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The effect of inorganic nitrogen speciation on primary production in the San Francisco Estuary

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ABSTRACT

We describe the results of a series of 96-h enclosure experiments conducted using water from stations in the northern San Francisco Estuary (SFE) along a gradient in ammonium (NH_4) and nitrate (NO_3) concentrations. Using dual-labeled $^{13}C/^{15}N$ tracers, we followed the timing and sequence of primary (carbon, C) production and phytoplankton nitrogen (N) use during experimental phytoplankton blooms. Our results show that diatoms consistently drive the phytoplankton blooms in the enclosures. By tracing both C and N uptake we provide clear evidence that high rates of C uptake are linked to phytoplankton NO_3, and not NH_4, use. Results from kinetics experiments demonstrated higher specific uptake rates (V_{MAx}) for NO_3 compared to NH_4 in the SFE. Finally, dissolved inorganic carbon and nutrient drawdown ratios in the enclosures from the chronically high NH_4 regions of the SFE were substantially lower than predicted from the Redfield ratio, suggesting suppressed C uptake, in relation to other elemental uptake. Our conceptual model of the DIN interactions that lead to higher primary production and phytoplankton blooms in the SFE suggests that higher rates of primary production that accompany phytoplankton NO_3 uptake are sufficient to outpace phytoplankton losses, leading to blooms, compared to the lower rates associated with NH_4 uptake (only 20% of that based upon NO_3). Historical changes in wastewater practices have increased the proportion of NH_4 to the DIN pool in the SFE leading to reduced access to NO_3 by phytoplankton. This may help to explain some of the reduced primary production and phytoplankton biomass observed there since the 1970s.

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1. Introduction

The San Francisco Estuary (SFE) is the major west coast estuary of the U.S. and like many large estuaries worldwide has been modified as a result of urbanization (Nichols et al., 1986). Among the many manifestations of population growth and development are the diversion of freshwater from the Sacramento and San Joaquin Rivers that feed the SFE to California's Central Valley for agriculture and to southern California's urban centers (Nichols et al., 1986), ballast water introductions of invasive species (Cohen and Carlton, 1998), and nutrient loading from agricultural and municipal wastewater sources (Nichols et al., 1986; Hager and Schemel, 1996; Jassby, 2008). In recent decades declines in phytoplankton (Jassby et al., 2002) and zooplankton (Orsi and Mecum, 1996; Kimmerer and Orsi, 1996; Kimmerer, 2005) have been observed and since the early 2000's several fish, including state and federally threatened species, have also declined (Feyrer et al., 2007). These changes have led to local concern that the estuary is experiencing a "pelagic organism decline" (POD) (Sommer et al., 2007).

Historically, primary production was low in the SFE compared to other estuaries (Boynton et al., 1982; Cloern, 2001), due to high suspended sediment loads resulting in reduced photic zone depth (Cole and Cloern, 1984, 1987; Alpine and Cloern, 1988). A decline to now chronically low chlorophyll-*a* (chl-*a*) concentrations occurred in the northern SFE in the late 1980s and was attributed mainly to grazing by the overbite clam, *Corbula amurensis* (Carlton et al., 1990), which was introduced to the estuary in 1986 (Alpine and Cloern, 1992). However, *C. amurensis* abundance alone may be insufficient to explain annual chl-*a* trends, as winter chl-*a* in the northern estuary began to decline before the clam's introduction (Jassby et al., 2002) and rare spring phytoplankton blooms have been observed in the northern SFE (Dugdale et al., submitted; Wilkerson et al., 2006; Glibert et al., 2011; Dugdale et al., submitted) since the clam's introduction even while clam biomass has been relatively stable.

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Until recently (Wilkerson et al., 2006; Dugdale et al., 2007) nutrients were eliminated as a factor in the low primary production condition in the SFE as they are always found in sufficient supply (Hager and Schemel, 1996; Jassby et al., 2002). Wilkerson et al. (2006) and Hogue et al. (2005) made the first direct measurements of phytoplankton nitrogen productivity in SFE using ^{15}N tracers and found that ammonium (NH_4) fueled primary production much of the time even though high nitrate (NO_3) was present. This has been described for other estuaries (e.g. Pennock, 1987) and is explained by NH_4 inhibition of phytoplankton NO_3 uptake (e.g. Conway, 1977; Dortch, 1990). Wilkerson et al. (2006) noted an exception to the dominance of phytoplankton NH_4 uptake during spring phytoplankton blooms when phytoplankton displayed high rates of NO_3 uptake allowing the larger pool of dissolved inorganic nitrogen (DIN) to be used for growth and chl-*a* accumulation. This occurred only under conditions of low NH_4. Biomass-specific NO_3 uptake rates during these periods were the highest phytoplankton N uptake observed in the estuary during the annual productivity cycle.

A limitation of the Wilkerson et al. (2006) study is that the authors considered phytoplankton N uptake only and did not measure primary production directly as C uptake or dissolved inorganic carbon (DIC) drawdown. To estimate C production the authors assumed a fixed C to N uptake ratio (i.e. Redfield stoichiometry; Redfield et al., 1963) or used chl-*a* biomass as a proxy of phytoplankton C biomass. These assumptions may not hold as C and N uptake have been shown to be uncoupled on shorter time scales in response to perturbations in light and time of day (Cochlan et al., 1991), nutrient concentrations (e.g. surge uptake; Harrison et al., 1977), and nutrient availability. Parker (2004) reported low C uptake in Delaware Bay enclosure experiments when the N nutrient supplied was NH_4. Yoshiyama and Sharp (2006) attributed a low productivity zone in the Delaware River to high ambient NH_4 concentrations.

To establish if the speciation of ambient DIN may result in differences in carbon uptake in the SFE (i.e. a secondary bottom-up control) we conducted enclosure experiments along a natural gradient of DIN concentrations with varying NO_3 to NH_4 ratios. The goal of this study was to link phytoplankton C and N uptake processes to more fully characterize productivity – nutrient dynamics along the DIN gradient by measuring carbon uptake and DIC use directly. We hypothesize that phytoplankton in the northern SFE show a physiological advantage to growth supported by NO_3 such that higher C uptake and biomass accumulation are linked with NO_3 uptake. Observed low rates of primary production in the northern SFE may be exacerbated by a lack of access to the high ambient concentrations of NO_3 as a result of suppression of NO_3 uptake by increased anthropogenic NH_4 supply.

2. Methods and materials

2.1. Experimental design

A series of enclosure experiments were conducted in the northern SFE during 2005. Experiments were designed specifically to remove light limitation by exposing phytoplankton to 50% of surface photosynthetically active radiation (PAR) (Lorenzi, 2006) and eliminate benthic grazing by *C. amurensis*. No attempt was made to remove zooplankton grazers. Water for enclosures was collected at three stations during March, July and September. Stations were selected to represent the three subembayments of the northern estuary, Suisun (SUI; 38°2.1′ N, 122° 5.8′ W), San Pablo (SPO; 38°1.7′ N, 122° 22.2′ W), and Central Bays (CEN; 37° 53.8′ N, 122° 25.5′ W) (Fig. 1). We relied on differences in initial ambient concentrations of NO_3 and NH_4 at the three stations to create experimental treatment conditions (Table 1).

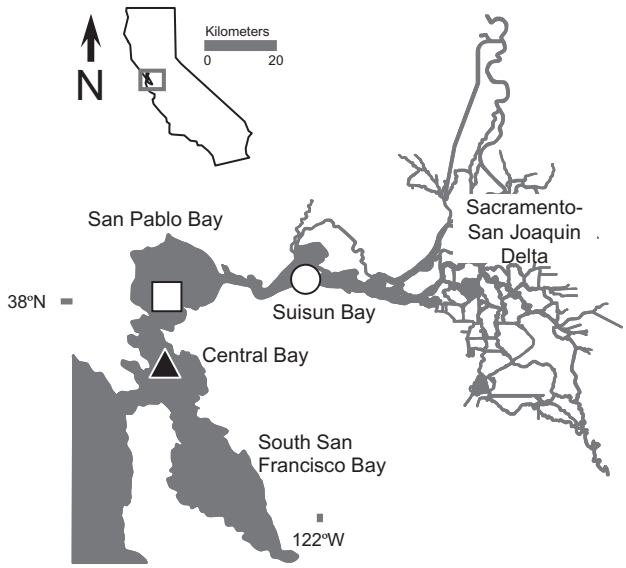

Fig. 1. Map of study site, indicating the sampling locations within the three subembayments of the northern San Francisco Bay.

Near-surface water (<1°m depth) was collected by clean bucket at each station and dispensed into three replicate 20-L low density polyethylene (LDPE) cubitainers (=enclosures), stored in the dark in coolers, and returned to the laboratory within 2 h of collection. Temperature and salinity were measured using a YSI 85 conductivity/temperature probe (Yellow Springs Instruments, Inc.). The enclosures were placed in baywater-cooled incubation tanks with surface photosynthetically active radiation (PAR) reduced by 50% with window screening and incubated for up to 96 h. Water flow within the incubation tanks was sufficient to keep the enclosures in gentle motion during the incubation period, homogenizing the light field experienced by replicate enclosures and allowing continuous mixing with little discernable accumulation of organic matter inside enclosure walls. The nine enclosures were sampled once daily around 10:00 h local time to track changes in DIC and inorganic nutrient concentrations. Phytoplankton were assessed daily by sampling for size-fractionated chl-*a* into two fractions (GF/F 0.7 μm nominal pore size and 5.0 μm polycarbonate filters). Primary production (C uptake) and phytoplankton NO_3 and NH_4 uptake were measured daily using stable isotope tracer techniques (Slawyk et al., 1977). Phytoplankton urea uptake was measured during one enclosure experiment conducted in April 2006, representing at most 20% of NO_3 uptake (data not shown).

Water was also collected in April 2005 at CEN to study phytoplankton N uptake kinetics. After collection, water was held for 48-h at 50% PAR to allow phytoplankton to reduce the ambient N concentration prior to studying phytoplankton N uptake with increasing NH_4 or NO_3 concentrations. After 48-h, NH_4 was reduced to <1 μmol N L^{-1}. However, ambient NO_3 concentrations were still too high (>12 μmol NO_3 L^{-1}) to be able to carry out Michaelis–Menten type kinetics experiments. Consequently, additions of NO_3 were made to a series of bottles in order to determine V_{MAx} NO_3 at NO_3 concentrations in excess of 12 μmol L^{-1}.

2.2. Routine analytical methods

DIC was measured in 20-ml samples using a Monterey Bay Research Institute-clone DIC analyzer with acid-sparging and non-

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Table 1
Hydrographic data and initial chemistry at stations in Suisun Bay (SUI), San Pablo Bay (SPO) and Central Bay (CEN) during 2005. Temperature and salinity data were not determined (NA) in SUI and SPO stations in September.

Experiment	Station	Sal. (psu)	Temp. (°C)	NO_3 μmol N L^{-1}	NH_4 μmol N L^{-1}	Urea μmol N L^{-1}	PO_4 μmol P L^{-1}	$Si(OH)_4$ μmol Si L^{-1}	Chl-*a* μg L^{-1}	Chl-*a* % >5-μm
March	SUI	2.7	15.4	39.43	9.18	1.93	1.76	281	1.3	70
	SPO	15.3	15.6	35.12	5.71	1.81	1.61	165	2.4	71
	CEN	19.4	15.2	31.37	4.91	1.57	1.55	128	1.9	79
July	SUI	7.2	20.0	22.60	8.61	1.18	2.92	182	0.9	33
	SPO	17.8	19.5	21.51	5.54	1.19	3.12	115	1.0	60
	CEN	24.1	18.4	17.30	5.21	0.82	2.58	69	1.5	33
September	SUI	NA	NA	23.99	5.65	0.76	3.00	182	1.9	48
	SPO	NA	NA	18.58	5.25	1.00	3.19	105	2.3	56
	CEN	NA	16.4	15.41	4.60	0.90	2.94	80	2.0	60

dispersive infrared (NDIR) analysis (Friederich et al., 2002; Parker et al., 2006) following preservation with 200 μL of 5% w/v $HgCl_2$ (Sharp et al., 2009).

Samples for inorganic nutrients were passed through a GF/F filter to remove particulate matter before nutrient analysis (Wilkerson et al., 2006). Twenty-ml filtered water samples were analyzed using a Bran and Luebbe AutoAnalyzer II with MT-19 manifold chemistry module for $NO_3 + NO_2$ and NO_2 according to Whitledge et al. (1981) and Bran and Leubbe (1999a,b) Method G-172-96, phosphate (PO_4) according to Bran and Luebbe Method G-175-96 and silicate ($Si(OH)_4$) by Bran and Luebbe Method G-177-96. $NO_3 + NO_2$ is referred to as NO_3 throughout the text as NO_2 concentrations were very low (<1.0 μmol L^{-1}). Urea concentrations were measured in all experiments using the method of Revilla et al. (2005) with concentrations rarely exceeding 1 μmol L^{-1} (representing <3% of the DIN pool). Separate 25-ml samples were collected for manual colorimetric determination of NH_4 according to Solorzano (1969) using a 10-cm path length cell. Sample water (50-ml–100-ml) was filtered for determination of *in vitro* chl-*a* using the extraction protocol of Arar and Collins (1992) and read on a Turner Designs fluorometer calibrated with commercially available chl-*a* (Turner Designs).

2.3. Carbon and nitrogen assimilation

Dual-labeled $^{13}C/^{15}N$ stable isotope tracer incubations were carried out to estimate hourly C and N uptake rates (Slawyk et al., 1977). Trace additions of $NaH^{13}CO_3$ and either $K^{15}NO_3$ or $^{15}NH_4Cl$ (99 atom %) were added to samples to approximately 10% of the ambient concentration. Samples were incubated in 180-ml polycarbonate bottles for 4-h around local noon, held in baywater-cooled incubator tables screened to 50% of surface PAR. Incubations were terminated by gentle vacuum filtration onto pre-combusted (450 °C for 4 h) 25-mm Whatman GF/F filters. Filters were frozen until analysis for ^{13}C and ^{15}N enrichment and particulate organic carbon and nitrogen concentration with a Europa 20/20 isotope ratio-mass spectrometer system. Nitrogen uptake rates (ρ, μmol L^{-1} h^{-1}) and biomass-specific uptake (V, h^{-1}) were calculated according to Dugdale and Wilkerson (1986). Carbon uptake was calculated in the same manner, using measured DIC concentrations to calculate substrate enrichment (Legendre and Gosselin, 1996; Parker, 2005). We report both V and ρ because while the two rates are related (ρ is derived from V), V provides an indication of phytoplankton physiology, while ρ provides information on C and N flux and cycling. The particulate carbon and nitrogen retained on GF/F filters likely contained particle-associated and some fraction of free-living heterotrophic bacteria (Hoch and Kirchman, 1995). Recent measurements suggest that between 76 and 90% of bacteria in the northern SFE are free-living minimizing their contribution to the organic matter captured on the filters (Parker, Unpublished data). Because of the potential for bias in V due to detrital particulate N (Garside, 1991), specific C and N uptake were also estimated by normalizing uptake rates to chl-*a* and cells L^{-1} (Kudela et al., 1997) and showed the same trends as the traditional measure of V, normalized to PON. We report here V, normalized to PON to be consistent with previous work in the SFE (i.e. Hogue et al., 2005; Wilkerson et al., 2006; Dugdale et al., 2007). An *f*-ratio was calculated as $\rho NO_3/(\rho NO_3 + \rho NH_4)$ to evaluate the relative importance of NO_3 uptake in phytoplankton N uptake.

No correction for NH_4 regeneration and isotope dilution was made. This may result in underestimation of NH_4 uptake. However, by keeping incubation times to 4-h we have lessened the importance of NH_4 regeneration (LaRoche, 1983). In addition, the high NH_4 (ca. 10 μmol N L^{-1}) conditions and relatively low ρNH_4 (ca. 0.10 μmol N L^{-1} h^{-1}) characteristic of the northern SFE (Wilkerson et al., 2006; Dugdale et al., 2007; Parker et al., 2012) all minimize the potential impact of NH_4 regeneration on isotope dilution. Assuming an initial ^{15}N isotopic enrichment of 10% and NH_4 regeneration equivalent to uptake (0.1 μmol N L^{-1} h^{-1}) the isotope enrichment would be reduced to 9.80% after 4-h resulting in an understate of NH_4 uptake by 2% (Dugdale and Wilkerson, 1986).

3. Results

3.1. Conditions in the embayments at time of sampling for enclosures

Salinity increased moving from SUI to SPO and CEN while water temperature was similar between locations, varying by ≤0.4 °C in March and 1.6 °C in July (Table 1). Initial nutrient and chl-*a* concentrations revealed a gradient in conditions from SUI to SPO and CEN (Table 1) with the highest inorganic nitrogen concentrations found always at SUI compared to other locations. The greatest difference in initial NO_3 and NH_4 concentrations between SUI and the other stations occurred in March. During July and September NO_3 and NH_4 concentrations in the stations were more similar but with the same trends of decreasing concentrations in the seaward direction. Initial urea concentrations were <2 μmol N L^{-1} with higher urea measured in March compared to July and September. PO_4 concentrations increased from March to September with no consistent spatial pattern. In contrast, $Si(OH)_4$ was highest in March compared to July and September and consistently declined in the seaward direction during each sampling date. Chl-*a* concentrations were similar between stations during each sampling date but consistently lowest at SUI (Table 1). The absolute differences in initial chl-*a* between stations for a given sampling date were ≤1.1 μg L^{-1}. The percentage of chl-*a* in cells >5-μm varied systematically by date but not location. The majority of chl-*a* was found in cells >5 μm during March whereas during July, smaller sized cells (<5-μm) accounted for as much as 70% of the initial chl-*a* in SUI and CEN. Chl-*a* was most evenly divided between cells <5-μm and >5-μm in September (Table 1).

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3.2. Enclosure experiments: dissolved inorganic carbon, chlorophyll and inorganic nitrogen concentrations

During the enclosure time series chl-*a* increased and was always greatest in enclosures collected at SPO and CEN compared to SUI for the first 72 h (Fig. 2A) with the absolute chl-*a* concentrations highest in March compared to July and September. In each experiment the maximum chl-*a* in CEN enclosures was always observed at 72-h and began to decline by 96-h, likely in response to nutrient exhaustion (Fig. 3 shows March data). Chl-*a* in SPO enclosures continued to increase throughout the 96-h incubation period, while SUI enclosures consistently lagged SPO and CEN enclosures with no significant increases in chl-*a* during the initial 48-h. Common to all enclosures, the chl-*a* produced was mostly in the >5-μm size fraction (% >5 μm; ca. 72–100%; Table 2). The decrease in dissolved inorganic carbon concentrations in SPO and CEN enclosures (Fig. 2B, Table 2) was substantially greater than in SUI enclosures during all experiments. For example, the decrease in DIC concentration was 5- and 6-fold greater in CEN and SPO enclosures, respectively, compared to SUI during March (Fig. 2B, Table 2). The larger drawdown of DIC in SPO and CEN suggests that higher primary production was occurring in those enclosures compared to SUI.

Nutrients declined less in SUI than SPO and CEN enclosures during the 96-h incubation period (Fig. 3, Table 2). In each of the experiments NH_4 concentrations in SPO and CEN enclosures declined within the first 24-h and were reduced to < 1 μmol N L^{-1} within 48-h (Fig. 3A for March, Table 3). In contrast, NH_4 concentrations in SUI enclosures required 72-h to reach <1 μmol N L^{-1} in March and September and 96-h in July (Table 3). NO_3 concentrations began to decrease in SPO and CEN enclosures during the first 24-h in March (Fig. 3B) and within 48-h in July and September (data not shown); NO_3 exhaustion occurred by 96-h in these enclosures (Table 3). NO_3 in SUI enclosures remained largely unchanged for 72-h in March (Fig. 3B) and July and declined by 1 μmol N L^{-1}–4 μmol N L^{-1} by 96-h (Table 3). During September, NO_3 in SUI enclosures decreased by ~5 μmol L^{-1} by 72-h (data not shown) with a further decline by 96-h.NO_3 was never exhausted in any of the SUI enclosures (Table 3).

Nutrient drawdown ratios, based on the disappearance of nutrients over the 96-h incubation period, show major deviations from the Redfield ratio for SUI enclosures (Table 2). C:N drawdown ratios in SUI were 3.7, 3.7 and 4.3 for March, July, and September, respectively. In contrast, C:N ratios in CEN were 6.7, 10.8, and 10.6 and in SPO were 5.5, 8.2, and 7.5. N and $Si(OH)_4$ drawdown was similar (i.e. N:Si ≈ 1) in all enclosures during March, in CEN enclosures in July, and SPO and CEN in September (Table 2).

3.3. Enclosure experiments: carbon and nitrogen uptake

Carbon uptake (V_C, h^{-1} and ρC, μmol C L^{-1} h^{-1}) supports the patterns observed for chl-*a* increase and DIC decrease in enclosures, with SUI enclosures revealing lower carbon uptake compared to SPO and CEN (Fig 2, Figs. 3C, D, Table 3). During March, both V_c and ρC showed little change in the first 24-h and then increased in SPO and CEN by 48-h, reaching maximal values at 72-h (Fig. 3C, D, Table 3). The maximum C uptake in SUI enclosure represented ~30% of the maximum value at SPO and CEN (Fig. 3C,D). Similarly, in July, C uptake in SPO and CEN enclosures increased after 48-h and peaked at 96-h. C uptake in SUI enclosures lagged SPO and CEN by 24-h (Table 3). During September, C uptake in excess of 4 μmol C L^{-1} h^{-1} were observed in enclosures from all three bays by 48-h (data not shown). However, the maximum V_C and ρC in SUI enclosures was lower than that observed in SPO and CEN enclosures (Table 3) Overall, the time series of C uptake that was observed in the enclosures (Fig. 3C, D) resembled the pattern observed for NO_3 uptake rather than NH_4 uptake (Fig. 3E, F, G, H).

Phytoplankton N uptake was dominated initially by NH_4 uptake followed by NO_3 uptake in all enclosure experiments (e.g. March time series, Fig. 3E, F, G, H). During March, VNH_4 reached maxima within 24 h in SPO and CEN enclosures and ca.72-h in SUI (Fig. 3G). Although the time of peak VNH_4 (V_{MAx} NH_4) in SUI enclosures was

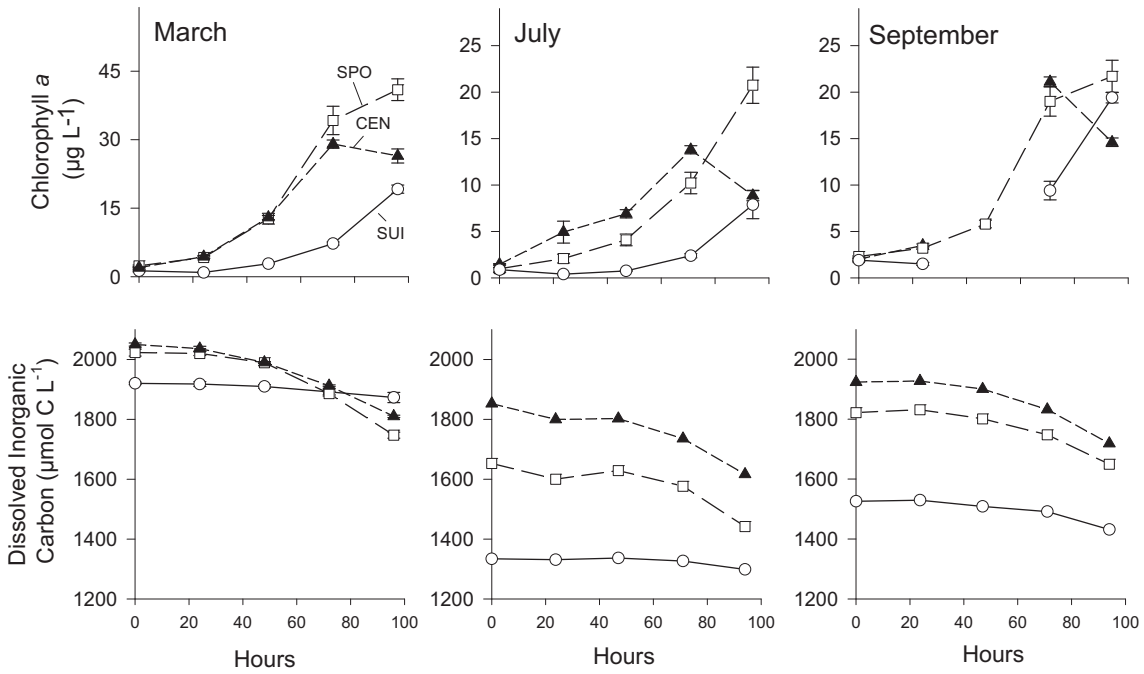

Fig. 2. Time series of chl-*a* and dissolved inorganic carbon concentrations in enclosure experiments from Suisun Bay (open circles), San Pablo Bay (open squares) and Central Bay (closed triangles) during March, July and September. Error bars represent one standard deviation based on three replicate 20-L enclosures. Note different *y*-axis scale for chl-*a* for March experiment.

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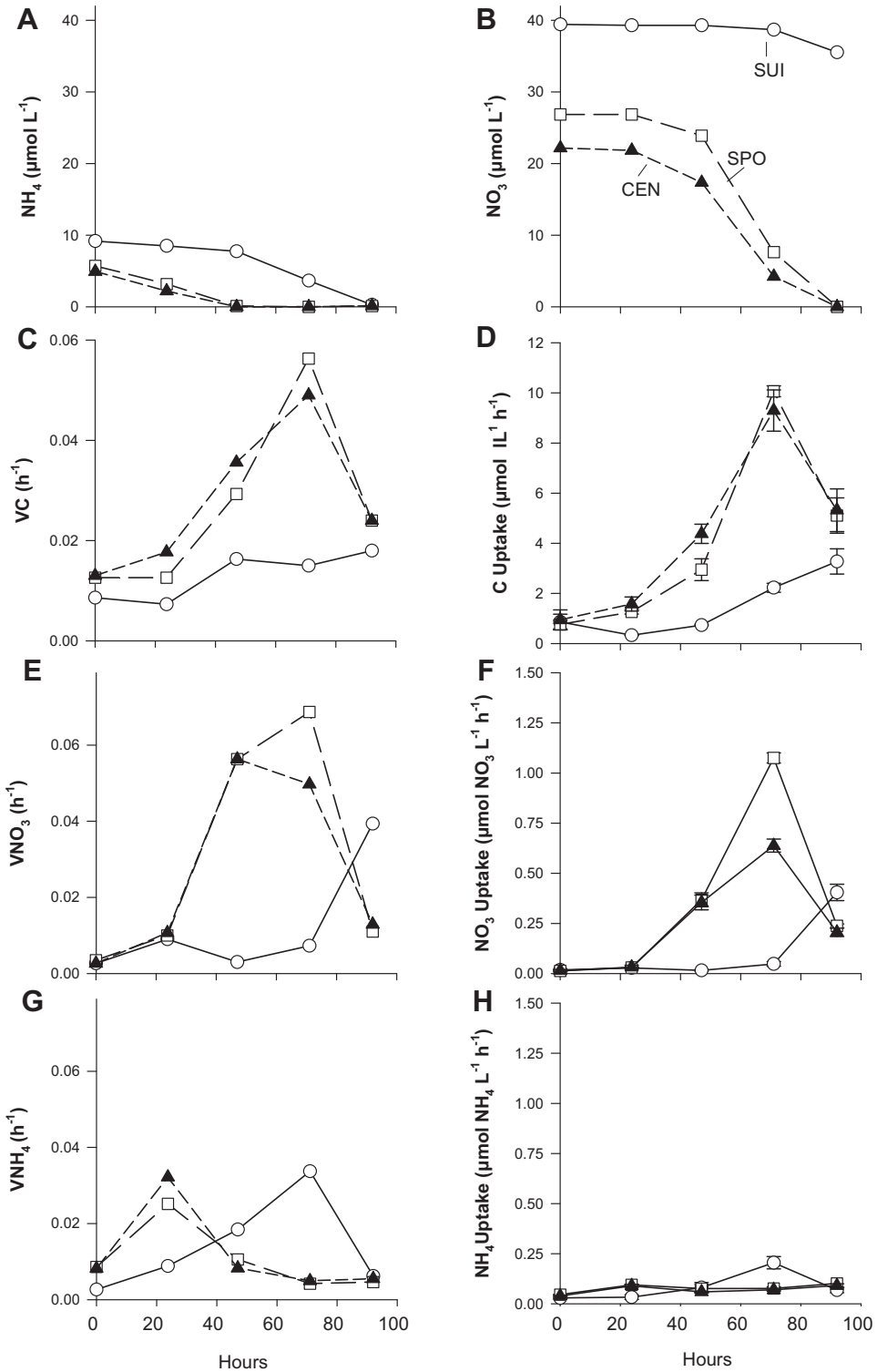

Fig. 3. Time series of NH_4 and NO_3 concentrations, specific uptake, and transport rates during 4-h daily incubations for C, NH_4 and NO_3 during March enclosure experiments conducted in Suisun Bay (open circles), San Pablo Bay (open squares) and Central Bay (closed triangles). A) NH_4, B) NO_3, C) specific C uptake, V_C, D) C uptake rate, ρC, E) specific NO_3 uptake, VNO_3, F) NO_3 uptake rate, ρNO_3, G) specific NH_4 uptake rate, VNH_4, H) NH_4 uptake rate, ρNH_4.

always 1–2 days later than in CEN or SPO enclosures, the V_{MAx} NH_4 values were similar to those of CEN and SPO in the three experiments (0.025–0.46 h^{-1}) (Table 3). ρNH_4 was low in both March (Fig. 3H) and July (Table 3) but higher in September. There was essentially no measurable NO_3 uptake in CEN and SPO enclosures for the first 24-h but a rapid increase in both VNO_3 and ρNO_3 was observed at 48-h (matching the increase in C uptake), reaching maximal ρNO_3 by 72-h (Fig. 3F, Table 3). ρNO_3 in SUI enclosures remained low up to 72-h, and only increased at 96-h. V_{MAx} NO_3 was almost always greater than V_{MAx} NH_4, often by a factor of >2

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Table 2
Elemental drawdown of dissolved inorganic carbon (DIC), dissolved inorganic nitrogen (DIN, NO_3 and NH_4), phosphate and silicate reported as total change in nutrient concentrations over 86–90-hr. Ratios of ΔC: ΔN: ΔP: ΔSi are shown normalized to P. In March PO_4 and NH_4 concentrations were exhausted by 72-hr in SPO and CEN enclosures (*); elemental drawdown ratios are given based on 72-hrs in these cases. %Chl-a >5 is the percentage of chl-*a* that was measured in cells >5-μm in diameter after 96-hr.

Experiment	Element (μmol L^{-1})	SUI	SPO	CEN
March	ΔDIC	47	270	240
	ΔDIN	12.8	48.51	36.16
	ΔP	0.74	1.61*	1.55*
	ΔSi	10.89	33.24	26.59
	C:N:P:Si	63:17:1:15	167:30:1:21	154:23:1:17
	C:N	3.7:1	5.5:1	6.7:1
	%DIN uptake as NH_4	72	12	12
	%Chl-*a* >5-μm	90	100	81
July	ΔDIC	35	211	236
	ΔDIN	9.39	26.92	22.42
	ΔP	0.75	2	1.82
	ΔSi	0.58	4.3	NA
	C:N:P:Si	46:12.5:1:1	106:13:1:2.2	129:12:1:NA
	C:N	3.7:1	8.2	10.8
	%DIN uptake as NH_4	92	21	23
	%Chl-*a* >5-μm	100	72	100
September	ΔDIC	94	172	205
	ΔDIN	22.07	23.64	19.74
	ΔP	1.43	1.91	1.93
	ΔSi	5.96	16.29	15.84
	C:N:P:Si	65:15:1:4	90:12:1:9	106:10:1:8
	C:N	4.3	7.5	10.6
	%DIN uptake as NH_4	26	22	23
	%Chl-*a* >5-μm	89	88	79

(Table 3). The exception to this was in SUI enclosures where phytoplankton had little access to NO_3 due to the high NH_4. In these cases V_{MAx} for NO_3 and NH_4 were comparable (Table 3).

3.4. Uptake kinetics

VNH_4 versus NH_4 concentration showed a hyperbolic relationship, with K_s for NH_4 of 1.3 μmol L^{-1} and V_{MAx} of 0.033 ± 0.003 h^{-1} (Fig. 4). Because ambient NO_3 was not reduced <12 μmol L^{-1}, we were unable to fit a Michaelis–Menten type curve or derive K for NO_3. However, saturating NO_3 uptake was V_{MAx} at 0.044 ± 0.002 (±sd) h^{-1} over a range of NO_3 from 12 μmol N L^{-1} to 35 μmol N L^{-1}. The difference in V_{MAx} for NO_3 represented ~33% increase over the V_{MAx} than achieved for NH_4.

4. Discussion

The maximum primary production reported here for enclosures are higher than rates reported previously for the northern SFE (Cole and Cloern, 1984; Kimmerer et al., 2012) reflecting the fact that light limitation was eliminated through the experimental design. The sequence of phytoplankton nutrient use and patterns of phytoplankton C and N uptake described here likely reflect periods in the estuary when light limitation is eliminated through vertical stratification of the water column. Higher C and N uptake were measured in Central and San Pablo Bays compared to Suisun Bay. Our hypothesis that phytoplankton populations in the northern SFE show a physiological advantage to growth when they use NO_3 is supported by these findings as all experiments showed elevated phytoplankton C uptake and chl-*a* accumulation associated with phytoplankton NO_3 use. Even allowing for possible isotope dilution and an underestimation of NH_4 uptake, maximum NO_3 uptake rates were consistently higher than maximum phytoplankton NH_4 uptake rates, ensuring effective use of the high ambient NO_3 concentrations in the northern SFE.

Three lines of evidence suggest that the phytoplankton group that responded most favorably to the enclosures conditions were diatoms. Results of size-fractionated chl-*a* showed that the phytoplankton community was dominated by larger cells which have been interpreted previously as diatom biomass in the SFE (Cloern

Table 3
Summary of enclosure experiments in March. July and September 2005. V_{MAx} and ρM_{Ax} (maximum V and ρ observed in the enclosure) and time to reach V_{MAx} for C, NO_3, and NH_4. Initial NO_3 and NH_4 and time to NO_3 exhaustion and NH_4 <1 μmol L^{-1} also provided.

	March			July			September		
	SUI	SPO	CEN	SUI	SPO	CEN	SUI	SPO	CEN
V_{MAx} C (h^{-1})	0.018	0.056	0.049	0.041	0.043	0.040	0.055	0.085	0.102
ρ_{MAx} C (μmol C L^{-1} h^{-1})	3.27	10.06	9.29	10.53	10.53	9.67	8.89	13.38	15.97
Time to V_{MAx} C (h)	96	72	72	96	72	72	72	48	48
Initial NO_3 (μmol N L^{-1})	39.43	35.12	31.37	22.60	21.51	17.30	23.99	18.58	15.41
Time to exhaustion, (h)	>96	96	96	>96	96	96	>96	96	96
V_{MAx} NO_3 (h^{-1})	0.039	0.069	0.056	0.041	0.046	0.075	0.030	0.088	0.116
Time to V_{MAx} NO_3 (h)	92	71	71	92	47	47	72	47	47
ρ_{MAx} NO_3 (μmol N L^{-1} h^{-1})	0.40	1.08	0.64	0.24	0.35	0.76	1.35	1.18	0.41
Initial NH_4 (μmol N L^{-1})	9.18	5.71	4.19	8.61	5.54	5.21	5.65	5.25	4.60
Time to <1 μmol L^{-1}	72	48	48	96	48	48	72	48	48
V_{MAx} NH_4 (h^{-1})	0.034	0.025	0.032	0.046	0.040	0.031	0.028	0.032	0.038
ρ_{MAx} NH_4 (μmol N L^{-1} h^{-1})	0.21	0.03	0.07	0.28	0.30	0.27	0.72	0.72	0.72

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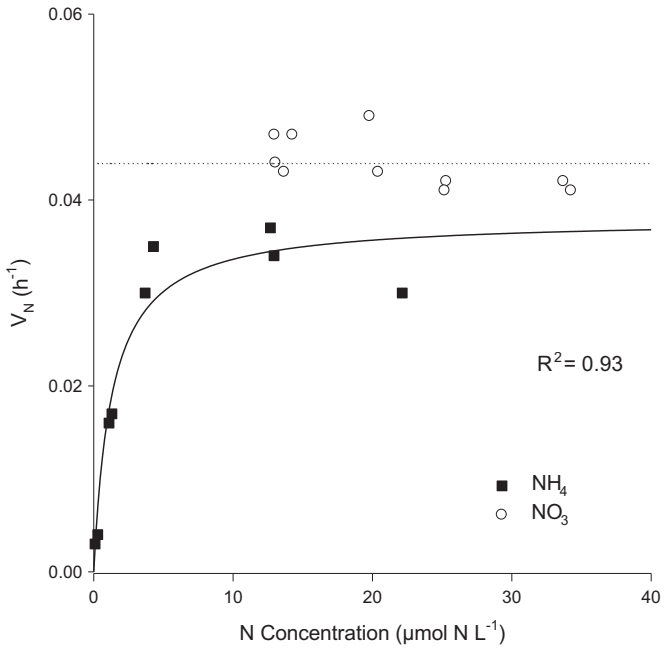

Fig. 4. Michaelis–Menten kinetic curves for NO_3 (open circles) and NH_4 (closed squares) in central San Francisco Bay in April 2005. Data for VNH_4 vs. [NH_4] were fit to a hyperbolic function. Dotted line is average V_{NO3}.

and Dufford, 2005; Wilkerson et al., 2006). N to Si nutrient drawdown ratios approached 1:1, as would be expected if diatoms contributed significantly to phytoplankton production (Brzezinski, 1985). Limited microscopy conducted on samples from our enclosure experiments identified several diatom species including *Skeletonema costatum, Leptocylindrus minimus* and small centric diatoms making up the bulk of the phytoplankton biomass (E. Carpenter, pers. comm.). Diatom dominance in the experimental enclosures is consistent with previous field studies in the northern SFE that have shown diatom dominance during phytoplankton blooms (Dugdale et al., submitted; Cloern, 1979; Cloern and Dufford, 2005; Lidström, 2009; Dugdale et al., submitted) and as an important food source within the pelagic foodweb of the SFE (Peterson et al., 1985).

4.1. Anomalously low phytoplankton carbon and nitrogen assimilation in Suisun Bay

In many ways the Suisun Bay enclosures showed different responses compared to Central and San Pablo enclosures. Suisun Bay enclosures had the highest initial NH_4 and phytoplankton showed a lagged response to the improved light conditions afforded by the experimental design. The timing of the maximal phytoplankton C and NH_4 uptake and the initiation of phytoplankton NO_3 uptake and chl-*a* accumulation was delayed by at least 24-h in Suisun Bay compared to San Pablo and Central Bay enclosures. The observed lag in phytoplankton NH_4 uptake in Suisun Bay was previously unappreciated. This lag and lower rate of NH_4 uptake, together with elevated ambient NH_4 acts to further delay the initiation of phytoplankton NO_3 use and the accompanied accumulation of chl-*a*. In nature, the delay in the timing of phytoplankton bloom initiation would likely result in fewer observed blooms in Suisun Bay (Dugdale et al., 2007). The delayed NO_3 uptake and the apparent link between carbon uptake and NO_3 uptake in Suisun Bay (Fig. 3D, F) results in lower C:N drawdown ratios than would be predicted by the Redfield Ratio (Redfield et al., 1963), with DIC drawdown only 40–60% of the carbon uptake predicted based on DIN drawdown (Table 2). We interpret these anomalous responses by Suisun Bay phytoplankton to reflect some stress on growth processes. The high NH_4 condition, the result of wastewater loading to the northern SFE (Jassby, 2008), is potentially exacerbated by some additional stress that results in low NH_4 uptake rates. Owing to its proximity to the Sacramento/San Joaquin Delta, which receives nearly half of California's surface water, there are a large number of potential contaminants including herbicides and pesticides (Kuivila and Hladik, 2008; Weston and Lydy, 2010; Werner et al., 2010), and metals (Johnson et al., 2010).

4.2. Ammonium effects on phytoplankton production

Investigators working in other systems have suggested that anthropogenic NH_4 concentration above some value may inhibit phytoplankton primary production. MacIsaac et al. (1979) investigated the effect of sewage effluent on coastal productivity and found that at >20 μmol NH_4 L^{-1}, C uptake was depressed, resulting in C:N uptake ratios of 2:1–3:1, similar to what was observed here for enclosures from Suisun Bay. Yoshiyama and Sharp (2006) examined a 26-yr dataset form the Delaware estuary and observed a "striking decline in production at NH_4 levels above a low threshold (10 μmol L^{-1}) suggesting a strongly negative influence of NH_4 itself, or something that accompanies high NH_4 concentrations, or both". Depression of primary production and phytoplankton NH_4 uptake was recently reported for the Sacramento River, immediately downstream of the Sacramento Regional Wastewater Treatment Plant (SRWTP) (Parker et al., 2012). Suisun Bay chronically experiences high ambient NH_4 concentrations with 90% of NH_4 in Suisun Bay originating at the SRWTP (Jassby, 2008). During the three year time series of Wilkerson et al. (2006) only one phytoplankton bloom with chl-*a* >30 μg L^{-1} was observed in Suisun Bay that occurred during a period of anomalously low NH_4 and substantial phytoplankton NO_3 uptake. Similarly, Dugdale et al. (submitted) documented two spring phytoplankton blooms in Suisun Bay during 2010 (the first known blooms in Suisun Bay since 2000), with their initiation attributed to low initial NH_4 concentrations as a result of freshwater dilution. In April 2007, we also observed a similar low NH_4 period in Suisun Bay and conducted an enclosure experiment. In this case, Suisun Bay phytoplankton dynamics followed the sequence typically observed in San Pablo and Central Bays (Fig. 5) and the phytoplankton were able to use all of the available NO_3 and accumulate chl-*a* within the 96-h incubation period. The present findings and those of Wilkerson et al. (2006) suggest that there are situations when Suisun Bay phytoplankton have the capacity to grow as well as those in Central Bay when NO_3 is made available by low NH_4 concentrations.

We observed NH_4 inhibition of NO_3 uptake in the enclosure experiments using water collected in all three embayments of the northern SFE and plotting ρNO_3 vs NH_4 concentrations (Fig. 6A, B), as seen in previous studies (Dugdale et al., 2007; their Fig. 2). We found that in the enclosure experiments with >1 μmol NH_4 L^{-1}, NO_3 uptake was relatively low and uniform. NH_4 inhibition of NO_3 uptake at low NH_4 concentrations (<1 μmol L^{-1}) has been known for some time in oceanic studies (e.g. Eppley et al., 1969; Conway, 1977) and at higher concentrations for several estuaries (e.g. Glibert et al., 1982; Pennock, 1987; Collos, 1989). While the phenomenon of NH_4 inhibition of NO_3 uptake is accepted universally, as pointed out in the review by Dortch (1990), its ubiquity in natural systems is less clear This may be particularly true in high NO_3 eutrophic systems where in some cases NO_3 uptake does not appear to be influenced by NH_4 concentration. At high NO_3 concentrations NO_3 may even inhibit phytoplankton NH_4 uptake (Dortch, 1990).

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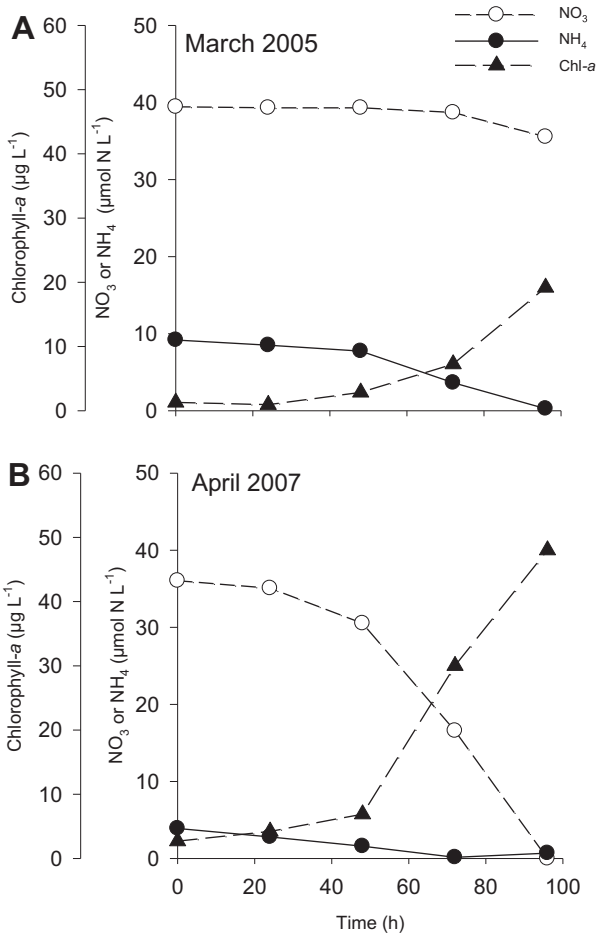

Fig. 5. NH_4, NO_3 and Chl-*a* concentrations over 96-h time series in enclosures collected at the same location in Suisun Bay during 2005 (A) and 2007 (B). Initial NH_4 concentrations in 2005 were 9.2 µmol L^{-1} and 3 µmol L^{-1} in 2007.

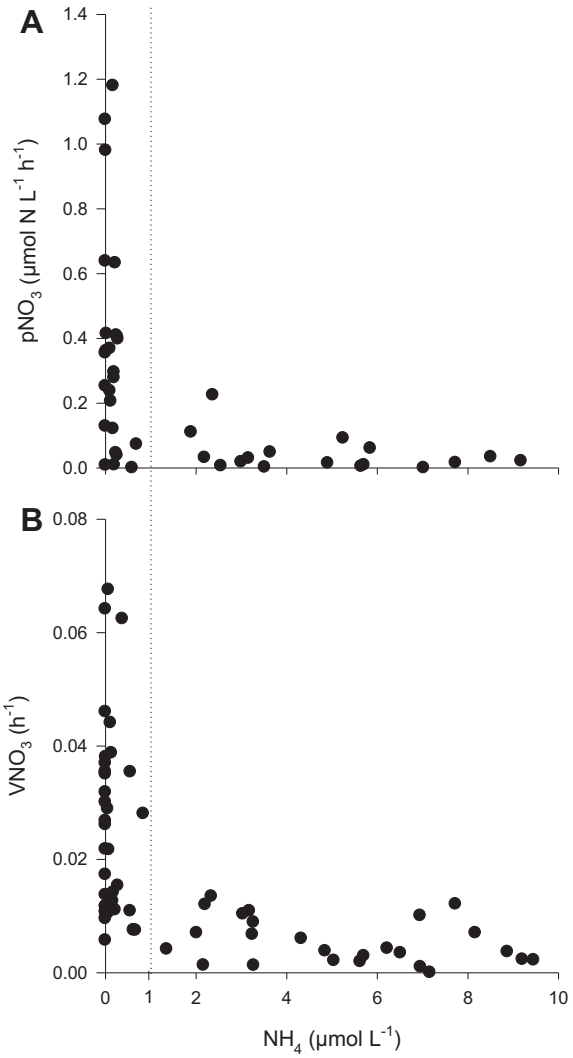

Fig. 6. A) NO_3 uptake rates versus NH_4 concentration. B) Biomass-specific NO_3 uptake versus NH_4 concentration. Results from enclosure experiments conducted in March, July and September ($n = 120$).

The sequential use of first NH_4 and then NO_3 as a result of the inhibition of phytoplankton use of NO_3 by NH_4 is often interpreted as a "preference for NH_4" (McCarthy et al., 1977). However, some phytoplankton, particularly diatoms, may display an increased capacity for NO_3 assimilation compared to NH_4 and may grow as well, or better on NO_3 (Thompson et al., 1989; Cochlan et al., 1991) and could equally be interpreted as a "preference" for NO_3 (Lomas and Gilbert, 1999a). The significance of this interaction between NH_4 and NO_3 in this study is that at low NH_4 concentrations, NO_3 uptake and high rates of primary production and chlorophyll accumulation can occur.

4.3. Maximal NO_3 uptake exceeds maximal NH_4 uptake

In these enclosures, as in the enclosures described in Dugdale et al. (2007) maximal rates on NO_3 uptake achieved (once NH_4 inhibition was alleviated) were always greater than those of NH_4. This may be due to different uptake kinetics with linear, not Michaelis–Menten hyperbolic, NO_3 uptake and to acceleration (or shift-up) of NO_3 but not NH_4 uptake. In this study we observed classical Michaelis–Menten kinetics for NH_4 but were unable to determine N uptake kinetics for NO_3, although comparing rates obtained with saturating level of the two DIN species, there was higher V_{MAx} for NO_3 compared to NH_4. Deviation (to linear or biphasic) from the hyperbolic relationship for NO_3 uptake at saturating to supersaturating concentrations have been described in many algal species including diatoms (Serra et al., 1978; Watt et al., 1992; Collos et al., 1992, 1997, 2005; Lomas and Glibert, 1999b) and upwelled phytoplankton (Dugdale et al., 2006). Two studies (Huntsman and Barber, 1977; Lancelot and Billen, 1985) showed that C and N uptake were coupled during linear NO_3 uptake at high concentrations. We are aware of only one study demonstrating deviations from Michaelis–Menten kinetics for NH_4 uptake, associated with the paralytic-shellfish poison-producing dinoflagellate *Alexandrium catenella* (Collos et al., 2006). Acceleration of NO_3 uptake (V_{MAx} NO_3) as a function of NO_3 concentrations (termed "shift-up") was described in recently upwelled waters (Dugdale et al., 1990) and shipboard enclosures (Wilkerson and Dugdale, 1987) with the consequence that all of the initial NO_3 was exhausted in 4–5 days, regardless of initial NO_3 concentration. V_{MAx} NH_4 uptake does not appear to accelerate linearly with NH_4 concentration. Consequently, the ratio of V_{MAx} NO_3:V_{MAx} NH_4 is variable and, as in this study, almost always >1 after 24-h to 48-h of incubation under favorable irradiance. Although the shift-up phenomenon for NO_3 uptake was originally described for the coastal ocean, it appears to also occur in estuaries, and may help to explain how the maximal uptake of NO_3 is greater than that of NH_4 in the experimental enclosures.

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4.4. Phytoplankton C Uptake and biomass accumulation linked to phytoplankton NO_3 use

Phytoplankton specific carbon uptake, normalized to either POC or chl-*a*, was higher during periods of phytoplankton NO_3 uptake compared to periods of phytoplankton NH_4 uptake (Fig. 3C, Table 3). Few published studies exist showing enhanced phytoplankton growth with NO_3 versus NH_4 (Thompson et al., 1989; Cochlan et al., 1991; Lomas and Gilbert, 1999a). Parker (Unpublished data) conducted mesocosm experiments in the Delaware Estuary in which phytoplankton were supplied with either NO_3 or NH_4 and found 2-fold higher increases in POC and chl-*a* increase in the NO_3 treatment over 56-h. Serra et al. (1978) showed that *S. costatum* growth rates were initially higher with NH_4 compared to NO_3 and NO_2 but NO_3 growth eclipsed NH_4 growth later in the experiment; the difference attributed to inducible adaptive enzymes required for NO_3 uptake (e.g. nitrate reductase).

The carbon drawdown and nutrient drawdown ratios observed in these enclosures (Table 2) show that when NH_4 is the major source of DIN being used by the phytoplankton (i.e. in SUI enclosures) C drawdown is low and the C:N drawdown ratio is roughly half of the Redfield ratio (Table 2). Conversely when both NO_3 and NH_4 are being used (as exemplified by the CEN and SPO enclosures) then the C drawdown is high and C:N drawdown approaches or exceeds the Redfield ratio. For example in March, DIC drawdown in SUI was only 17% of that observed in SPO. An NH_4 based system will likely exhibit a primary production of <20% of that where NO_3 is fully used. It is true that heterotrophic bacteria in our enclosures likely contribute to some fraction of DIN disappearance, complicating this interpretation. However, the large phytoplankton biomass in the experimental enclosures (>20 μg chl-a L^{-1}) probably far exceeds bacterial biomass and nutrient cycling processes.

4.5. An evolving conceptual model of phytoplankton bloom development in high NH_4 estuaries with Implications for management

The classical view is that phytoplankton blooms in SFE are controlled by the availability of light, with spring blooms occurring as a result of brief periods of water column stratification (Alpine and Cloern, 1988). However, anthropogenic NH_4 may play a modulating role in bloom formation by limiting access to the NO_3 pool reducing the potential for enhanced phytoplankton C and NO_3 uptake once light conditions improve. We suggest the following scenario of C, NO_3 and NH_4 uptake and phytoplankton bloom development for northern SFE (Fig. 7A). Phase 1 is characterized by low NH_4 uptake and low C uptake; there is virtually no NO_3 uptake due to NH_4 inhibition and C:N uptake ratios are low. Once NH_4 is almost exhausted, phase 2 begins with a rapid uptake of NO_3 coupled with high C uptake; C:N uptake ratios increase over this period. Finally, as NO_3 is exhausted, the system enters phase 3 of bloom development, and phytoplankton become N-limited, relying primarily on recycled NH_4 or intracellularly stored NO_3; relatively high C:N uptake ratios are observed.

Results from the Central Bay enclosure experiments completed in September (Table 3) are plotted for comparison with this conceptual model (Fig. 7B). C:N ratios calculated from ^{13}C and ^{15}N tracer results and the f-ratio are provided (Fig. 7C). C:N ratios confirm the progression from low C:N to balanced growth and finally high C:N ratios as the f-ratio increased, reflecting a greater dependence on NO_3 over NH_4 uptake. In general, phytoplankton remain in phase 1 of the scenario much of the time in the northern SFE as a result of poor light conditions and high ambient NH_4. These conditions result in low primary production and low biomass. In

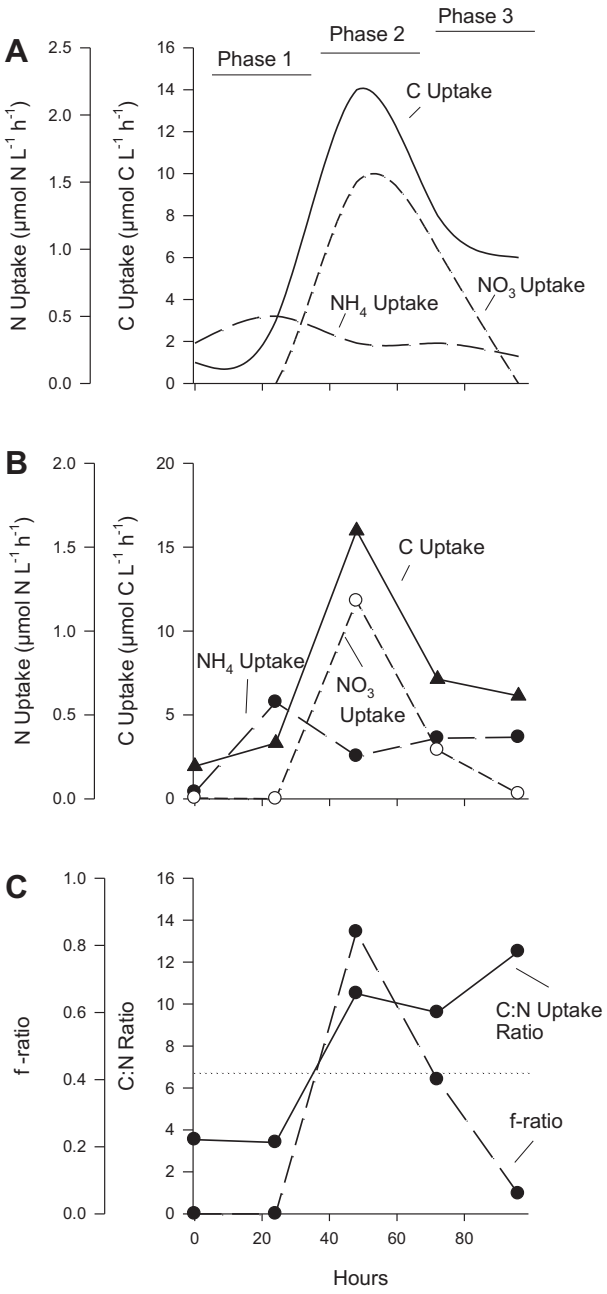

Fig. 7. A) Idealized sequence of carbon, NH_4 and NO_3 uptake in enclosure experiments. B) Data from central Bay enclosures during September. C) C:N uptake ratios and f-ratio for September.

recent years this has prevented spring blooms occurring in Suisun Bay except twice in 2000 and 2010 when NH_4 concentrations were low (Dugdale et al. submitted; Wilkerson et al., 2006) and phytoplankton shifted to phase 2 and 3 of the bloom progression.

NH_4 concentrations have steadily increased in the northern SFE since 1979 (California DWR; Jassby, 2008) as a result of human population increase; the major input of NH_4 being wastewater discharge (Hager and Schemel, 1992; Jassby, 2008). In Suisun Bay, the 4 μmol NH_4 L^{-1} threshold (Dugdale et al., 2007) for the inhibition of phytoplankton NO_3 uptake is generally exceeded during spring and summer (Jassby, 2008). We speculate that changing wastewater management practices to favor the discharge of NO_3 rather than NH_4 may increase primary production in the northern

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SFE. NO_3 stimulated primary production would likely enhance secondary production, which may be beneficial to fisheries in the Bay and nearshore (Pacific) waters. Understanding the phytoplankton response to nutrient enrichment is a major challenge to estuarine scientists and will require more sophisticated models of coastal eutrophication (i.e. Cloern, 2001; Sharp, 2001). We suggest that careful consideration of not only DIN loading but also N speciation of the DIN must also be considered for effective nutrient management strategies. As well illustrated by this study, enabling NO_3 utilization by phytoplankton will increase the rate of carbon uptake (i.e. primary production), and chl-*a*, whereas contaminant levels of NH_4 will keep carbon uptake low and may even be sufficiently toxic to decrease productivity directly.

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