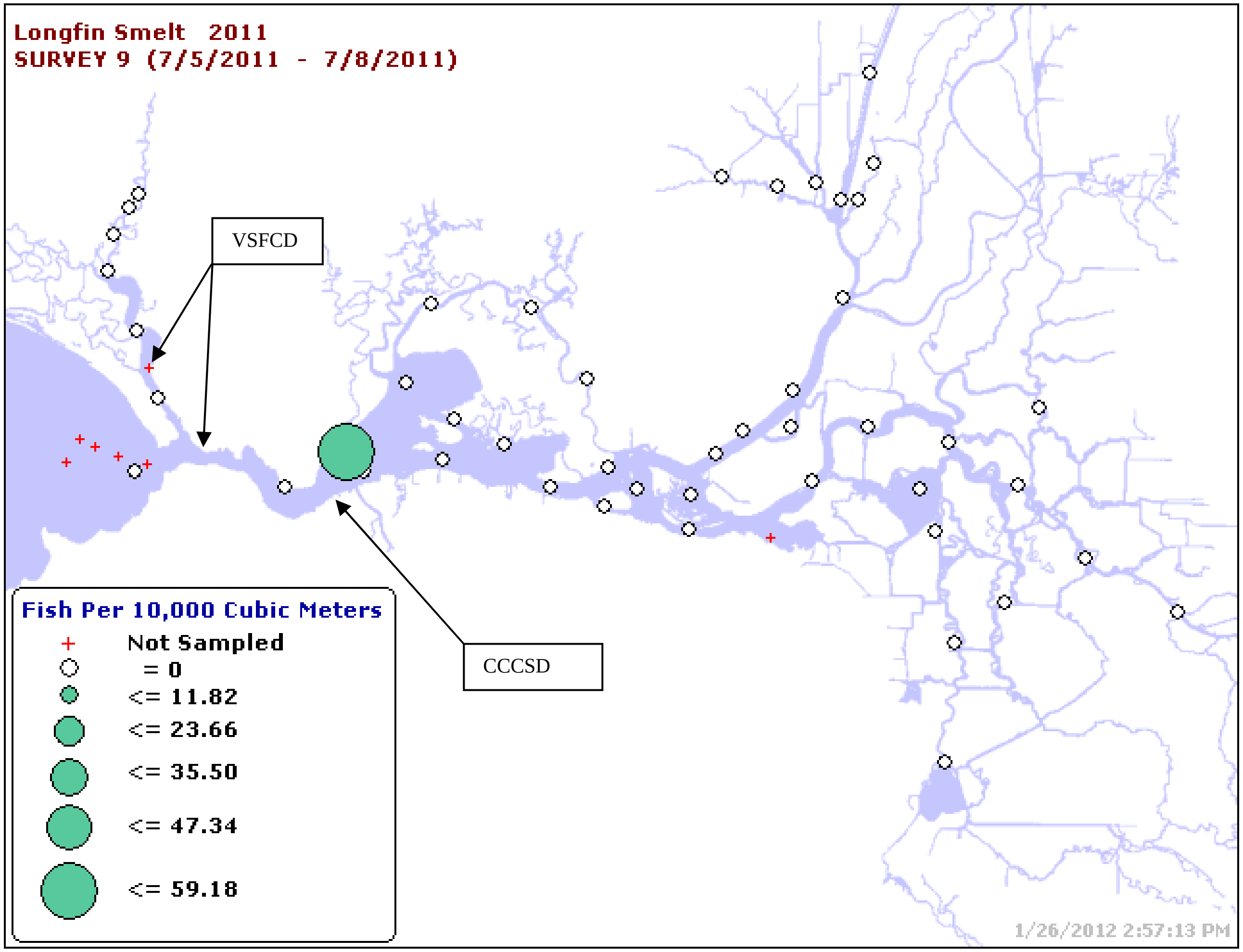
Survey maps and underlying data from Cal. Dept. of Fish & Game, available at http://www.dfg.ca.gov/delta/projects.asp?ProjectID=20mm.

Figure 4. Longfin Smelt 2011 20 mm Surveys 5-8.



Survey maps and underlying data from Cal. Dept. of Fish & Game, available at http://www.dfg.ca.gov/delta/projects.asp?ProjectID=20mm.

Figure 4B. Longfin Smelt 2011 20 mm Survey 9.



Survey maps and underlying data from Cal. Dept. of Fish & Game, available at http://www.dfg.ca.gov/delta/projects.asp?ProjectID=20mm.

ATTACHMENT 2

Parker, A. E., et al. (2012)
Elevated ammonium concentrations from wastewater discharge depress primary productivity in the Sacramento River and the Northern San Francisco Estuary

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Elevated ammonium concentrations from wastewater discharge depress primary productivity in the Sacramento River and the Northern San Francisco Estuary

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ABSTRACT

Primary production in the Northern San Francisco Estuary (SFE) has been declining despite heavy loading of anthropogenic nutrients. The inorganic nitrogen (N) loading comes primarily from municipal wastewater treatment plant (WTP) discharge as ammonium (NH_4). This study investigated the consequences for river and estuarine phytoplankton of the daily discharge of 15 metric tons NH_4–N into the Sacramento River that feeds the SFE. Consistent patterns of nutrients and phytoplankton responses were observed during two 150-km transects made in spring 2009. Phytoplankton N productivity shifted from NO_3 use upstream of the WTP to productivity based entirely upon NH_4 downstream. Phytoplankton NH_4 uptake declined downstream of the WTP as NH_4 concentrations increased, suggesting NH_4 inhibition. The reduced total N uptake downstream of the WTP was accompanied by a 60% decline in primary production. These findings indicate that increased anthropogenic NH_4 may decrease estuarine primary production and increase export of NH_4 to the coastal ocean.

1. Introduction

Nutrient loading is increasing globally due to population growth and intensification of agriculture. Cultural eutrophication and the loading of aquatic systems with nitrogen (N) and phosphorus (P) have long been recognized as important drivers of ecosystem change. Generally, eutrophication is thought to degrade food webs and lead to increases in autotrophic biomass, including nuisance algal species, inefficient trophic transfer, stimulation of microbial activity and hypoxia. However, study of estuarine eutrophication globally for more than three decades has revealed a range of ecosystem responses to nutrient enrichment (Sharp, 2001). Increased nutrients may lead to eutrophication with undesirable consequences, but not in all cases (Cloern, 2001; Sharp et al., 2009). Rather than stimulating algal processes, negative effects on phytoplankton physiology have been observed (MacIsaac et al., 1979; Wilkerson et al., 2006). Reduction in primary productivity associated with anthropogenic ammonium (NH_4) loading has been reported, for example in the Delaware Estuary (Yoshiyama and Sharp, 2006) and a wastewater-dominated Canadian river (Waiser et al., 2011). The San Francisco Estuary (SFE) has also experienced declining primary productivity (Jassby et al., 2002) while receiving increased nutrient loading (Jassby, 2008). It is the largest estuary on the west coast of the US and highly impacted by the urban centers of the San Francisco Bay Area (San Francisco, Oakland and San Jose) and the City of Sacramento and receives nutrient inputs from more than 80 municipal wastewater treatment plants (WTPs) with varying levels of effluent treatment.

Increased loading of NH_4 to the SFE is largely the product of the Clean Water Act requiring the conversion of WTP's to secondary treatment resulting in discharge of N as NH_4. With the exception of Stockton, major cities in the Northern SFE and Delta do not carry out advanced secondary treatment and discharge N primarily in the form of NH_4 rather than NO_3. As of 2006, 75% of the effluent released by Delta treatment plants was processed only to the secondary level (Brooks et al., 2011). Approximately 90% of the total N in the Northern SFE originates from a single point source, at the Sacramento Regional WTP (SRWTP), which discharges approximately 15 metric tons of N per day, largely as NH_4, to the Sacramento River (Jassby, 2008).

Primary productivity in the SFE ranks towards the bottom of river-dominated estuaries (Boynton et al., 1982) and is thought to be regulated by turbidity and not nutrient supply (Cole and Cloern, 1984; Alpine and Cloern, 1988). However, recent studies suggest that in addition to light availability, increased nutrient loading (especially NH_4 loading) acts as an additional estuarine "filter" (Cloern, 2001) that modulates primary production and results in alterations to the food web (Glibert, 2010; Glibert et al., 2011). Spring and summer phytoplankton blooms (traditionally diatoms; Cloern and Dufford, 2005) were previously a regular feature in the Northern SFE but rarely occur now (Kimmerer, 2006;

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Wilkerson et al., 2006; Jassby, 2008). Suppression of SFE spring blooms was linked to elevated NH_4 concentrations (Wilkerson et al., 2006; Dugdale et al., 2007). When NH_4 concentrations were above 4 μmol N L^{-1}, high chlorophyll-a concentrations were not observed. Only when NH_4 was decreased below 4 μmol N L^{-1}, either through phytoplankton assimilation or through freshwater dilution, did phytoplankton access NO_3, the larger pool of dissolved inorganic nitrogen (DIN) and accumulate chlorophyll-a biomass (Dugdale et al., 2007). A bloom sequence consists of two phases and only occurs when irradiance conditions are favorable for phytoplankton growth. In the first phase, NH_4 is taken up by the phytoplankton resulting in reduction of ambient NH_4 concentrations to below about 4 μmol N L^{-1}. In the second phase, as NO_3 is taken up, chlorophyll-a biomass accumulates and blooms result (Dugdale et al., 2007).

The requirement for use of NO_3 to enable bloom formation in SFE, rather than NH_4 seems counter-intuitive to the classical paradigm that phytoplankton "prefer" NH_4 over NO_3 as a result of lower energetic costs to the cell associated with protein synthesis (McCarthy et al., 1977). While the energetic argument is correct and applies in most batch culture experiments in the laboratory, in the SFE NH_4 concentrations (e.g. winter mean in the Northern SFE = 6.8 μmol N L^{-1}; Wilkerson et al., 2006) are insufficient to fuel blooms. So for elevated chlorophyll-a concentrations, NO_3 (e.g. 27.5 μmol N L^{-1}; Wilkerson et al., 2006), the larger DIN pool, must be accessed. This can only be accomplished once NH_4 is below some threshold above which it is inhibitory to NO_3 uptake and assimilation. Raven et al. (1992) described how when both NO_3 and NH_4 are present (as in the SFE), phytoplankton will almost invariably use NH_4 with complete suppression of NO_3 uptake at NH_4 concentrations of as little as 1–2 μmol N L^{-1}. The suppression of phytoplankton NO_3 uptake by NH_4 has been documented in phytoplankton isolates (e.g. Cochlan and Harrison, 1991; Dortch, 1990; Lomas and Glibert, 1999; Maguer et al., 2007) and in natural communities (e.g. McCarthy et al., 1977; Collos et al., 1989; Cochlan and Bronk, 2003; L'Helguen et al., 2008).

The impact of NH_4 suppression of NO_3 uptake and the reduction of phytoplankton blooms and primary production is particularly important for the Northern SFE, where food limitation has been demonstrated for zooplankton (Mueller-Solger et al., 2002) and fish species (Bennett and Moyle, 1996) and may be in part responsible for an overall "pelagic organism decline" (Sommer et al., 2007). Glibert (2010) described how the decline in fish may be closely linked to historical changes in nutrient loadings, especially of NH_4 and P (Van Nieuwenhuse, 2007). Although the Sacramento River that feeds the Northern SFE has been considered a significant source of organic matter for the Northern SFE (Jassby et al., 2002; Sobczak et al., 2005), little is known or documented about productivity of the phytoplankton in the river and the impact of N loading on their physiology. The goals of this study were to: (1) understand the distribution and biological processing of different forms of DIN in the Sacramento River and (2) describe how discharge of wastewater NH_4 effluent influences phytoplankton biomass and primary productivity in the Sacramento River and downstream to the Northern SFE.

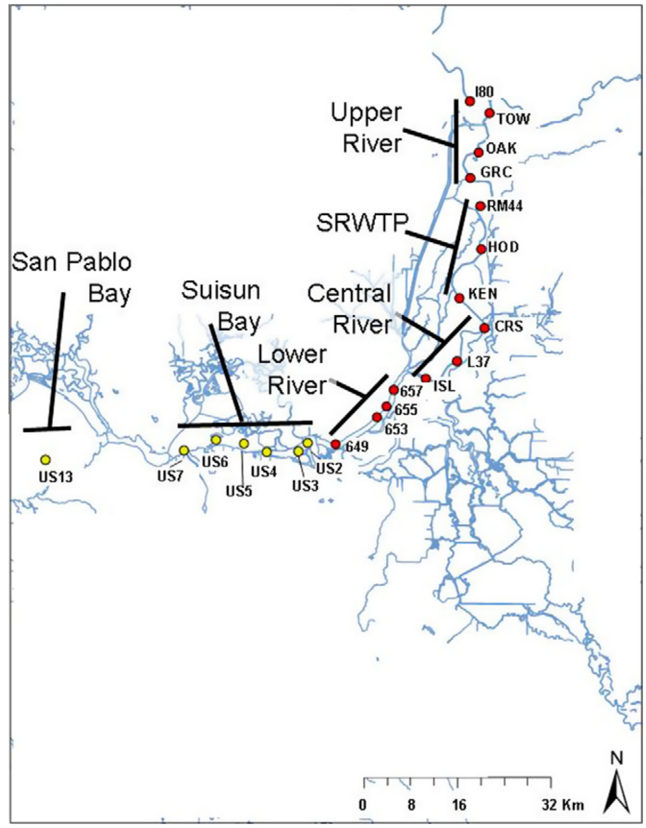

Fig. 1. Study region of the Sacramento River and San Francisco Estuary, CA showing sampling stations and river and Northern estuary transect regions.

2. Materials and methods

2.1. River and estuary surveys

Two, 150-km surveys of the Sacramento River and Northern San Francisco Estuary were made on 26–27 March and 23–24 April 2009 using the R/V *Questuary*. During each survey 21 geographically fixed stations were sampled on the outgoing tide from upstream to downstream (Fig. 1 and Table 1). For analysis the transect was divided into six regions based on geographic location, ambient NH_4 and chlorophyll-a concentrations. The Upper River region included the four stations (I80, TOW, OAK and GRC) above the Sacramento Regional Wastewater Treatment Plant (SRWTP) and was characterized by low NH_4 concentrations (⩽1 μmol N L^{-1}). The SRWTP region included three stations (RM44, HOD and KEN), that were the closest geographically to the SRWTP and had elevated NH_4; RM44 is the station closest to the SRWTP discharge. The Central River region encompassed three stations (CRS, L37 and ISL) and also exhibited high NH_4 concentrations. The Lower River region included four stations (657, 655, 653 and 649) and was marked by declines in both NH_4 and chlorophyll-a concentrations. In the Northern estuary, Suisun Bay included six stations (US2, US3, US4, US5, US6 and US7) and San Pablo Bay was represented by a single station (US13). Stations south of Isleton (ISL) were identical to monthly water quality monitoring stations sampled by the US Geological Survey (USGS) (Jassby et al., 1997; http://sfbay.wr.usgs.gov/access/wqdata/index.html). River distances (km) were calculated from the SRWTP (i.e. at 0 km) with stations upstream of the SRWTP being negative. Sacramento River discharge was obtained from the California Department of Water Resources Dayflow algorithm (http://www.water.ca.gov/dayflow/). SRWTP daily effluent discharge was obtained from the California Central Valley Regional Water Quality Control Board.

At each station, a Seabird Electronics SB-32 rosette mounted with six 3-L Niskin bottles and fitted with a Seabird SBE-19 plus CTD was deployed to collect vertical profiles of temperature and salinity and collect surface water samples. In the freshwater regions the salinity was reported as electrical conductivity (μS cm^{-1}) while in the Northern SFE salinity was reported using the practical salinity scale (pss). Turbidity was measured with a D&A Instruments Optical Backscatter (Model OBS-3, S/N 937) sensor

Table 1
Salinity, light attenuation coefficient and nutrient concentrations (mean ± SD) in Sacramento River and SF Estuary by river region (number of stations) for March and April 2009.

River Region	EC ($\mu S\ cm^{-1}$)	k (m^{-1})	NO_3 ($\mu mol\ L^{-1}$)	NO_2 ($\mu mol\ L^{-1}$)	NH_4 ($\mu mol\ L^{-1}$)	DIN ($\mu mol\ L^{-1}$)	NH_4 as %DIN (%)	Urea ($\mu mol\ L^{-1}$)	SRP ($\mu mol\ L^{-1}$)	$Si(OH)_4$ ($\mu mol\ L^{-1}$)
March 2009										
Upper River (4)	86 ± 8	2.5 ± 0.5	13.08 ± 0.59	0.12 ± 0.02	0.25 ± 0.09	13.81 ± 0.60	1.8	0.36 ± 0.07	1.37 ± 0.12	343 ± 19
SRWTP (3)	85 ± 5	3.2 ± 0.1	13.85 ± 1.46	0.15 ± 0.08	29.58 ± 10.24	43.87 ± 12.05	64.2	0.29 ± 0.38	2.94 ± 0.95	336 ± 4
Central River (3)	86 ± 2	3.5 ± 0.2	17.21 ± 2.16	0.35 ± 0.09	34.50 ± 8.29	52.43 ± 9.04	66.8	0.44 ± 0.38	3.14 ± 0.39	333 ± 11
Lower River (4)	117 ± 1	1.8 ± 0.3	29.07 ± 1.24	0.95 ± 0.10	13.76 ± 3.17	44.26 ± 3.93	31.2	0.44 ± 0.22	2.98 ± 0.16	350 ± 4
Suisun Bay (6)	0.9 ± 1.3*	1.3 ± 0.1	32.94 ± 0.5	1.19 ± 0.29	8.54 ± 1.20	43.23 ± 1.70	19.7	0.56 ± 0.40	2.96 ± 0.11	327 ± 14
San Pablo Bay (1)	23.1*	2.5	21.85	1.03	2.24	26.01	8.6	0.84	2.33	138
April 2009										
Upper River (4)	113 ± 11	1.0 ± 0.4	2.06 ± 0.54	0.14 ± 0.01	0.58 ± 0.23	2.78 ± 0.73	20.4	0.10 ± 0.20	0.44 ± 0.10	270 ± 34
SRWTP (3)	123 ± 4	1.4 ± 0.3	4.57 ± 0.95	0.21 ± 0.10	36.02 ± 13.47	40.80 ± 14.38	86.9	0.26 ± 0.25	1.70 ± 0.20	276 ± 13
Central River (3)	123 ± 4	1.1 ± 0.2	7.73 ± 2.08	0.42 ± 0.10	31.84 ± 13.35	39.99 ± 15.19	81.4	0.24 ± 0.14	1.81 ± 0.43	271 ± 10
Lower River (4)	144 ± 2	2.5 ± 0.7	18.29 ± 1.96	0.93 ± 0.07	14.57 ± 1.46	33.79 ± 0.58	44.6	0.08 ± 0.06	1.84 ± 0.15	276 ± 16
Suisun Bay (6)	2.6 ± 2.5*	3.0. ± 0.4	30.71 ± 2.35	1.35 ± 0.30	7.72 ± 0.96	39.78 ± 3.15	19.4	0.46 ± 0.46	2.32 ± 0.23	259 ± 19
San Pablo Bay (1)	24.6*	1.7	28.00	0.78	3.13	31.13	10.0	0.10	2.32	72

* Indicated salinity (dimensionless) reported on the practical salinity scale.

and reported as nephelometric turbidity units (ntu). The rosette was also equipped with a LiCor 4Π photosynthetically active radiation (PAR) sensor. Light attenuation, k (m^{-1}), was calculated by linear regression of log transformed PAR versus depth.

2.2. Detailed methods

20-ml dissolved inorganic carbon (DIC) samples were collected in glass scintillation vials, preserved according to Sharp et al. (2009) with 200 µL 5% w/v $HgCl_2$ and stored in the dark. These data were used for calculating ^{13}C uptake rates. DIC analysis was completed within 1 week using a Monterey Bay Research Institute – clone DIC analyzer with acid-sparging and a LiCor nondispersive infrared detector (Model 6252) (Friederich et al., 2002; Parker et al., 2006). Water samples for nutrient analysis were immediately filtered through Whatman GF/F filters using a 50-ml syringe and stored on dry ice in 20-ml HDPE scintillation vials or 50-ml centrifuge tubes. All nutrient analyses, except for NH_4 and urea–N, were performed on a Bran and Luebbe AutoAnalyzer II. NO_3, NO_2 and soluble reactive phosphorus (SRP) were analyzed using Whitledge et al. (1981) and $Si(OH)_4$ using Bran and Luebbe (1999) and MacDonald et al. (1986). Twenty-five milliliter samples for NH_4 determination were collected separately into 50-ml centrifuge tubes after filtration (Wilkerson et al., 2006). These samples were also immediately frozen for later analysis by the colorimetric method of Solorzano (1969) using a Hewlett Packard diode array spectrophotometer and 10-cm path length cell. Samples for urea were prepared in the same manner as NH_4 samples with analysis performed according to Revilla et al. (2005).

Two size fractions were collected for analysis of extracted chlorophyll-a concentration using 25-mm Whatman GF/F filters (nominally cells >0.7-µm, referred to here as the ''whole community'' fraction) and 25-mm diameter 5.0-µm Nuclepore pore-sized polycarbonate filters. Sample volumes were selected to minimize filtration times to <10 min using a low vacuum (<250 mm Hg) and varied between 50 and 200 ml. Filters were stored dry at 4 °C for up to one week. Prior to analysis, chlorophyll-a was extracted from the filters in 90% acetone for 24-h at 4 °C according to Arar and Collins (1992). Analysis was performed fluorometrically with a Turner Designs Model 10-AU using 10% hydrochloric acid to correct for and measure phaeophytin. The fluorometer was calibrated with commercially available chlorophyll-a (Turners Designs chlorophyll-a standard). Phaeophytin concentrations were calculated according to Holm-Hansen and Riemann (1978).

Phytoplankton carbon productivity and nitrogen (NO_3 and NH_4) uptake rates were estimated using dual-labeled $^{13}C/^{15}N$ tracer incubations (Legendre and Gosselin, 1996; Parker, 2005; Parker et al., submitted for publication). Two, 160-ml clear polycarbonate incubation bottles were filled with sample water at each station; to one incubation bottle $H^{13}CO_3$ and $^{15}NH_4Cl$ were added and to the other, $H^{13}CO_3$ and $K^{15}NO_3$ (all stable isotope stocks contained 99 at%, Cambridge Isotope Laboratories). Isotope additions were kept to ca 10% of ambient concentrations. Incubations were performed over 24-h on board in a flowing river water incubator covered with one layer of window screening to simulate 50% of ambient surface PAR. A 24-h period was selected so that incubations could be started throughout the day. Because DIN concentrations were generally high (>>2 µmol N L^{-1}) N-substrate limitation during incubations was unlikely at most stations as phytoplankton N uptake rates were generally <2 µmol N L^{-1} d^{-1}. We did not attempt to account for NH_4 regeneration and reported NH_4 uptake rates should be considered conservative. Incubations were terminated by gentle vacuum filtration onto pre-combusted (450 °C for 4-h) 25-mm diameter GF/F filters. Phytoplankton ^{13}C and ^{15}N enrichment, concentrations of particulate carbon (POC) and nitrogen (PON) were measured on a PDZ Europa 20/20 gas chromatograph – mass spectrometer. Carbon and nitrogen uptake rates (ρ, µmol L^{-1} d^{-1}) and biomass-specific uptake (normalized to either POC or PON, V, d^{-1}) were calculated according to Dugdale and Wilkerson (1986). Phytoplankton carbon uptake rates (ρC) are referred to as ''primary production'' as is the convention for carbon uptake studies.

During this study phytoplankton C and N uptake rates were measured only on surface samples incubated at 50% of surface PAR. To estimate a maximum depth-integrated NH_4 uptake rate for the SRWTP region, we multiplied the average surface NH_4 uptake rate by the euphotic zone depth. This procedure assumes a constant uptake throughout the euphotic zone and is likely an overestimate. The depth integrated water column NH_4 concentration at the SRWTP region was calculated using the mean surface concentration for the SRWTP region multiplied by the depth at the SRWTP station RM44 (8 m), assuming full vertical mixing.

To estimate microbial nitrification rates, a mass balance approach was used that calculated the increase in NO_3 concentrations measured between the SRWTP region (at KEN, Fig. 1) (NO_3 = 15.62 µmol N L^{-1}) and downstream in Suisun Bay at the location with the maximum NO_3 concentration (US5 = 34.00 µmol N L^{-1}). Using the mean March 2009 Sacramento River flow rate (850 m^3 s^{-1}, Fig. 2), the calculated river flow speed was ~13 km d^{-1}. Assuming no algal uptake of NH_4 and quasi-steady state conditions, the difference in NO_3 concentrations divided by the transit time between the locations was used to calculate a rate

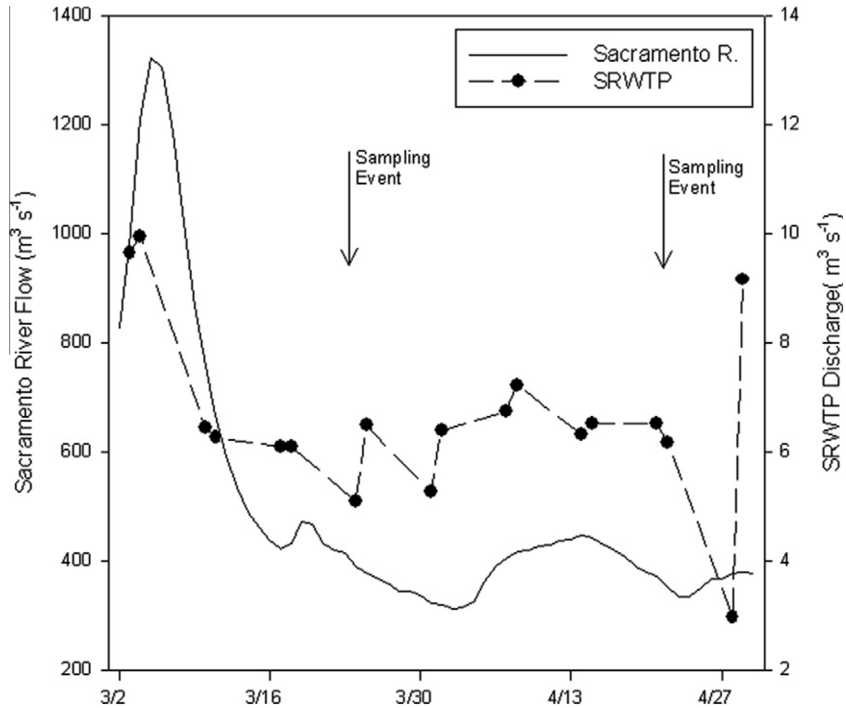

Fig. 2. Sacramento River flow (solid line) and Sacramento Regional Wastewater Treatment Plant (SRWTP) discharge (dashed line) during March and April 2009. Sampling event dates are indicated with arrows.

of NO_3 appearance (i.e. nitrification). An alternative approach from Yool et al. (2007) used an average specific nitrification factor to predict the μmol NO_3 L^{-1} produced per μmol NH_4 L^{-1} per day. This factor was applied to the maximal NH_4 concentration (40 μmol N L^{-1}) in the Sacramento River (at KEN).

3. Results

3.1. River and SRWTP discharge, temperature, salinity, turbidity and light attenuation

Based on the California Water Year Hydrologic Classification Index (http://cdec.water.ca.gov/cgi-progs/iodir/wsihist), 2009 was classified as a "dry" year. Sacramento River flow during March and April varied between 311 and 1322 $m^3\ s^{-1}$ with higher flow at the beginning of March (Fig. 2). SRWTP discharge represented roughly one percent of river flow (3–10 $m^3\ s^{-1}$). Mean nitrogen load from the SRWTP was 15.5 ± 2.9 tons N d^{-1} during the study period (Central Valley Regional Water Quality Control Board, personal communication). Surface water temperature was similar between stations during the March survey, with an average (±SD) water temperature of 14.2 ± 0.3 °C (data not shown). During April, surface water temperatures were warmest in the Upper River, SRWTP and Central River regions (averaging 18.9 ± 0.4 °C; $n = 10$) and in the Lower River region (18.4 ± 0.6 °C, $n = 4$) and coldest in Suisun and San Pablo Bays (16.8 ± 1.0 °C, $n = 7$). In April, mean electrical conductivity (EC) was 113 ± 11 μS cm^{-1} in Upper River and 123 ± 4 μS cm^{-1} for both SRWTP, and Central River regions and then increased within the Lower River (144 μS cm^{-1}) and into Suisun Bay (2.6 psu) (Table 1). The downstream decrease in water temperatures with increased salinity during April was due to mixing with ocean water. During March, EC showed a similar pattern although values were generally lower. Vertical profiles of temperature, salinity and turbidity suggest a well mixed water column in the Upper River (I-80), SRWTP (RM44), Central River (L37) and Lower River (US657) regions (Fig. 3). Stations within Suisun Bay (US4) and San Pablo Bay (US13) showed some vertical structure, with slightly colder temperatures and higher salinity with depth. Turbidity showed increases at depth at these two stations suggesting higher suspended sediment loads.

Light attenuation coefficients for the different regions varied between 1.3–3.5 m^{-1} for March and 1.0–3.0 m^{-1} for April (Table 1). Using all data from March and April transects, k and turbidity were strongly correlated ($k = 12.2 * \text{ntu} + 0.62$; $r^2 = 0.91$, $p < 0.0001$, $n = 42$; data not shown). Similar analysis of k versus chlorophyll-a did not show a significant relationship ($r^2 = 0.02$, $p = 0.65$, $n = 42$, data not shown), indicating that phytoplankton biomass and light attenuation were not related. Because sampling was generally restricted to the main navigational channel of the estuary and river, the ratio of water column depth to euphotic zone depth (i.e. to 1% of surface PAR) was relatively high indicating generally poor average light conditions for phytoplankton throughout the well mixed water column. This ratio averaged 2.5 for the Upper River, SRWTP and Central River regions, 5.9 for the Lower River region, 10.8 for Suisun Bay and 4.8 for San Pablo Bay. At two locations (I80 and ISL) during April the water column depth (<5 m) was less than the euphotic zone depth such that sunlight likely penetrated to the river bottom, providing a more favorable light environment for phytoplankton.

3.2. Nutrient concentrations

The effect of the SRWTP effluent on NH_4 concentrations was apparent during March and April, first as a large step increase in NH_4 between the Upper River and the SRWTP region at station RM44 followed by peak values in the Central River region (Fig. 4A, B). NH_4 concentrations declined going downstream to the Lower River region and remained relatively low through Suisun Bay. NO_3 concentrations remained relatively constant from the Upper River, SRWTP and Central River regions, and then increased rapidly to the Lower River. Dissolved inorganic nitrogen (DIN) concentrations were lower in all transect regions during April

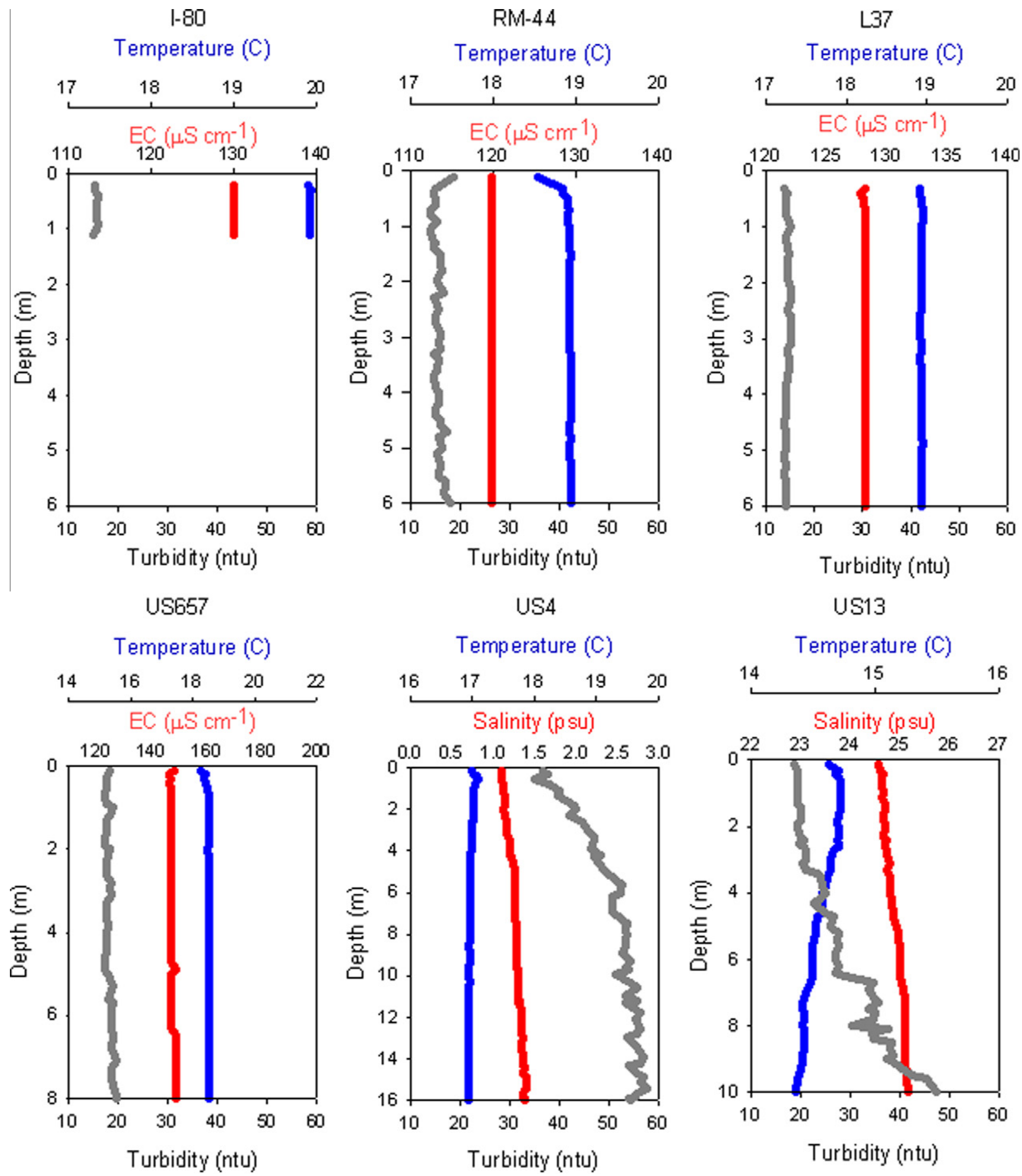

Fig. 3. Vertical profiles of temperature (blue), electrical conductivity or salinity (red) and optical backscatter (gray) in April 2009 from stations representing six regions in the Sacramento River and the Northern San Francisco Estuary.

compared to March except for San Pablo Bay. This difference between months was most pronounced in the Upper River Region where the DIN concentration (mostly NO_3) in March was 4-fold greater than April (Table 1 and Fig. 4A, B). In the Upper River during both months, NH_4 was low (<1 µmol N L^{-1}), but since NO_3 varied between months in the Upper River, NH_4 contributed between 1.8% in March to 20.4% in April to the DIN pool (Table 1). In the SRWTP and Central River regions the percent NH_4 increased from 64.2% to 86.9%. The contribution of NH_4 to total DIN decreased to 31.2% to 44.6% in the Lower River region, to <20% in Suisun Bay and to $\leqslant$10% in San Pablo Bay.

NO_2 concentrations were generally low (<2 µmol N L^{-1}) relative to NO_3 and NH_4 along both surveys (Table 1 and Fig. 4A, B). However, a consistent increase in NO_2 occurred within the Lower River and Suisun Bay (Table 1 and Fig. 4A, B). The highest region-mean NO_2 concentrations (1.19 and 1.35 µmol N L^{-1}, for March and April, respectively) were observed within the Suisun Bay region (Table 1). Urea concentrations were always <1.0 µmol N L^{-1} (Table 1). A large increase in SRP concentration was observed during both surveys at RM44, suggesting that the SRWTP was a significant source of SRP for the river (Table 1 and Fig. 4A, B). Downstream SRP concentrations followed the downstream changes in DIN during both

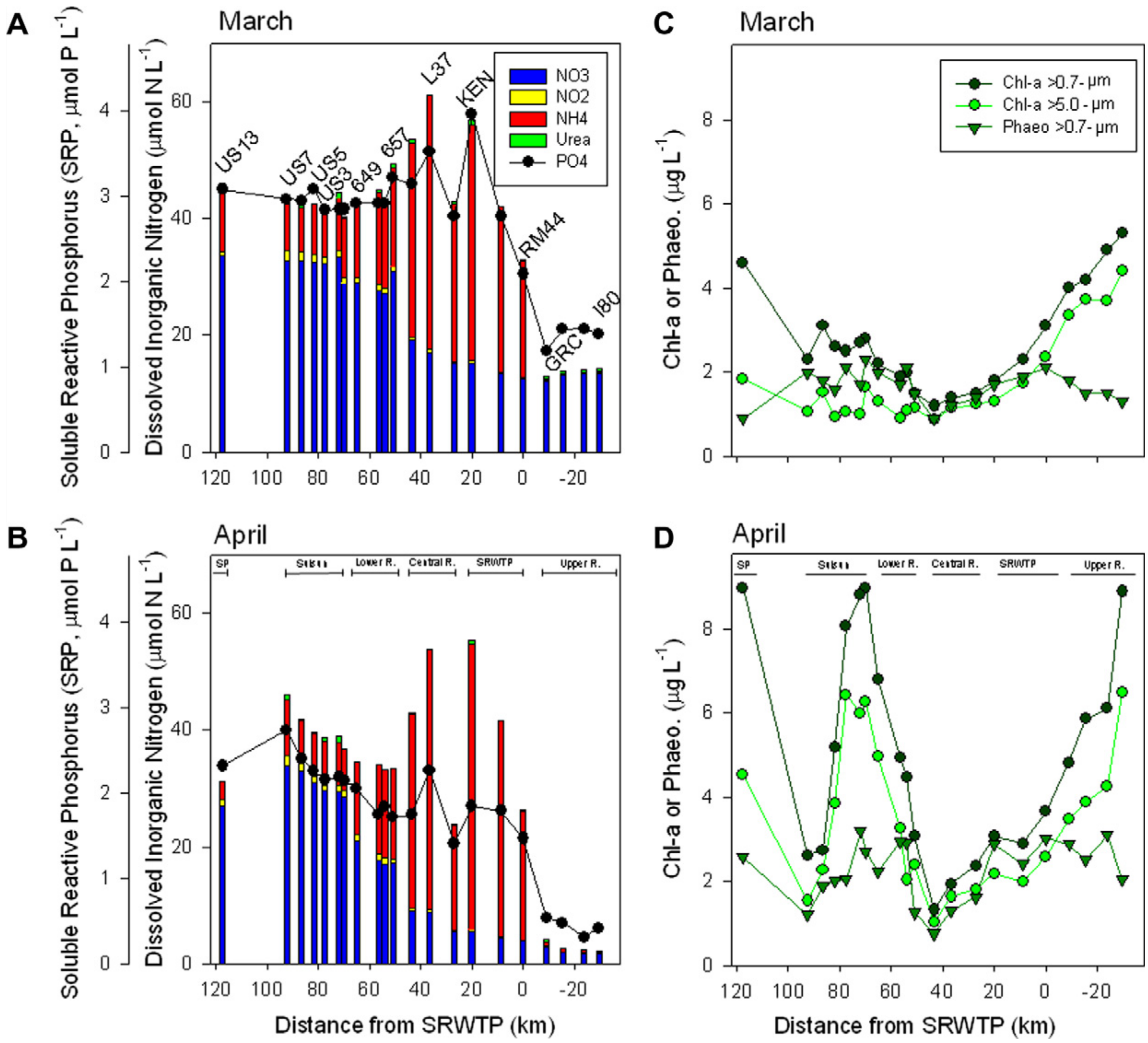

Fig. 4. Inorganic nutrient concentrations measured in the Sacramento River and Northern SFE in (A) March and (B) April 2009 (NO_3; blue, NO_2; yellow, NH_4; red, urea–N; green, SRP; black). Concentrations of chlorophyll-a in cells >0.7-μm diameter (closed circle) and >5.0-μm (open circles) and phaeophytin >0.7-μm (inverted triangles) during (C) March and (D) April 2009.

months. Silicate concentrations declined with distance along the transect, and were generally inversely related to salinity.

3.3. Chlorophyll-a concentrations

The downstream distribution of chlorophyll-a followed similar patterns for both surveys (Fig. 4C and D) but concentrations were higher during April compared to March (Table 2 and Fig. 4C, D). Chlorophyll-a for the whole community (>0.7-μm fraction) decreased downstream from the Upper River region (4.6 ± 0.6 and 6.4 ± 1.7 μg L^{-1} in March and April, respectively) through the Central River region where the lowest chlorophyll-a concentrations were observed (1.4 ± 0.2 and 1.9 ± 0.5 μg L^{-1}; Table 2 and Fig. 4C, D). Chlorophyll-a then increased in the seaward direction from the Lower River region to Suisun Bay and San Pablo Bay (maximum values of 4.6 and 9.0 μg L^{-1} at San Pablo Bay, Table 2 and Fig. 4C, D). Chlorophyll-a in the larger cells (i.e. >5-μm in diameter) showed a similar pattern to whole community chlorophyll-a along both surveys (Fig. 4C and D). At most locations the larger cell-sized fraction contributed more than 60% to the total chlorophyll-a (Table 2). However, in March, in the Lower River region and seaward, the percentage of chlorophyll-a in the larger cells was lower (Table 2). Phaeophytin concentrations paralleled that of chlorophyll-a throughout most of the surveys except in the Upper River region where they decreased as chlorophyll-a increased upstream (Fig. 4C and D).

3.4. Primary production and nutrient uptake

Consistent with chlorophyll-a concentrations, rates of primary production (ρC) were lower during the March survey compared to April likely in response to the seasonal increase in solar irradiance (Table 2 and Fig. 5A, B). The primary production pattern followed the changes in the nitrogen source being accessed and taken up (Fig. 5A and B). The highest river primary production rates were observed in the Upper River region where NO_3 was being taken up (Fig. 5A and B) and.NH_4 concentrations were low (Fig. 5C and D). Accompanying elevated NH_4 concentrations in the SRWTP region, phytoplankton NO_3 uptake ceased and phytoplankton NH_4 uptake increased (Fig. 5A and B). With the elevated NH_4 concentrations downstream of the SRWTP (Fig. 5C and D), phytoplankton NO_3 uptake was negligible (Fig. 5A and B). Primary production and phytoplankton NH_4 uptake declined downstream to minima within the Lower River region in March and the Central River region during April. Primary production increased in Suisun Bay (Table 2) as NH_4 concentrations declined (Fig. 5C and D) and both

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Table 2
Chlorophyll concentrations and carbon uptake (mean ± SD) in Sacramento River and SF Estuary by river region (number of stations) for March and April 2009.

River Region	Chl-a in cells >0.7-μm (μg L^{-1})	Chl-a in cells >5.0-μm (μg L^{-1})	% Chl-a in cells >5.0-μm (μg L^{-1})	ρC (μmol L^{-1} d^{-1})	Assimilation.Number (μmol $L^{-1}d^{-1}$(μg chl-a)$^{-1}$)	ρC as % of Upper River (%)	VC (d^{-1})
March 2009							
Upper River (4)	4.6 ± 0.6	3.8 ± 0.4	83	14.13 ± 1.34	3.07		0.15 ± 0.03
SRWTP (3)	2.4 ± 0.6	1.8 ± 0.5	75	8.47 ± 1.77	3.53	60	0.08 ± 0.02
Central River (3)	1.4 ± 0.2	1.1 ± 0.2	79	5.38 ± 0.59	3.87	38	0.06 ± 0.00
Lower River (4)	1.9 ± 0.3	1.1 ± 0.1	58	4.47 ± 1.30	2.35	32	0.03 ± 0.06
Suisun Bay (6)	2.7 ± 0.3	1.2 ± 0.3	44	9.39 ± 1.26	3.47	64	0.05 ± 0.01
San Pablo Bay (1)	4.6	1.8	39	24.11	5.24	171	0.29
April 2009							
Upper River (4)	6.4 ± 1.7	4.5 ± 1.3	70	36.32 ± 8.50	5.68		0.31 ± 0.07
SRWTP (3)	3.2 ± 0.4	2.3 ± 0.3	72	18.02 ± 4.62	5.63	50	0.13 ± 0.04
Central River (3)	1.9 ± 0.5	3.4 ± 0.4	69	11.01 ± 1.52	5.79	30	0.11 ± 0.00
Lower River (4)	4.5 ± 1.5	2.9 ± 1.2	64	13.66 ± 3.58	3.03	38	0.08 ± 0.02
Suisun Bay (6)	6.1 ± 3.0	4.4 ± 2.2	72	21.59 ± 9.19	3.50	59	0.09 ± 0.03
San Pablo Bay (1)	9.0	4.5	50	36.07	4.00	99	0.30

phytoplankton NO_3 and NH_4 uptake also increased (Table 3 and Fig. 5A, B). Primary production was highest in San Pablo Bay (24.11 and 36.07 μmol C L^{-1} d^{-1} for March and April, respectively) relative to other locations along the survey (Table 2 and Fig. 5A, B). Primary productivity showed a U-shaped pattern with peaks at each end of the transect. Nitrogen uptake showed the same downstream U-shaped pattern with peak NO_3 uptake rates in the Upper River and San Pablo Bay (Table 3 and Fig. 5A, B).

Additional insight into the underlying physiological mechanisms of the phytoplankton can be obtained from the biomass-specific C and N uptake rates (VC or VN) from the Upper River region to San Pablo Bay (Fig. 5C and D). Unlike ρC and ρN, VC and VN do not reflect any changes in biomass as seen with chlorophyll-a along the surveys but indicate physiological changes. Still, similar U-shaped patterns, consistent with that observed for chlorophyll-a concentrations and phytoplankton C and N uptake rates (ρC and ρN), were observed for VC and VN. This U-shape was an inverse pattern to that of NH_4 concentration. The transition from a NO_3 uptake-based phytoplankton population to one based on NH_4 uptake is seen in the progression from Upper River to the SRWTP region. In the Upper River region, high VNO_3 of 0.3 d^{-1} implies a doubling time of the phytoplankton population of about 3 days, based on NO_3 uptake. At the SRWTP region, VNO_3 decreased dramatically to near-detection limits and VNH_4 increased, accompanying increased NH_4 concentration. VNH_4 then declined downstream as NH_4 concentrations increased further. From the Lower River region to Suisun Bay, VNO_3 remained low and unchanged, and VNH_4 was either unchanged (March) or increased (April). Peak specific carbon uptake (VC) coincided with peak VNO_3 in the Upper River region and in San Pablo Bay where NH_4 concentrations were lowest. Within the Sacramento River downstream of the Upper River region, VC rates declined, reaching near zero in the Lower River during March, paralleling the decrease in VNH_4.

The elevated NH_4 concentrations introduced in the SRWTP region were related negatively to both phytoplankton NO_3 and NH_4 uptake (Fig. 6A and B). Biomass-specific NO_3 uptake decreased exponentially with increasing NH_4 concentrations, starting at <2 μmol NH_4 L^{-1} (Fig. 6A). Biomass-specific NH_4 uptake versus NH_4 concentration showed a complex pattern with indications of inhibition of VNH_4 at both low and high NH_4 concentrations (Fig. 6B). Within the SRWTP and Central River regions where effluent is first introduced to the Sacramento River, linear regression analysis shows VNH_4 was negatively correlated with NH_4 concentration for both transects, with nearly identical regression slopes (−0.0031 and −0.0039) and high r^2 values, indicating that effluent NH_4 decreased NH_4 uptake (Fig. 6B). At other locations within the river, there was no correlation between VNH_4 and NH_4 concentration.

Estimates of depth-integrated phytoplankton NH_4 uptake (4.65 mmol NH_4 m^{-2} d^{-1}) and water column NH_4 concentration (288.16 mmol N m^{-2}) in the SRWTP region were calculated for April 2009 using the mean surface ρNH_4 uptake of 1.41 μmol N L^{-1} d^{-1}; Table 3) multiplied by a euphotic zone depth of 3.3 m and the mean surface NH_4 concentration of 36. 02 μmol N L^{-1} multiplied by 8 m (the depth at RM44). The proportion of the water column NH_4 taken up by the phytoplankton was then estimated to be 4.65 mmol N m^{-2} d^{-1}/288.16 mmol N m^{-2} = 0.016 d^{-1} or 1.6% of the water column NH_4 each day. A river nitrification rate, estimated using the mass balance approach for increasing NO_3 downstream, was 4.0 μmol N L^{-1} d^{-1}. Using the average specific nitrification factor, nitrification was estimated to be 6.4 μmol N L^{-1} d^{-1}. Assuming a fully mixed water column of 8 m depth translates to a depth integrated rate of 32.0–51.2 mmol N m^{-2} d^{-1}.

4. Discussion

4.1. Depressed primary production in the Sacramento River

The Sacramento River has been thought to be a source of organic carbon to the Northern SFE (Jassby et al., 2002; Sobczak et al., 2005; Lehman et al., 2008). However the data reported here, similar to the limited primary production estimates for the main channel provided by Lehman et al. (2008), indicate that primary production and phytoplankton biomass in the Sacramento River in spring are actually lower than rates and stocks found in the Northern SFE (including in the well-described low productivity region of Suisun Bay, e.g. Kimmerer, 2005; Wilkerson et al., 2006).

Primary production in the Upper River region was relatively high (equivalent to <70% to ca. 100% of the rates measured in San Pablo Bay) but was strongly depressed in the middle section of the river. At the SRWTP region, primary production decreased by more than 50% compared to the Upper River region. Primary production in the Central River and Lower River regions were the most strongly depressed but began to increase again through Suisun Bay. This generalized U-shaped downstream spatial pattern of primary production was consistent between the two surveys. Clearly, the river is not a significant source of phytoplankton derived organic carbon to Suisun Bay as both primary productivity and chlorophyll-a concentrations are higher in Suisun Bay than in the inflowing river water. These results are in stark contrast to historic phytoplankton surveys of the Sacramento River made during the 1960's when phytoplankton stocks gradually increased moving downstream with highest abundances found at Isleton (ISL). At that time the phytoplankton community in the river was dominated by diatoms (Greenberg, 1964). While phytoplankton

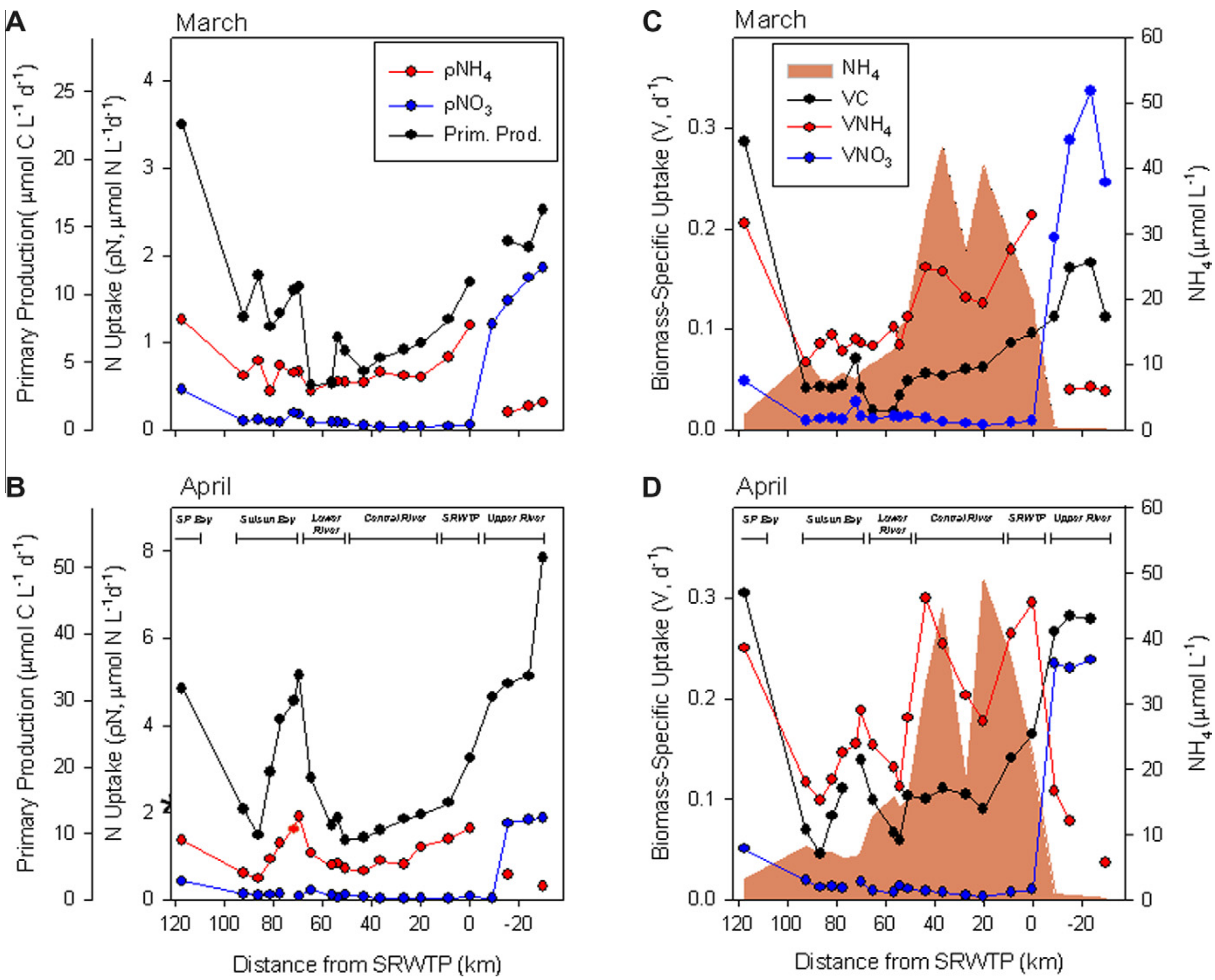

Fig. 5. Primary production and phytoplankton nitrogen uptake in the Sacramento River and Northern SFE during (A) March and (B) April 2009. Biomass-specific carbon uptake and phytoplankton nitrogen uptake and NH_4 concentrations (shaded area) during (C) March and (D) April 2009. *Y*-axes for phytoplankton C and N uptake are scaled at 6.6 C:1 N (i.e. the Redfield ratio).

Table 3
Ammonium and nitrate uptake (mean ± SD) in Sacramento River and SF Estuary by river region (number of stations) for March and April 2009.

River Region	ρNH_4	ρNO_3	% NO_3 uptake %	VNH_4	VNO_3
	μmol N L^{-1} d^{-1}			d^{-1}	
March 2009					
Upper River (4)	0.26 ± 0.06	1.57 ± 0.29	86	0.04 ± 0.00	0.27 ± 0.06
SRWTP (3)	0.88 ± 0.30	0.04 ± 0.01	4	0.18 ± 0.05	0.01 ± 0.00
Central River (3)	0.61 ± 0.06	0.04 ± 0.01	6	0.15 ± 0.02	0.01 ± 0.00
Lower River (4)	0.50 ± 0.08	0.08 ± 0.04	14	0.10 ± 0.02	0.01 ± 0.00
Suisun Bay (6)	0.65 ± 0.13	0.12 ± 0.05	16	0.11 ± 0.05	0.01 ± 0.01
San Pablo Bay (1)	1.26	0.46	27	0.21	0.05
April 2009					
Upper River (4)	0.44 ± 0.19	1.82 ± 0.05	81	0.06 ± 0.03	0.23 ± 0.00
SRWTP (3)	1.41 ± 0.21	0.06 ± 0.03	4	0.25 ± 0.06	0.01 ± 0.00
Central River (3)	0.80 ± 0.12	0.03 ± 0.01	4	0.25 ± 0.05	0.01 ± 0.00
Lower River (4)	0.86 ± 0.15	0.08 ± 0.03	9	0.14 ± 0.03	0.01 ± 0.00
Suisun Bay (6)	1.15 ± 0.56	0.14 ± 0.05	11	0.14 ± 0.03	0.02 ± 0.00
San Pablo Bay (1)	1.36	0.43	24	0.25	0.05

species were not enumerated during this study, the same stations were occupied during spring 2010 and showed a mixed phytoplankton community in the upper river (with diatoms comprising ~40% of the cells) to a community dominated (~80%) by small flagellates and green algae below the SRWTP region (Kress, personal communication) and with diatoms in Suisun and San Pablo Bays (Dugdale et al., submitted for publication).

Because light attenuation is largely explained by turbidity, the potential role that turbidity plays in the present results can be explored using euphotic zone depth. The ratio of river depth to

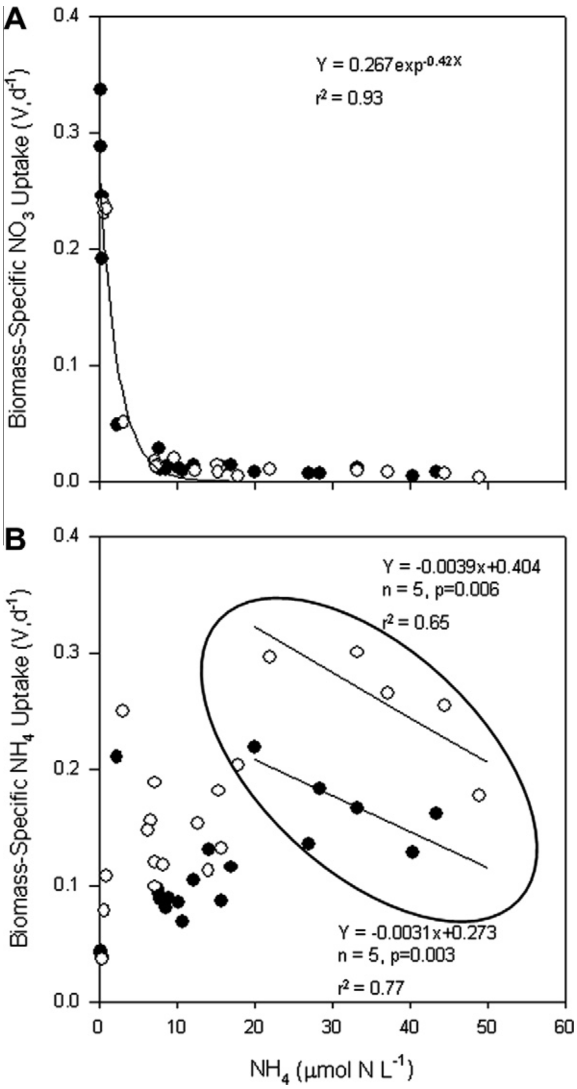

Fig. 6. Effect of NH_4 concentration on phytoplankton N uptake processes in the Sacramento River and Northern Sacramento River. (A) Biomass-specific NO_3 uptake rate (VNO_3) and (B) biomass-specific NH_4 uptake rate (VNH_4) versus NH_4 concentrations measured during March (closed circles) and April (open circles) 2009. Linear regression shown in panel B is based on the five stations occupied in the SRWTP and Central River regions (RM44, HOD, KEN, L37, ISL).

euphotic zone depth (i.e. critical depth, Sverdrup, 1953) does not explain chlorophyll-a trends in the Sacramento River. For example, within the Central River region, the photic zone extended to >70–100% of the river depth (i.e. phytoplankton-received solar energy throughout the water column), yet neither chlorophyll-a or primary production increased there. In contrast, in the eastern end of Suisun Bay water column depth increased significantly (up to 20-m), increasing the ratio of water depth to euphotic zone. This should result in decreased productivity and chlorophyll-a, yet chlorophyll-a and primary production were higher at these locations compared to shallower regions.

The declining productivity and NH_4 uptake conditions in the Sacramento River and Suisun Bay is comparable to observations in other river, estuarine and coastal ecosystems impacted by wastewater effluent (Waiser et al., 2011; Yoshiyama and Sharp, 2006; MacIsaac et al., 1979). In the Delaware Estuary which exhibits a similar range in both primary productivity and NH_4 concentrations (Yoshiyama and Sharp, 2006) a decline in the assimilation number (carbon uptake per unit chlorophyll-a) was associated with NH_4 concentrations >10 μmol N L^{-1} (Yoshiyama and Sharp, 2006). In the Sacramento River, assimilation number declined by 43–47% from the Upper River to the Lower River and in March mean primary production (Table 2) decreased by a factor of ~3 from the highest values at the Upper River region to the lowest value in the Lower River region.

4.2. Effect of NH_4 on river primary production and nutrient uptake

The U-shaped spatial pattern of chlorophyll-a, primary production and phytoplankton N uptake are the mirror of NH_4 concentrations, and appear to be linked to the form of DIN being used by phytoplankton for growth, and by inhibition of NO_3 uptake by NH_4. The overall pattern that emerges is (1) high productivity at the upper end of the transect, associated with NO_3 uptake, (2) a mid-river region (Central River) in which primary production follows NH_4 uptake and NO_3 uptake is shut-down and NH_4 uptake is inhibited (by the high NH_4 concentrations), (3) elevated productivity in Suisun Bay and San Pablo Bay where both NO_3 and NH_4 fuel productivity.

This pattern and its relation to ambient NH_4 are better visualized in plots (Fig. 7A–F) of mean uptake rates for the different transect regions (Tables 2 and 3) versus mean NH_4 concentration (Table 1). The patterns for ρNO_3 versus NH_4 for March and April transects (Fig. 7A) are similar with an immediate decline in uptake from the relatively high levels in the Upper River to very low levels at the SRWTP and the Central River as NH_4 concentrations increase to 30–35 μmol N L^{-1}. ρNO_3 remains low in Lower River as NH_4 concentrations decrease and then increases in Suisun Bay and San Pablo Bay with further decreases in NH_4. When NO_3 uptake is normalized to the mean Upper River value for March (Fig. 7B), the patterns are virtually identical for the two transects sampled one month apart. The progression of ρNH_4 (Fig. 7C) shows an opposite pattern to ρNO_3 uptake, initially low in the Upper River at low NH_4 concentration, increasing to a peak at SRWTP with effluent NH_4 input, decreasing to Central River and Lower River, and finally increasing at Suisun Bay and San Pablo Bay at the lowest NH_4 concentration. The pattern is similar for March and April, especially apparent when normalized to mean Upper River ρNH_4 values for March (Fig. 7D). Carbon uptake, ρC (based upon the combined uptake of NH_4 and NO_3) when plotted against NH_4 concentration (Fig. 7E), decreases 50–60% from the Upper River to the SRWTP region with high effluent NH_4 (Table 2). A further decrease (to 30–38% of Upper River values) occurs in the Central River with increased NH_4. Carbon uptake remains low in the Lower River as NH_4 declines. Finally, ρC increases in Suisun Bay to 59–64% of the Upper River carbon uptake as NH_4 declines further (Fig. 7E) and NO_3 uptake begins to increase (Fig. 7A). The normalized plot for ρC versus NH_4 shows that the patterns for March and April are almost identical (Fig. 7F). The result is little assimilatory capacity of the river DIN by the phytoplankton and flux of NH_4 and NO_3 and little organic carbon to the Northern estuary.

Diminished estuarine productivity and the lack of spring phytoplankton blooms in Suisun Bay was attributed to the inability of the phytoplankton to access the largest inorganic N pool that was NO_3, due to NH_4 inhibition (Wilkerson et al., 2006; Dugdale et al., 2007). This apparently occurred also in the Sacramento River (Fig. 5) where there was high primary production at low NH_4 concentrations and phytoplankton N demand was satisfied by NO_3. Although phytoplankton use NH_4 before NO_3, sometimes referred to as a "preference" for NH_4 (McCarthy et al., l977), some diatoms require NO_3 over NH_4 under some conditions (Glibert et al., 2004, 2006). Reduced primary production was associated with high NH_4 concentrations and the inhibition of phytoplankton NO_3 uptake. The decrease in phytoplankton NO_3 uptake with increasing river NH_4 concentration is consistent with many previous studies

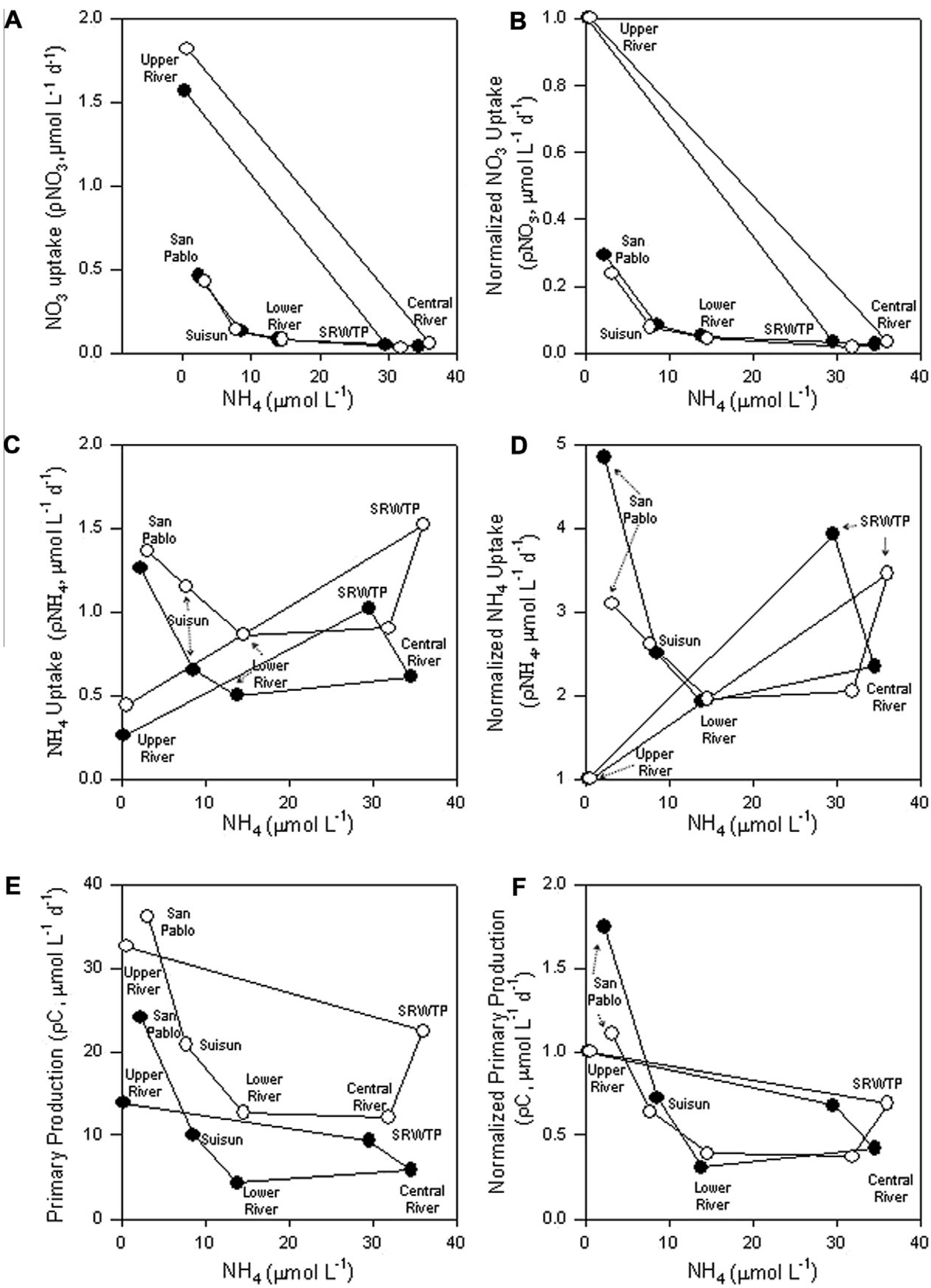

Fig. 7. River and estuary region means of C, NO_3 and NH_4 uptake versus NH_4 concentrations in the Sacramento River and the Northern SFE. (A, C, E) nitrate uptake (ρNO_3), ammonium uptake (ρNH_4), and carbon uptake (ρC). (B, D, F) The same data with uptake rates normalized to Upper River region mean uptake (ρ) rates.

(Dortch, 1990), including those made in the SFE (Dugdale et al., 2007), Hong Kong waters (e.g. Xu et al., 2011) and coastal waters (Dugdale et al., 2006). An exponential function is often used to describe the inhibition of NO_3 uptake by NH_4 (e.g. Cochlan and Harrison, 1991) and this approach fit the data well here suggesting that NH_4 is the major factor in the reduced NO_3 uptake (Fig. 6A).

Another contribution to the depression in primary production and the decrease in chlorophyll-a in the river may be NH_4 inhibition of phytoplankton NH_4 uptake (Syrett, 1981). Suppression of VNH_4 immediately downstream of the SRWTP discharge was related to increased NH_4 concentrations (Fig. 6B). Two situations apparently exist within the Sacramento River. In the SRWTP and Central River regions where wastewater NH_4 discharge is most pronounced, phytoplankton NH_4 uptake is negatively correlated with NH_4 concentration. At other locations this does not occur. We are aware of at least one study that showed inhibition of both phytoplankton NH_4 uptake and primary production with additions of sewage effluent containing primarily NH_4 (MacIsaac et al., 1979). It is unclear in the present study whether NH_4 or some other component of the sewage effluent (of which NH_4 concentrations act as a "tracer") is responsible for the relationship observed here between VNH_4 and NH_4 concentrations although experimental additions of SRWTP effluent into Sacramento River water collected upstream of SRWTP influence showed the same result (Parker et al., 2009). The combination of these effects and resultant depression in primary production result in unused nutrients passing downstream of the Sacramento River and into Suisun Bay.

4.3. Effect of phytoplankton assimilation and nitrification on Sacramento River NH_4 concentrations

The extent to which phytoplankton NH_4 assimilation contributes to the decline in NH_4 concentrations downstream from the SRWTP can be estimated, as can microbial transformations such as nitrification (ammonia oxidation). With a river transport time of about 4 days from the SRWTP to the entrance of Suisun Bay, phytoplankton NH_4 uptake would account for only 6% of the water column NH_4 concentrations found in the SRWTP region. Based on this analysis, using a maximal estimate of the vertically integrated NH_4 uptake, phytoplankton have only a negligible influence on river NH_4 concentration as it flows downstream.

An additional, potentially important sink for anthropogenic NH_4 entering the Sacramento River is nitrification. This is the sequential oxidization of NH_4 to NO_2 and NO_3 to support chemosynthesis and is carried out in estuaries by NH_4-oxidizing bacteria and some archaea (e.g. AOA, Francis et al., 2005; Caffrey et al., 2007). Hager and Schemel (1992) showed that increases in NO_3 were correlated with decreases in NH_4 in the Sacramento River and inferred that nitrification might be a cause. A similar pattern was observed during this study, with elevated NH_4 at the SRWTP region that decreased, while NO_3 increased toward Suisun Bay. In the region where there was the greatest decrease in NH_4 and increase in NO_3, the intermediate inorganic N form, NO_2 was observed also suggesting that nitrification was occurring (Fig. 4A and B). Dark incubations using water collected at RM44 showed little conversion of NH_4 to NO_3 on time scales of seven days but appreciable NO_3 increase after 14 days (data not shown); the time lag for conversion of NH_4 to NO_3 may reflect low initial populations of AOA in the river upstream of the SRWTP region (Pauer and Auer, 2000). Using variation in the natural abundance of ^{15}N in NO_3 and NH_4, Kendall observed declining $\delta^{15}N–NO_3$ and increasing $\delta^{15}N–NH_4$; in the river below the SRWTP; evidence of nitrification with indications of strong nitrification in the vicinity of US657 (Kendall, personal communication).

Our two estimates of Sacramento River nitrification rates give a range (4.0–6.4 $\mu mol\ N\ L^{-1}\ d^{-1}$) comparable to other eutrophic systems that translates to a depth integrated rate of 32–51.2 $mmol\ N\ m^{-2}\ d^{-1}$ assuming a fully mixed water column of 8 m depth. Lipschultz et al. (1986) estimated July–September nitrification in the highly eutrophic region of the Delaware River of 0.08–0.47 $\mu mol\ N\ L^{-1}\ h^{-1}$ (or 1.9–11 $\mu mol\ N\ L^{-1}\ d^{-1}$). Feliatra and Bianchi (1993) measured nitrification rates of 0.23–2.15 $\mu mol\ N\ L^{-1}\ d^{-1}$ in the Rhone River where NH_4 concentrations varied between 1 and 10 $\mu mol\ N\ L^{-1}$. While the present estimates of nitrification for the Sacramento River are crude, the measured water column NH_4 uptake rate by phytoplankton is 9.1–14.5% of the inferred nitrification rate, indicating that nitrification may be the more significant biological process affecting the fate of NH_4 in the Sacramento River. Direct measurements of water column nitrification for the Sacramento River are needed.

Both nitrification and phytoplankton N uptake processes influence the concentrations of NH_4 downstream in the river. However, the sum of the two processes, at most 8 $\mu mol\ N\ L^{-1}\ d^{-1}$, are insufficient to prevent the export of substantial effluent-derived NH_4 to Suisun Bay and other seaward embayments of the Northern SFE. The NH_4 resulting from SRWTP effluent combined with phytoplankton nutrient assimilation and potential nitrification results in a mirror pattern of NH_4 concentration to the downstream U-shaped pattern of phytoplankton uptake and productivity. The delivery of NH_4 to the Northern SFE potentially impacts the pelagic food web and the success of pelagic fishes in this ecosystem.

5. Conclusions

Wastewater discharge from the Sacramento Regional Wastewater Treatment Plant fundamentally changes the microbial processes and biogeochemistry of the river as well as the receiving waters of the San Francisco Estuary and Delta. This study shows the importance of the effluent NH_4 contribution to the DIN pool used by river and estuarine phytoplankton. Three observations have been identified that show how wastewater discharge has changed the chemistry and biology of the river: (1) The secondary-level treatment in the wastewater results in substantial NH_4 concentrations in the Sacramento River downstream of the sewage discharge point. (2) Elevated NH_4 concentrations prevent access by the phytoplankton to high concentrations of NO_3 by inhibiting uptake, suppressing NH_4 uptake and depressing primary production downstream to Suisun Bay. (3) Phytoplankton NH_4 uptake rates and nitrification rates within the Sacramento River are insufficient to appreciably reduce NH_4 concentrations within the river, resulting in significant NH_4 loading to the Northern SFE, suppressing phytoplankton blooms and high primary productivity there. These results indicate that control of river nutrients, especially NH_4 loading, is essential to management efforts to restore the river/estuary to a productive condition.

Acknowledgments

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ATTACHMENT 3

California Department of Fish and Game Staff Analysis of 2000 - 2010 Zooplankton Data for Suisun Bay

Spring Suisun Bay (station D7, D8, D10)- average of all calanoid copepod adults

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
March	4	159	223	2054	566	116	42	264	330	235	137
April	168	419	79	1650	235	273	24	59	305	199	131
May	859	442	165	902	866	571	210	210	414	187	1698
Total	366	340	156	1521	555	320	92	178	349	207	655

Spring Suisun Bay (station D7, D8, D10)- average of Sinocalanus adults

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
March	4	14	1	39	383	35	6	15	8	50	76
April	21	5	15	33	133	62	9	11	31	29	33
May	358	97	73	812	435	373	71	25	92	70	1299
Total	141	39	30	327	317	157	29	17	44	50	469

Spring Suisun Bay (station D7, D8, D10)- average of Sinocalanus copepodids

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
March	2	107	13	110	2235	85	9	35	35	524	107
April	345	58	101	51	262	185	19	45	103	215	175
May	209	97	205	193	183	746	20	62	110	294	2455
Total	166	87	106	126	893	339	16	47	83	344	912

Spring Suisun Bay (station D7, D8, D10)- average of Eurytemora adults

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
March	0	139	133	122	95	71	4	88	316	157	34
April	143	13	38	10	42	148	2	41	191	126	54
May	114	23	41	76	17	79	61	29	160	32	246
Total	79	58	70	77	51	99	22	53	222	105	111

May- average of Sinocalanus adults

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
D6	0	23	0	6	15	7	32	5	10	4	
D7	84	127	74	488	50	661	42	25	171	67	164
D8	31	29	20	1806	43	203	166	5	51	7	2254
D10	960	134	125	142	1213	254	6	46	54	138	1479
D4	229	581	712	9	1061	285	13	237	418	2852	1179
D22	547	2062	1235	14	6051	0	6	591	6432	212	777

June- average of Sinocalanus adults

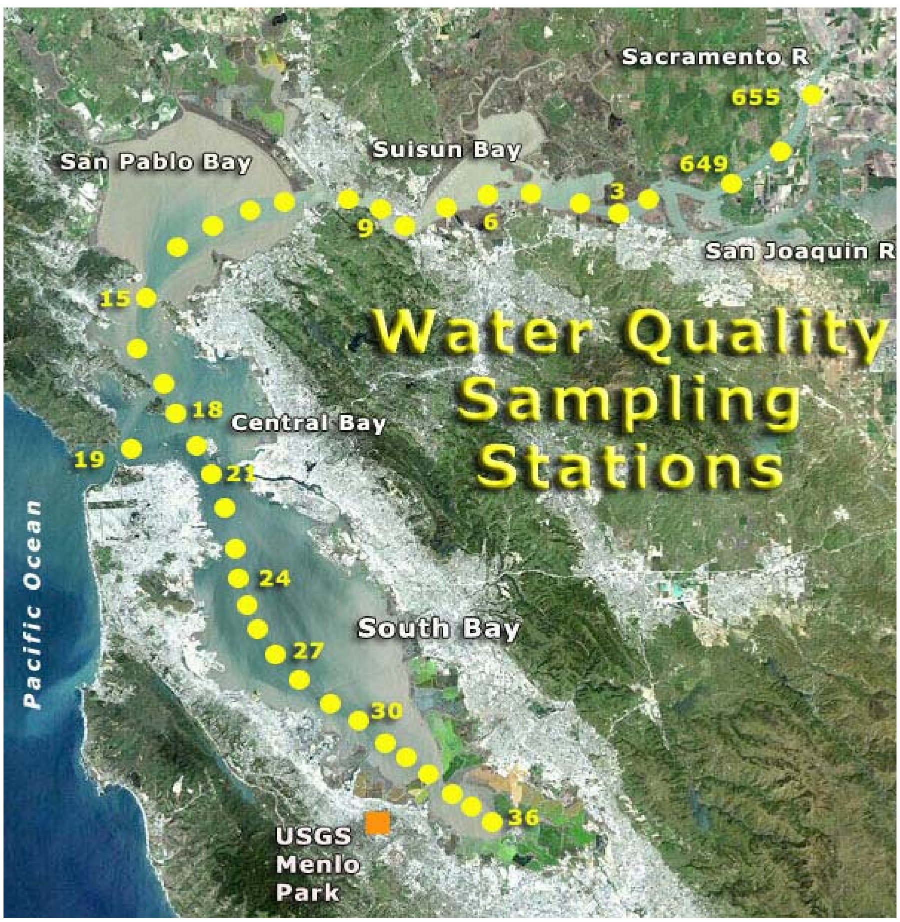
	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
D6	2	3	0	12	0	1	15	4	3	0	19
D7	75	4	94	514	3	50	208	6	18	5	195
D8		11	148	503	1	79	267	6	14	4	1204
D10	198	8		1637	4	283	174	39	154	54	1676
D4	812	1273	8019	1775	0	350	163	440	442	1000	1273
D22	824	2245	661	140	618	7	468	881	1845	248	2258
Total	382	591	1784	763	104	128	216	229	413	218	1104

ATTACHMENT 4

USGS Water Quality Sampling Stations

USGS Water Quality Sampling Stations List

Station Number	General Location	North Latitude	West Longitude	Depth MLW (meters)
657	Rio Vista	38° 8.9'	121° 41.3'	10.1
649	Sacramento River	3.7'	48.0'	10.1
2	Chain Island	3.8'	51.3'	11.3
3	Pittsburg	3.0'	52.7'	11.3
4	Simmons Point	2.9'	56.1'	11.6
5	Middle Ground	3.6'	58.8'	9.8
6	Roe Island	3.9'	122° 2.1'	10.1
7	Avon Pier	2.9'	5.8'	11.6
8	Martinez	1.8'	9.1'	14.3
9	Benicia	3.0'	10.4'	34.4
10	Crockett	3.6'	12.5'	17.7
11	Mare Island	3.7'	15.8'	15.5



ATTACHMENT 5

Lancelot, C., et al. (Nov. 28, 2011)

Rejoinder to "Perils of correlating CUSUM-transformed variables to infer ecological relationships (Breton et al. 2006; Glibert 2010)"

L&O 11-252 - November 28, 2011 - 2nd revision

Rejoinder to "Perils of correlating CUSUM-transformed variables to infer ecological relationships (Breton et al. 2006; Glibert 2010)."

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In their comment, Cloern et al. (2011) develop theoretical evidence that cumulative sum of variability (CUSUM)-transformed variables should not be used to lead to inferences due to the increase of auto-correlation. Indeed the use of statistical tools based on the independency between variables is misleading. The *p*-value associated to the tests described in Breton et al. (2006) and Glibert (2010) as well as in earlier papers (Ibanez et al. 1993; Le Fevre-Lehoerff et al. 1995; Choe et al. 2003) should be disregarded.

We however, do not support the concluding remark of the paper that advises against any comparison of CUSUM-transformed variables. Indeed, such comparisons are useful as they visually accentuate transitions in time between independent variables, a task for which the CUSUM transformation is particularly efficient (Ibanez et al. 1993; Nichols 2001; Breaker and Flora 2009). If CUSUM-transformations of two independent series show transitions at the same time periods, there is a basis for assuming a direct or indirect relationship between those variables; there is most likely a common underlying mechanism (or mechanisms) that is (are) responsible for the similar transitions in the two series. As with any correlative approach, hypotheses resulting from such relations ultimately must be demonstrated by alternate methods.

For instance, the synchronism between CUSUM of diatom biomass and of the North Atlantic Oscillation (NAO) suggested in fig.3A, B of Breton et al. (2006) is supported by a large set of observational (Lancelot et al. 1987, 1995) and modeling (Gypens et al. 2007; Lancelot et al. 2007) papers all showing the importance of meteorological conditions and human activity on the watershed in driving the interannual variations of diatom and *Phaeocystis* colonies in the central Belgian coastal zone.

Similarly, long-term trends between nutrient concentrations and nutrient ratios and changes in abundances of multiple trophic levels, including fish, inferred from CUSUM analysis by Glibert (2010) in San Francisco Estuary, have been further shown using bivariate analyses with original data as well as data adjusted for autocorrelation (Glibert et al. 2011). Glibert (2010) interpreted the change in delta smelt abundance, as well as changes in other fish species, along with other trends in nutrients, phytoplankton, and zooplankton, as an indirect effect due to multiple changes in the food web over time driven by bottom-up changes in both nitrogen and phosphorus loading, not as a singular or as a direct effect of ammonium on delta smelt.

In ecology, the application of CUSUM transformations for identifying links between meteorological, hydrological and ecological patterns has been recently increasing (Adrian et al. 2006; Molinero et al. 2008; Breaker and Flora 2009; Briceño et al. 2010) and the combination of CUSUM charts and bootstrapping has been identified as an important tool in regime shift analysis (Andersen et al. 2008). Therefore, while supporting the Cloern et al. (2011)'s cautious comment, we agree with those who have previously used CUSUM in ecological analysis, that comparisons of transitions in time, using CUSUM transformations, are useful for the identification of synchrony between time series.

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ATTACHMENT 6

Proposed Revisions to Tentative Order NO. R2-2011-XXXX
NPDES NO. CA0037648
For the Central Contra Costa Sanitary District
Wastewater Treatment Plant

Proposed Revisions

At p. 12 of the Tentative Order, revise introductory sentence at VI.C.1 as follows:

> The Regional Water Board ~~may~~ shall modify or reopen this Order prior to its expiration date…

At p. 12 of the Tentative Order, revise existing VI.C.1.a as follows:

> a. If present or future investigations demonstrate that the discharges governed by this Order have or will have a reasonable potential to cause or contribute to, or will cease to have, adverse impacts on water quality or beneficial uses of the receiving waters~~.~~, including if the data, results or other information developed in studies conducted pursuant to VI.D of this Order or any other information demonstrate at any time that effluent limitations, including for Total Ammonia as N (in Table 7), should be reduced.

At p. 13 of the Tentative Order, delete existing VI.C.1.g and replace with the following:

> g. Within 30 months after the effective date of this Order, to reassess the effluent limitations, including for Total Ammonia as N (in Table 7), based on available information, unless the Regional Board makes a finding that more time is required before making that reassessment.
>
> ~~g.~~h. Or as otherwise authorized by law.

At p. 20 of the Tentative Order, insert the following new sections, VI.D and VI.E:

D. **Nutrient discharge Work Plan, Studies and Reports**

1. **Work Plan.** The Discharger shall submit to the Regional Water Board a Work Plan to conduct studies to evaluate further the effects of Total Ammonia as N (or ammonium), and other nutrients, in its discharge. The proposed Work Plan shall be submitted by March 1, 2012. The Work Plan shall be open for public comment and shall be finalized by June 1, 2012. The data collected in the course of the studies shall be made available to the public for review. The Work Plan shall provide that the studies shall be completed by no later than July 1, 2014 and that a Final Report shall be submitted to the Regional Water Board by the Discharger by no later September 1, 2014. The studies may be completed by CCCSD or in conjunction with others, including the Bay Area Clean Water Agencies (BACWA).

2. **Work Plan Elements.** The Work Plan shall include schedules and commitments to fund studies that address the following:

   a. The Surface Water Ambient Monitoring Plan (SWAMP) sampling and associated studies outlined in the existing approved SWAMP plans shall be completed. Taberski, Dugdale, et al., SWAMP Monitoring Plan 2011-2012, *San Francisco Bay Region Work Plan, Monitoring Spring Phytoplankton Bloom Progression in Suisun Bay* (Dec. 2010). The 2011 data with a report shall be provided to the Regional Water Board by June 1, 2012. The 2012 data with a report shall be provided to the Regional Water Board by June 1, 2013.

   b. In addition to other effluent characterization required elsewhere (including Provision VI.C.2 of this Order), the Discharger shall collect representative samples of the discharge sufficient to characterize fully and adequately the nutrient concentrations, loadings, and fate of nitrogen and phosphorus in the discharge. The data provided shall include the form and ratios of nitrogen and phosphorus, including organic and inorganic forms, in the effluent and receiving waters. The data shall be collected and provided to the Regional Water Board by July 31, 2013.

c. A study of the full life cycle toxicity of Total Ammonia as N on copepods in the receiving waters, including Suisun Bay. The study shall use the methodology followed by Dr. Swee Teh or other method accepted by the Regional Water Board. Swee Teh, et al., FINAL REPORT, Full Life-Cycle Bioassay Approach to Assess Chronic Exposure of *Pseudodiaptomus forbesi* to Ammonia/Ammonium Submitted to: Chris Foe and Mark Gowdy State Water Board / UC Davis Agreement No. 06-447-300 SUBTASK No. 14 (August 31, 2011). The study shall be completed by March 1, 2013.

d. Participate in studies evaluating the role of Total Ammonia as N (ammonium) in primary productivity and zooplankton abundance, the significance of nutrient ratios, and the role of sediment biogeochemistry in nutrient fluxes.

3. **Final Report.** The Discharger shall submit a Final Report to the Regional Water Board on the results of the studies done pursuant to the Work Plan by September 1, 2014.

E. **Pre-Design and Site Characterization**

1. **Pre-Design of Ammonium Removal.** The Discharger shall undertake and complete an evaluation of alternative treatment technologies to remove Total Ammonia as N (ammonium) from the discharge, including nitrification technologies. The evaluation shall include pre-design planning and conducting necessary pilot scale systems analyses. By April 1, 2012, the Discharger shall provide a Pre-Design Work Plan for this work to the Regional Water Board. The Pre-Design Work Plan shall provide that the work shall be completed and a Final Report submitted to the Regional Water Board by the Discharger by no later December 31, 2013.

2. **Site Characterization.** The Discharger shall evaluate suitability of the Facility and property owned or controlled by the Discharger to provide land necessary for treatment and removal of ammonium, including nitrification. As part of this evaluation, the Discharger shall conduct sufficient sampling to characterize fully the portion of the property where materials previously placed for disposal would have to be managed to develop a nitrification treatment train. By May 1, 2012, the Discharger shall provide a work plan for the Site Characterization study to the Regional Water Board. The Site Characterization Work Plan shall provide that the sampling shall be completed and a Final Report submitted to the Regional Water Board by the Discharger by no later December 31, 2012.

3. **Final Reports.** The Discharger shall submit (1) a Final Report to the Regional Water Board on the results of the Pre-Design Work Plan for Ammonium Removal by December 31, 2013, and (2) a Final Report to the Regional Water Board on the results of the Site Characterization Work Plan and associated sampling by December 31, 2012.