

Suisun Bay Ammonium Synthesis Report

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Executive Summary

Observations made since the early 2000's have noted declining abundances of important, pelagic members of the Suisun Bay and Sacramento/San Joaquin Delta food webs. In response, numerous investigations have been launched, aimed at identifying the underlying cause(s) of what is referred to regionally as the Pelagic Organism Decline (POD). The conceptual model for the POD recognizes that multiple factors may act in concert to degrade habitat and contribute to the POD (Baxter et al., 2010; NRC 2012), including: changes in flow regime, physical alterations to habitat, land use changes, invasive species, contaminants, and nutrients. Understanding the underlying causes of habitat degradation and the POD in Suisun Bay and the Delta requires an integrated analysis across the range of potential drivers. This report focuses on one set of these issues: elevated loadings and concentrations of ammonium (NH_4^+) in Suisun Bay and a subset of the proposed mechanisms by which NH_4^+ may adversely impact ecosystem health.

Recent studies have hypothesized that anthropogenic nutrient loads over the past few decades, in particular NH_4^+ , are negatively impacting food webs in Suisun Bay and the Delta. Elevated NH_4^+ concentrations are hypothesized to be inhibiting primary productivity in Suisun Bay, San Pablo Bay, and the Sacramento River (Dugdale et al., 2007; Parker et al., 2012), and indirectly contributing to the POD by decreasing the potential food supply. Other investigators hypothesize that changes in nutrient ratios and forms of N are exerting additional bottom-up pressures on Delta and Suisun food webs by altering the phytoplankton community composition and the N:P composition of individual cells (e.g., Glibert et al., 2011; Glibert et al., 2012). In addition, a recent study reported evidence that NH_4^+ , at concentrations observed in some areas of the Delta and Sacramento River, can exert chronic toxicity on a copepod species (*Pseudodiaptomus forbesi*) that is an important food resource (Teh et al., 2011).

The purpose of this report is to provide an overview of the state of the science and identify science gaps related to a subset of the hypothesized adverse impacts of NH_4^+ in Suisun Bay, and characterize NH_4^+ loads, concentrations, and fate. The report's specific goals are to

1. Synthesize the scientific literature on nitrogen utilization by marine and estuarine phytoplankton, with a particular focus on factors and mechanisms that regulate the N form utilized by phytoplankton, and the effect of different N sources on primary production rates. (Section 2)
2. Through the perspective of the broader scientific literature, evaluate the results and interpretations of recent studies that hypothesize that elevated NH_4^+ levels inhibit primary production rates. (Section 3)
3. Summarize the scientific literature related to NH_4^+ toxicity to copepods. (Section 4)
4. Synthesize the scientific literature on copepod ecology and changes in community composition and abundance in Suisun Bay (Section 5)
5. Quantify NH_4^+ loads to Suisun Bay, evaluate long-term changes and seasonal variations in ambient NH_4^+ concentrations, and characterize NH_4^+ fate. (Section 6)

6. Summarize key observations and identify next steps. (Section 7)

Although additional pathways of nutrient-related impairment have been proposed in Suisun Bay and the Delta, this report is narrowly focused on the above goals. The report was developed under the assumption that it would be used in conjunction with complementary reports (including reports already developed, e.g., Baxter et al., 2010; Meyer et al., 2009) that address other factors affecting ecosystem health in Suisun Bay and the Delta to help identify the outstanding science questions whose answers will inform management decisions. For additional background and context on nutrient related issues in San Francisco Bay, the reader is referred to a recent nutrient conceptual model report (Senn et al. 2014).

The report is organized into individual sections that address each of the six main goals, and the overall findings are summarized below.

NH₄⁺ inhibition of primary production

The NH₄⁺ inhibition hypothesis was developed through multiple studies by researchers at San Francisco State University's Romberg Tiburon Center (RTC) for Environmental Studies over the past decade (e.g., Wilkerson et al., 2006; Dugdale et al., 2007; Parker et al., 2012a, 2012b; Dugdale et al., 2012). The conceptual model for the ecological impacts of the NH₄⁺ inhibition hypothesis is built around three main points:

- P.1** The presence of NH₄⁺ at elevated levels (>1-4 μmol L⁻¹) inhibits the uptake of nitrate by phytoplankton.
- P.2** The rate of NO₃⁻ uptake (when NH₄⁺ is absent or less than 1-4 μM) is greater than the rate of NH₄⁺ uptake. Thus, when NO₃⁻ uptake is suppressed, and only NH₄⁺ is being taken up by phytoplankton, the overall rate of N uptake is lower.
- P.3** The lower rate of N uptake resulting from this mechanism translates into lower rates of primary production.

Dugdale et al (2012) refer to the suppression of bloom development by elevated NH₄⁺ as “the NH₄⁺ paradox”. The NH₄⁺-inhibition conceptual model that is based on P.1-P.3 argues that phytoplankton uptake of NO₃⁻, the largest pool of N in the San Francisco Estuary, is necessary for phytoplankton bloom development. Under this model, bloom initiation is dependent on lower NH₄⁺ concentrations combined with certain river flow and loading conditions (assuming sufficient irradiance), and three criteria must be met: 1) NH₄⁺ loading must not exceed the capacity of the phytoplankton to assimilate the inflow of NH₄⁺; 2) NH₄⁺ concentration must be equal to or less than 4 μmol L⁻¹ to enable phytoplankton NO₃⁻ uptake; 3) The dilution rate of the phytoplankton biomass, set by river flow, must not exceed the phytoplankton growth rate to avoid washout.

There is strong support in the scientific literature for P.1, with numerous studies demonstrating either that multiple species of phytoplankton exhibit a strong preference for NH_4^+ or that NO_3^- uptake is actively inhibited by elevated NH_4^+ concentrations. RTC studies offer convincing support for P.1, with NO_3^- uptake by phytoplankton strongly inhibited when NH_4^+ concentrations exceed $1\text{--}4\ \mu\text{mol L}^{-1}$.

P.2 is not well-supported by the broader scientific literature on N uptake rates by phytoplankton. Few well-controlled studies have actually investigated N uptake rates during experiments in which both NO_3^- and NH_4^+ were available over a range of concentrations. Thus, there remains a critical gap in the literature on this topic. While there are limited studies that explicitly compare NO_3^- vs. NH_4^+ uptake kinetics, the more broadly accepted conceptual model is that, when nutrients are abundant, cells access whichever N source is most readily available, and that uptake rates of NO_3^- and NH_4^+ are similar. The RTC studies provide some support for P.2 through enclosure experiments carried out with Bay water and using ambient phytoplankton community assemblages (Parker et al., 2012a), and with one set of uptake kinetic experiments using ambient community assemblages. However, RTC studies also yield some experimental evidence that suggests NH_4^+ uptake rates can be comparable to or even greater than NO_3^- uptake rates. In addition, uncertainty remains about whether experimental artifacts or other reasonable explanations could explain some of the observations used as evidence in support of P.2. While P.2 remains a plausible hypothesis, additional research is needed to more rigorously establish NO_3^- and NH_4^+ kinetics under a range of conditions (temperature, light levels), including experiments carried out with mono-cultures of phytoplankton species or taxa commonly present in Suisun Bay. .

P3 is not well supported by the broader scientific literature. As with P2, the more broadly accepted concept is that most phytoplankton taxa grow equally well when using NH_4^+ or NO_3^- as their nitrogen source (see Section 2 for further discussion). Multiple studies have found similar growth rates (rates of carbon fixation) across a range of taxa when using NH_4^+ or NO_3^- . While the rate of growth varies with different levels of light, experiments in which monocultures of phytoplankton were grown under different light regimes and different N sources found that growth rate was not strongly dependent on whether NO_3^- or NH_4^+ was provided (see Section 2). As with P.2, few studies have done growth experiments in which phytoplankton have the choice between NH_4^+ and NO_3^- , so there also remains a critical gap in the literature on this related topic. RTC field and enclosure experiments provide some evidence that is consistent with the hypothesis that primary production rates (using rates of C uptake) are slower at high NH_4^+ levels, and that growth rates increase when NH_4^+ is depleted and phytoplankton begin utilizing NO_3^- (Parker et al., 2012a, 2012b). In other studies, primary production rates are inferred from changes in chl-a or assumed to be proportional to the N uptake rate, both of which are prone to considerable uncertainty (due to variations in C:chl-a and C:N). In addition, in some components of RTC studies, experimental artifacts (e.g., acclimation time to light conditions in enclosures) or competing explanations have not been sufficiently ruled out, including the potential role of other

contaminants, either co-occurring in treated wastewater effluent, or other sources such as agricultural runoff. Even if P.2 and P.3 are occurring, N uptake and primary production in Suisun Bay appear to behave differently compared to the conceptual model, which was developed largely based on observations in San Pablo and Central Bay (Dugdale et al., 2007; Parker et al., 2012). Dugdale et al. (2007) and Parker et al (2012a) acknowledge the potential role of other factors, such as other contaminants. However, their conclusions about Suisun Bay do not sufficiently address this nuance, or the extent to which the NH_4^+ -based explanations can be readily applied in Suisun Bay. Finally, NH_4^+ levels are present at comparable levels in South San Francisco Bay, and examples of NH_4^+ inhibition of primary production rates have not been documented there.

Similar to P.2, P.3 remains a plausible hypothesis. Inhibition of primary production by elevated NH_4^+ has been proposed as one possible mechanism to explain lower production rates elsewhere (e.g., Delaware Bay; Yoshihama and Sharp, 2006). The RTC studies have tackled the issue with field observations and experimental studies using ambient phytoplankton assemblages, as opposed to pure culture experiments. Their field studies and simulation of field conditions through enclosure experiments with Bay water and ambient phytoplankton communities provide an important perspective on net effects at the field scale. However, the complexity introduced by field conditions or simulated-field conditions, when multiple underlying factors are changing over space or time (e.g., phytoplankton community composition, grazing, acclimation to experimental light conditions, increases or decrease in light attenuation as a function of space in field studies, stratification) can make it difficult to directly evaluate the role of the NH_4^+ inhibition mechanism. Additional research is needed to:

- Determine whether statistically significant differences in primary production rates occur due to the N form utilized. Effort should be directed toward establishing NO_3^- and NH_4^+ uptake kinetics and phytoplankton growth kinetics under a range of conditions (e.g., varying temperature and light levels, varying proportions of NO_3^- and NH_4^+), including experiments carried out with mono-cultures of phytoplankton species or taxa commonly present in Suisun Bay.
- Determine the ecological significance of this mechanism at the ecosystem scale, including improved understanding of the conditions under which differences in growth rates occur, and the magnitude of the effect.
- Rule out competing explanations and experimental artifacts in field observations and enclosure experiments.

Some of these research needs are the focus of on-going or proposed studies by RTC researchers, their collaborators, and other research. Those studies have not been discussed in this report; therefore, this review may need to be revisited as that data becomes available.

Independent of whether the set of processes laid out in the NH_4^+ -inhibition conceptual model occur as proposed, their potential importance at the ecosystem scale has not been adequately

investigated. Other factors are known to play important, if not dominant, roles in limiting primary production rates (e.g., light limitation) or biomass accumulation (clam grazing, residence time) in Suisun Bay. The RTC studies acknowledge the roles of light limitation and clam grazing; they point out that NH_4^+ inhibition of primary production is an additional factor that limits production when conditions might otherwise allow for blooms to occur. However, this important point sometimes gets lost when the NH_4^+ -inhibition conceptual model is discussed in the context of its management implications. The potential ecosystem-scale importance of the NH_4^+ -inhibition conceptual model could be assessed using relatively basic biogeochemical models and existing data. Such modeling efforts would have benefits far beyond testing the NH_4^+ hypothesis, in that they will yield tools for quantitatively synthesizing existing nutrient and phytoplankton data in Suisun Bay and other embayments, identifying data and monitoring needs, and informing the broader modeling strategy for the Bay.

NH_4^+ toxicity to copepods

Changes in quality and abundance of food for pelagic fishes has been identified as one potential factor contributing to POD in the Delta and Suisun Bay. Zooplankton abundance and size have decreased over the last four decades, and these declines in food availability may be exerting bottom-up pressure on the food web (Baxter et al., 2010), since zooplankton are the primary prey for Delta smelt and other pelagic fishes whose decline lie at the center of the POD. High grazing rates by invasive benthos, low food abundance (i.e., low phytoplankton biomass), and direct toxicity of contaminants have been hypothesized to be acting in concert to keep zooplankton populations low. The unionized form, ammonia (NH_3), is the form that has most commonly been considered to be toxic to aquatic organisms. However, Teh et al. (2011) recently reported on chronic toxicity to the copepod *Pseudodiaptomus forbesi* at fairly low NH_4^+ concentrations. *P. forbesi* is of particular interest because during most times of the year, *P. forbesi* is considered the most important food source for all fish that have shown declining populations.

Teh et al. (2011) found that the survival of *P. forbesi* from early life stages to adult stages was reduced at NH_4^+ concentrations as low as $26 \mu\text{mol L}^{-1}$. The toxicity mechanism was hypothesized to be related to the fact that copepods excrete N waste as NH_4^+ , and that elevated NH_4^+ levels in the ambient surrounding water interfere with NH_4^+ excretion rates. Since NH_4^+ levels exceed $26 \mu\text{mol L}^{-1}$ in some parts of the northern Delta and the Sacramento River, it has been suggested that *P. forbesi* population levels may be impacted by elevated NH_4^+ loads to the system.

If toxicity to copepods from NH_4^+ may be among the issues that will inform nutrient management decisions in Suisun Bay, it would worthwhile to conduct further investigations. While the copepod toxicity study by Teh et al. (2011) was carefully executed, it has not yet been replicated. Furthermore, although there is some support for the proposed toxicity mechanism in the literature, only a handful of studies have been published on NH_4^+ toxicity to aquatic invertebrates, and none of those studies used copepods as the test organism. In addition, Teh et

al. (2011) observed an effect in the lowest dosed samples, and treatments at lower levels are needed to establish a no observed effect level (NOEL). Finally, studies at salinity and pH ranges relevant to Suisun Bay would be needed, in particular because toxicity is thought to be exerted through the Na^+/K^+ transporter and Na^+ and K^+ levels vary with salinity; therefore copepod sensitivity to NH_4^+ could vary with salinity.

Copepod ecology in Suisun Bay

Copepods are key links in the San Francisco Estuary (SFE) foodweb between microplankton and fish. As such, declines in the abundance and biomass of copepods and changes in the dominant copepod species over the past few decades in Suisun Bay, and the underlying causes of these changes, are of critical concern. Most of the copepods of the upper estuary are introduced species, some of which are not suitable as food for fish because of their small size. The biomass of the larger copepods is less than it was before the introduction of the clam *Corbula amurensis*, because of competition for food and grazing by clams on the early life stages of copepods. The resulting low abundance of copepods of suitable size, and the long food chain supporting them, may be contributing factors to the decline in abundance of several estuarine fish species.

Copepods live in a moving frame of reference and therefore are more closely tied to a particular salinity range than a geographic position. Some species use tidal vertical migration to maintain their position in the salinity field. Copepods have elaborate sensory, feeding, and swimming appendages that enable them to feed very selectively and to escape from predators. Some feed by scanning the water for particles and removing them with their feeding appendages (e.g., the calanoid copepod *Pseudodiaptomus forbesi*), while others attack individual motile prey (e.g., the tiny cyclopoid *Limnoithona tetraspina*). Most copepods will consume microzooplankton such as ciliate protozoans at higher rates than phytoplankton, but microzooplankton are not monitored in the estuary. Diatoms can be key primary producers in productive areas but copepods often feed on other particles even when diatoms are abundant, and there is some controversy about the suitability of diatoms as food. Common copepods in the upper SFE are severely food limited, which manifests as very low reproductive and growth rates. In the low-salinity zone (Suisun Bay and the western Delta) the combination of high grazing by clams and low food supply means that the *P. forbesi* population there must be subsidized through advection from their population center in freshwater.

Nutrient concentrations could have direct or indirect effects on copepods. As noted above, it has been hypothesized that ammonium could be exerting direct toxicity to copepods. Indirectly, elevated ammonium has also been hypothesized to slow diatom production, which could affect copepod growth and development and elevated NH_4^+ could also have a positive effect on growth of the toxic cyanobacteria *Microcystis*. However, so far there is no clear evidence documenting that these effects play an important role in regulating copepod populations in Suisun Bay and the Delta..

NH₄⁺ loads, ambient concentrations, and fate

Over the period 1975-2011, NH₄⁺ concentrations in Suisun Bay have increased 25-50% in some months, and exhibited strong seasonal variability, with 2-4 fold lower concentrations in summer and fall months than in higher flow months.

The major anthropogenic NH₄⁺ loads to Suisun Bay came from the Delta and from treated wastewater effluent discharged directly to Suisun Bay. Delta loads were estimated using an approach similar to Jassby and Cloern (2000), and, due to changes in data availability, we have the greatest confidence for the periods of 1975-1995 and 2006-2011, and describe those briefly here. Since 1975, NH₄⁺ loads from the Delta to Suisun have increased substantially with most of the increase occurring after 1995. On an annual basis, the mean (\pm 1 s.d.) loads entering Suisun Bay from the Delta were 5800 ± 1800 kg N d⁻¹ from 2006-2011, as compared to 4100 ± 2700 kg N d⁻¹ from 1975-1995. NH₄⁺ loads from the Delta varied seasonally, as did the magnitude in the increase between pre-1995 and post-1995. Estimated NH₄⁺ loads to Suisun Bay from the Delta increased the most during spring months (April-May) increasing by 5000-6000 kg d⁻¹ between over the entire period of 1975-2011, with most of this increase occurring after 1995. Most of the Delta-derived NH₄⁺ load entering Suisun was estimated to have come from the Sacramento River, as opposed to the southern Delta (i.e., San Joaquin), and most of the NH₄⁺ transported along the lower Sacramento River has been shown to originate at Sacramento Regional Wastewater Treatment Plant (SRWTP). SRWTP's NH₄⁺ loads increased by nearly a factor of 2 between 1985 and 2005, with most of that increase occurring after 1995 (Jassby 2008) and were presumably responsible for most of the increase in estimated loads from the Delta to Suisun Bay during this time. Other studies have found that much of SRWTP's NH₄⁺ load undergoes nitrification en route to Suisun Bay (Foe 2010; Parker et al., 2012). Our estimates are also consistent with substantial nitrification of effluent NH₄⁺ during its transit to Suisun Bay: present day loads from SRWTP (annual average = 13200 kg N d⁻¹ for 2006-2011) are much larger than the loads entering Suisun from the Delta (annual average = 5800 kg d⁻¹).

POTWs that discharge directly to Suisun Bay also contribute substantial NH₄⁺ loads to the system. Next to loads entering from the Delta, Central Contra Costa Sanitation District was the second largest NH₄⁺ source to Suisun Bay, with annual average loads that increased from 2600 kg d⁻¹ in the early 1990s to current loads of 3400 kg d⁻¹ (annual average for the years 2008-2011). Delta Diablo Sanitation District was the third largest NH₄⁺ source to Suisun Bay (1100 kg d⁻¹), and its NH₄⁺ loads have remained relatively constant since 1990. Initial estimates of stormwater loads suggest that they contribute less than 5% of NH₄⁺ loads during wet periods, and little if any NH₄⁺ during the dry season. The magnitude of internal NH₄⁺ sources (flux from the sediments) are poorly constrained but they could conceivably be as high as 1000 s of kg d⁻¹, and thus may be a quantitatively-important unknown.

Box model mass balance estimates, calculated using data for the months of May-October over the period 2006-2011, suggest that NH₄⁺ exhibits strong non-conservative behavior within

Suisun Bay. If NH_4^+ behaved conservatively, concentrations would have been on the order of $20 \mu\text{mol L}^{-1}$ based on monthly-average load estimates. Instead, spring, summer, and fall concentrations typically fell in the range of $3\text{-}6 \mu\text{mol L}^{-1}$. This large difference between predicted and measured concentration is especially relevant within this concentration range of $3\text{-}20 \mu\text{mol L}^{-1}$, considering the levels at which NH_4^+ is hypothesized to inhibit primary production ($>2\text{-}4 \mu\text{mol L}^{-1}$) and have toxic effects on copepods (LOEL = $26 \mu\text{mol L}^{-1}$). Based on box model estimates, on average only 25% of the NH_4^+ that was added to the system during these months was actually transported out of Suisun Bay through the Carquinez Straits. The remaining $\sim 75\%$ of the NH_4^+ must have been lost by transformation (e.g., nitrification) or uptake by phytoplankton. The first order rate constants required to explain the loss of NH_4^+ during low-flow periods was in the range of $0.1\text{-}0.3 \text{ d}^{-1}$, which is comparable in magnitude to nitrification rates typically used in water quality models. This mass balance analysis did not include NH_4^+ flux from the sediments, indicating that, if benthic fluxes were substantial, the calculated losses and rates are lower bound estimates.

Ambient NH_4^+ concentrations in Suisun Bay frequently exceeded the levels above which NH_4^+ inhibition of primary production has been hypothesized to occur. According to the conceptual model proposed by RTC researchers, at NH_4^+ concentrations of $2\text{-}4 \mu\text{mol L}^{-1}$ the uptake of NO_3^- by phytoplankton is substantially inhibited, resulting in lower primary production rates. RTC investigators note that $4 \mu\text{mol L}^{-1}$ is not a “bright-line” threshold, and that NO_3^- uptake and phytoplankton productivity are also inhibited at lower levels of NH_4^+ (down to $\sim 1 \mu\text{mol L}^{-1}$). The $4 \mu\text{mol L}^{-1}$ value is used here because it is the most commonly cited value. The $4 \mu\text{mol L}^{-1}$ threshold was compared to ambient concentrations in April-October, when high chlorophyll concentrations were most commonly observed prior to the mid-1980s. Between 1975-1986, NH_4^+ levels exceeded $4 \mu\text{mol L}^{-1}$ in 44% of the monthly observations. Between 1987-1997, the $4 \mu\text{mol L}^{-1}$ threshold was exceeded in 70% of monthly observations. Most recently, from 1998-2011, ambient NH_4^+ concentrations exceeded $4 \mu\text{mol L}^{-1}$ the vast majority of the time (87%). Thus, the frequency with which a $4 \mu\text{mol L}^{-1}$ threshold has been exceeded between April-October has approximately doubled over the past 35 years.

Teh et al (2011) found that the LOEL for chronic toxicity to copepods was $26 \mu\text{mol L}^{-1}$. Year-round ambient NH_4^+ concentrations at D6, D7, and D8 were compared to this value and found to exceed the LOEL only two times, once at each D6 and D7, and both times in 1977.

While considering the above comparisons of ambient concentrations with proposed effect concentrations, one should keep in mind the remaining uncertainties about the underlying mechanisms and in the concentrations at which effects may be observed. The underlying mechanisms of the NH_4^+ -inhibition hypothesis still require further testing; in addition, if it is found to be an important mechanism, the lowest level at which ecologically-meaningful effects occur needs to be determined. The copepod toxicity study by Teh et al. (2011) has not been replicated. In addition, Teh et al. (2011) observed adverse effects in the lowest dosed samples,

and treatments at lower NH_4^+ concentrations are needed to establish a no observed effect level (NOEL).

Recommended Next Steps

The recommendations identified here are not intended to be comprehensive, but rather communicate some broad suggestions that became clear during this report's writing.

1. General: A coordinated nutrient science plan should be established for Suisun Bay and the Delta, with clearly articulated scientific questions, recommended experiments or monitoring, and a prioritization of work. There are currently numerous nutrient-related studies being conducted in Suisun and the Delta. However, the work is being carried out in more of a patchwork fashion, funded or directed by different organizations, and with limited overarching prioritization and coordination. This does not necessarily require a new entity. Instead, the development of a Delta-Suisun nutrient science plan could be coordinated among the Bay-wide nutrient strategy participants, the IEP, and other entities. Developing such a coordinated nutrient science program is consistent with recent recommendations in the Delta Plan V6.0.

2. NH_4^+ inhibition hypothesis:

2.a To identify the specific science questions and the types of studies needed to better understand the hypothesized mechanisms of the NH_4^+ -inhibition conceptual model, it would be both helpful and efficient to convene a science panel. This panel should consist of regional scientists working on phytoplankton ecology and nutrient issues in the Bay, as well as outside experts. The panel would explore the detailed evidence from studies in San Francisco Bay and literature from other systems and identify: science issues on which there is consensus among the panelists; outstanding scientific questions; and studies that need to be carried out to address the outstanding questions. It is recommended that the panel develop a brief consensus document summarizing their observations and recommendations. Such a document could be a key component of the Suisun nutrient science plan mentioned above..

2.b. In parallel with any field or experimental studies, modeling work should be carried out to evaluate the potential quantitative importance of NH_4^+ -inhibition at the ecosystem scale, relative to other factors known to play important roles in limiting primary production rates (e.g., light limitation) or biomass accumulation (clam grazing, residence time) in Suisun Bay. Thus far this issue has not been adequately investigated. Such an analysis could be carried with relatively basic biogeochemical models and existing data, and using current parameterizations of the proposed mechanisms. These modeling efforts have benefits well beyond testing the NH_4^+ hypothesis, in that they will simultaneously yield tools for quantitatively synthesizing existing nutrient and phytoplankton data in Suisun Bay and other embayments, identifying data and monitoring needs, and informing the broader modeling strategy for the Bay.

3. NH_4^+ toxicity to copepods: If toxicity to copepods from NH_4^+ will be among the issues that will inform nutrient management decisions in Suisun Bay, it would be worthwhile to conduct further investigations. While the copepod toxicity study by Teh et al. (2011) was carefully executed, it has not yet been replicated. Furthermore, although there is some support for the proposed toxicity mechanism in the literature, only a handful of studies have been published on NH_4^+ toxicity to aquatic invertebrates, and none of those studies used copepods as the test organism. Prior to beginning work it would be valuable to have the study design peer reviewed, and to have broad buy-in among regulators and stakeholders (see recommendation #1). Teh et al. (2011) observed an effect in the lowest dosed samples, and treatments at lower NH_4^+ concentrations would be needed to establish a no observed effect level (NOEL). In addition, treatments using salinity and pH ranges relevant to Suisun Bay would be needed, since copepod sensitivity to NH_4^+ could vary with salinity. While other more nuanced questions and complex study designs may eventually be warranted (e.g., effect of food limitation plus NH_4^+), replicating the chronic toxicity experiment first, and determining if similar or different thresholds are observed, is a logical next step.

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1. Introduction

1.1. Background

Observations made since the early 2000's have noted declining abundances of important, pelagic members of the Suisun Bay and Sacramento/San Joaquin Delta food webs. In response, numerous investigations have been launched, aimed at identifying the underlying cause(s) of what is referred to regionally as the Pelagic Organism Decline (POD). The conceptual model for the POD recognizes that multiple factors may act in concert to degrade habitat and contribute to the POD (Baxter et al., 2010; NRC 2012), including: changes in flow regime, physical alterations to habitat, land use changes, invasive species, contaminants, and nutrients. Understanding the underlying causes of habitat degradation and the POD in Suisun Bay and the Delta requires an integrated analysis across the range of potential drivers. This report focuses on one set of these issues: elevated loadings and concentrations of ammonium (NH_4^+) in Suisun Bay and a subset of the proposed mechanisms by which NH_4^+ may adversely impact ecosystem health.

Recent studies have hypothesized that anthropogenic nutrient loads over the past few decades, in particular NH_4^+ , are negatively impacting food webs in Suisun Bay and the Delta. Elevated NH_4^+ concentrations are hypothesized to be inhibiting primary productivity in Suisun Bay, San Pablo Bay, and the Sacramento River (Dugdale et al., 2007; Parker et al., 2012), and indirectly contributing to the POD through decreasing the potential food supply. Other investigators hypothesize that changes in nutrient ratios and forms of N are exerting additional bottom-up pressures on Delta and Suisun food webs by altering the phytoplankton community composition and the N:P composition of individual cells (e.g., Glibert et al., 2011; Glibert et al., 2012). In addition, a recent study reported evidence that NH_4^+ , at concentrations observed in some areas of the Delta and Sacramento River, can exert chronic toxicity on a copepod species (*Pseudodiaptomus forbesi*) that is an important food resource (Teh et al., 2011).

1.2. Goals and Report Structure

The purpose of this report is to provide an overview of the state of the science and identify science gaps related to a subset of the hypothesized adverse impacts of NH_4^+ in Suisun Bay, and characterize NH_4^+ loads, concentrations, and fate. The report is organized around its six specific goals:

1. Synthesize the scientific literature on nitrogen utilization by marine and estuarine phytoplankton, with a particular focus on factors and mechanisms that regulate the N form utilized by phytoplankton, and the effect of different N sources on primary production rates. (Section 2)
2. Through the perspective of the broader scientific literature, evaluate the results and interpretations of recent studies that hypothesize that elevated NH_4^+ levels inhibit primary production rates. (Section 3)
3. Summarize the scientific literature related to NH_4^+ toxicity to copepods. (Section 4)
4. Synthesize the scientific literature on copepod ecology and changes in community composition and abundance in Suisun Bay (Section 5)

5. Quantify NH_4^+ loads to Suisun Bay, evaluate long-term changes and seasonal variations in ambient NH_4^+ concentrations, and characterize NH_4^+ fate. (Section 6)
6. Summarize key observations and identify next steps. (Section 7)

Although additional pathways of nutrient-related impairment have been proposed in Suisun Bay and the Delta, this report is narrowly focused on the above goals. The report was developed under the assumption that it would be used in conjunction with complementary reports (including reports already developed, e.g., Baxter et al., 2010; Meyer et al, 2009) that address other factors affecting ecosystem health in Suisun Bay and the Delta to help identify the outstanding science questions whose answers will informed management decisions. For additional background and context on nutrient related issues in San Francisco Bay, the reader is referred a recent nutrient conceptual model report (Senn et al. 2014).

1.3. References

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2. Literature review on factors influencing phytoplankton nutrient uptake and metabolism: molecular and phytoplankton ecology perspectives

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2.1. Introduction

The goal of this section is to describe the principal pathways for photosynthesis, carbon fixation, and transport and assimilation of nitrogen (N) inherent in phytoplankton. Because these pathways are complex and contain numerous enzymes only the key enzyme(s) in each pathway will be described. Investigations at the molecular and physiological levels characterizing the presence and activity of these enzymes (and mechanisms) are prioritized in this review. Emphasis is placed on recent information with respect to N transport and assimilation including 1) the types of enzymes and pathways available in marine phytoplankton and 2) investigations of their regulation based on gene expression. Historical data acquired using ^{15}N tracer experiments in mixed phytoplankton communities, and their interpretations, are discussed in relation to newer information acquired from molecular studies.

As noted in Section 1, the role of nutrients in shaping phytoplankton composition and succession will be the focus of another report, and will not be discussed here.

2.2. Photosynthesis and Carbon Fixation

Phytoplankton grow by turning CO_2 into carbohydrates that are subsequently used to create biomass and power the cell. The cost of fixing carbon (C) in this manner is high and it is made possible by using energy from the sun to extract electrons from water and produce ATP, the principal currency of energy used in cells. Both ATP and electrons (via NADPH) are needed to drive C-fixation. The process providing ATP and electrons for C-fixation is called oxygenic photosynthesis.

2.2.1. Oxygenic Photosynthesis

Photosynthesis proceeds in the same manner in terrestrial plants and aquatic algae because the components of the photosynthetic apparatus are very well conserved (Appendix A.2.1). Photosynthesis starts with the absorption of light by antenna pigments (chlorophyll and other pigments) that pass the energy on to a specialized chlorophyll *a* (Chl *a*) pigment molecule (Govindjee et al. 2010). This leads to the excitation of an electron in the Chl *a* molecule which is captured by an electron acceptor before being passed down a chain of acceptors embedded in the thylakoid membrane. As the electron is passed down the chain, a proton gradient is established that powers ATP synthesis. ATP is produced in a continuous manner as long as the proton gradient is maintained across the membrane (Govindjee et al. 2010). By far the largest sink for electrons and ATP produced in the light reactions is C-fixation, followed by nitrogen (N) assimilation.

The extent of photosynthesis performed by the cell can be adjusted in two ways; either by varying the amount of light energy that reaches Chl *a*, or by varying the Chl *a* pigment content of the cell (Ballottari et al. 2012). The former is used as a safety valve to prevent the photosynthetic apparatus from becoming damaged under sudden and large increases in light intensity, whereas the latter is used to acclimate to longer-term changes in irradiance. For example under persistent

high light, a cell will acclimate by shedding Chl *a* in order to decrease its antenna size and avoid photoinhibition, the loss of photosynthetic function due to damage in excess of cell's capacity of repair (Falkowski and LaRoche 1991, Falkowski et al. 1985). Adjustment of both antenna size and transfer of electrons occurs constantly in phytoplankton exposed to varying conditions (e.g. as a function of mixing, cloud cover, etc.) but phytoplankton are generally optimizing for the light environment experienced over the previous 24 hours.

2.2.2. Carbon Fixation

Carbon fixation is controlled by light because the energy needed to power the enzymes that convert CO₂ to carbohydrates comes entirely from photosynthesis. Therefore, changes in light level produce instant changes in the rate of C-fixation and growth (Fig. 2.1). The amount of light available to phytoplankton frequently limits their productivity. However, when irradiance is not at a level where it limits C-fixation, the rate-limiting factor is the Rubisco enzyme that catalyzes the first step in the C-fixation pathway known as the Calvin Cycle (See Appendix A.2.2 for more details on Rubisco and other C fixing enzymes). This is because Rubisco is notoriously slow and catalyzes 3 molecules per second compared with 1000 molecules per second for a typical reaction. To make up for this, photosynthetic cells produce large quantities of the enzyme. The amount of Rubisco produced per cell varies substantially depending on taxon. For example, Rubisco expression on a per cell basis is one to four orders of magnitude greater in heterokont algae, primarily diatoms, compared with cyanobacteria (Paul et al. 1999, John et al. 2007a). Within cyanobacteria, the *Synechococcus* clade exhibits greater expression of Rubisco compared to the *Prochlorococcus* clade at similar light intensities. In phytoplankton, Rubisco expression is directly proportional to C-fixation (Warwick et al. 2002, 2003, 2004, Corredor et al. 2004, Berg et al. 2011), and several-fold variation in Rubisco expression among phytoplankton taxa translates into several-fold variation in their intrinsic maximum growth rates, independent of light levels or nutrient abundance.

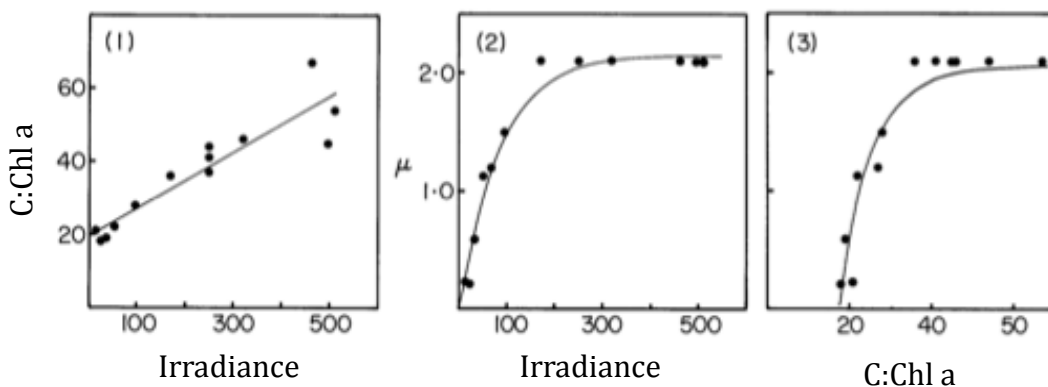


Figure 2.1. Reproduced from Geider 1987; Light dependence of C:Chl *a* for the diatom *Thalassiosira pseudonana*. (1) C:Chl *a* versus Irradiance ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and (2) is growth rate (d^{-1}) versus irradiance and (3) is growth rate versus C:Chl *a*.

2.2.3. Carbon:Chl *a* ratio

Because phytoplankton change the amount of Chl *a* they contain in response to light levels, the ratio of C to Chl *a* varies vary inversely with light intensity. As irradiance increases, C-fixation will increase but Chl *a* per cell will decrease. The carbon:chlorophyll *a* ratio (C:Chl *a*) of the cell also changes as a function of nutrient concentration and temperature (Geider 1987, LaRoche et al. 1993, Graziano et al. 1996). From a series of experiments with phytoplankton across a number of taxa, Geider (1987) generalized that C:Chl *a* tends to increase linearly with increased light level at constant temperature and decreases exponentially with increased temperature (and growth rate) at constant light level (Fig. 2.1). Despite its variability, the C:Chl *a* ratio is frequently used to infer phytoplankton C biomass from field Chl *a* measurements (Cloern et al. 1995, Behrenfeld and Falkowski 1997, Geider et al. 1998, Behrenfeld et al. 2002, 2005). This inference assumes a constant C:Chl *a* and therefore has the potential to be highly uncertain (Mateus et al 2012; Kimmerer et al. 2012).

2.3. Nitrogen assimilation

C and N assimilation are tightly linked because they share the flow of energy from light, and because fixed C provides skeletons for N assimilation. Additional energy for N reduction is supplied from respiration of fixed C (Fig. 2.2, Appendix A.2.3). Marine and estuarine phytoplankton use numerous sources of reduced and oxidized N for growth (Antia and Landymore 1974, Antia et al. 1975, Antia et al. 1991). With the exception of NH_4^+ , each of these N sources must first be reduced (as in the case with NO_3^-), or deaminated, to NH_4^+ before they can be assimilated into amino acids and protein. The discussion below addresses NO_3^- reduction and urea hydrolysis pathways before moving onto NH_4^+ assimilation and amino acid biosynthesis. Numerous other pathways exist for assimilation of alternative N substrates in marine phytoplankton that will not be discussed here (Antia et al. 1991, Berg et al. 2008).

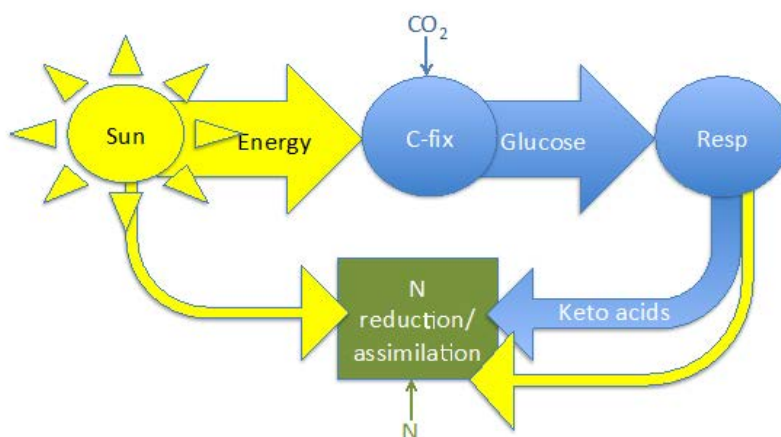


Figure 2.2. Flow of energy in the form of reductant and ATP (yellow arrows) and carbon (blue arrows) to N assimilation. C-fix=carbon fixation, Resp=respiration

2.3.1. Molecular perspective on nitrogen transport and assimilation

In order for phytoplankton to use N they have to transport it into the cell. By examining the activity of the various transporters phytoplankton possess, it is possible to characterize their propensity to use different forms of N and to obtain clues about the N sources that phytoplankton are using at any given time. Transport activity is regulated at two levels: the first is at the level of the gene and the second at the level of the protein. When genes are “turned on” their information is transcribed into mRNA (Fig. 2.3). The greater the mRNA abundance of a specific gene, the more it is being turned on, or “expressed”. The second level of regulation is at the point of protein synthesis, where the mRNA is translated into protein (Fig. 2.3). For transport proteins that turn over quickly, the level of mRNA corresponds fairly well with the amount of protein synthesized (Yoshimoto et al. 2007). However, for proteins with low turnover, such as the nitrate reductase enzyme (NR) which is typically synthesized once during a cell’s lifetime, the level of mRNA typically does not correspond with the level of enzyme. This is because the mRNA may code for various subunits of the enzyme that are translated but not assembled. The point in time when the protein subunits are assembled into a functional enzyme whose activity is measurable lags the time when mRNA was transcribed (Poulson and Kroger 2005). For the remainder of this discussion, the word “gene expression” or “expression” will be associated with mRNA abundance/transcript abundance and the word “activity” with protein abundance. Also, the name of a gene will be italicized whereas the name of the corresponding protein will not, i.e. the *NR* gene will be distinguished from the NR enzyme

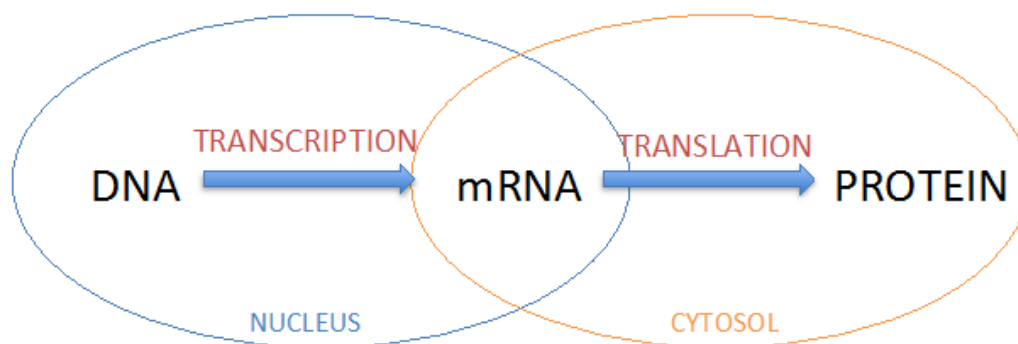


Figure 2.3. Information contained in an organism’s genome, housed in the nucleus, is transcribed into mRNA before it’s translated into protein in the cytosol. The amount of mRNA corresponds with the number of times a gene has been transcribed and is often called “gene expression” or just “expression”. Gene expression may or may not correlate with protein abundance depending on the turnover of the particular protein.

2.3.1.1. NO_3^- transport and reduction

Nitrate is actively transported into marine phytoplankton via the high-affinity transporter Nrt2 of the major facilitator superfamily (Navarro et al. 1996, Hildebrand and Dahlin 2000, Galvan and

Fernandez 2001, He et al. 2004) at N concentrations found in the marine environments. At concentrations above $\cong 60 \mu\text{moles NO}_3^- \text{ L}^{-1}$, low-affinity transporters of the *Nrt1* type may also be induced (Galvan and Fernandez 2001, Collos et al. 2005). Recent investigations demonstrate that marine and estuarine phytoplankton vary greatly in the number of *Nrt2* genes in their genomes. Some, like the diatom *Thalassiosira weissflogii* contain six nearly identical copies while others like the harmful alga *Aureococcus anophagefferens* contain only one copy (Song and Ward 2007, Berg et al. 2008). Additional copies may speed the rate at which cells can produce transcripts, potentially allowing them to take better advantage of NO_3^- (Hildebrand and Dahlin 2000). The *Nrt2* genes are transcribed in response to NO_3^- and N starvation, and inhibited in response to NH_4^+ (Navarro et al. 1996, Hildebrand and Dahlin 2000, Galvan and Fernandez 2001, He et al. 2004). In diatoms, irradiance does not appear to play a role in *Nrt2* transcription (Hildebrand and Dahlin 2000).

Following uptake into the cell, NO_3^- is reduced to NH_4^+ in a two-step process (Appendix A.2.3) via the enzymes nitrate reductase (NR) and nitrite reductase (NiR) requiring eight electrons (Huppe and Turpin 1994). Light plays a key role in the supply of electrons and in the daily regulation of NR synthesis and degradation (Huppe and Turpin 1994, Berges et al. 1995, 1997).

The combined requirement of eight electrons to reduce NO_3^- to NH_4^+ before it can be assimilated has been used to argue that NH_4^+ is preferred to NO_3^- as a N substrate for growth (Syrett 1981, Fernandez and Cardenas 1989, Huppe et al. 1994). Evidence for NH_4^+ preference over NO_3^- comes from culture investigations that demonstrate addition of NH_4^+ to cultures growing on NO_3^- can rapidly inhibit NO_3^- uptake and assimilation while concurrently stimulate uptake and assimilation of NH_4^+ (Creswell and Syrett 1979, Syrett 1988). This has subsequently been demonstrated at a molecular level where NH_4^+ represses NR activity (Berges et al. 1995, Berges 1997, Song and Ward 2004) and represses transcription of the *Nrt2* NO_3^- transporter gene (Navarro et al. 1996, Hildebrand and Dahlin 2000, Koltermann et al. 2003, He et al. 2004, Song and Ward 2007, Berg et al. 2008) for as long as NH_4^+ is available to satisfy the N growth requirement of the cell.

2.3.1.2. Urea transport and hydrolysis

Aside from NH_4^+ and NO_3^- , urea has been identified as an important source of N for growth of marine and estuarine phytoplankton (McCarthy 1972, Antia et al. 1977, Antia et al. 1991, Berman and Bronk 2003). Urea's availability as a N substrate for phytoplankton has received increasing attention recently because of higher urea levels observed in coastal systems due to agricultural runoff (Glibert et al. 2006), and because elevated urea may favor blooms of certain harmful algal bloom (HAB) species (Kristiansen 1983, Berg et al. 1997, Glibert and Terlizzi 1999, Kudela and Cochlan 2000, Solomon et al. 2010). The urea uptake and hydrolysis pathway is principally comprised of two proteins; the high-affinity urea transporter DUR3 (Liu et al. 2003, Wang et al. 2008) and the urease enzyme (URE) required to decompose urea ($\text{CO}(\text{NH}_2)_2$) to NH_4^+ and CO_2 (Solomon et al. 2010). There is also evidence that some marine phytoplankton

possess a low-affinity urea transporter that may be induced under very high concentrations of urea (Solomon et al. 2010).

2.3.1.3. NH_4^+ transport and assimilation

2.3.1.3.1 NH_4^+ transport: High affinity transport of NH_4^+ into plant cells occurs via the AMT1 transporter family (Loque et al. 2007, 2009). These transporters have a high affinity for NH_4^+ , low transport capacity, and have mechanisms for rapid-shut off to prevent NH_4^+ toxicity (Loque et al. 2007). In contrast with the high affinity NO_3^- transporter *Nrt2* which occurs in near-identical copies in phytoplankton, copies of AMT1 diverge substantially in their sequences and therefore functionality (Hildebrand 2005, Gonzalez-Ballester et al. 2004). Of the eight or so copies of the *AMT1* genes characterized to date, some are expressed preferentially during N starvation, some are depressed in the presence of NH_4^+ and NO_3^- , and some are expressed constitutively regardless of N sufficiency or source (Gonzalez-Ballester et al. 2004, Hildebrand 2005, Berg et al. 2008). In addition to the AMT1 transporters, low affinity transporters, passive ion channels and aquaporins also play important roles in the transport of NH_4^+ (Ullrich et al. 1984, Franco et al. 1988, Wang et al. 1993, Crawford and Forde 2002). Not all marine phytoplankton possess eight *AMT1* genes; as with *Nrt2*, the number of gene copies varies widely with taxon (Hildebrand 2005). Compared with the *Nrt2* genes, comparative expression of *AMT1* genes among different phytoplankton taxa has not been investigated to date.

2.3.1.3.2 NH_4^+ toxicity: Transport of NH_4^+ into the cell is shut down to prevent toxicity at a concentration ranging from micromolar to low millimolar NH_4^+ L^{-1} depending on the species (Loque et al. 2006, 2007, Yuan et al. 2007). Despite the built-in shut-down mechanism of the AMT1 transporter, it's been demonstrated that influx of NH_4^+ can occur above the millimolar L^{-1} range, mediated by potassium (K^+) channels, in vascular plants (Britto et al. 2001, 2002). This influx at high external NH_4^+ concentrations is accompanied by efflux pumping of NH_4^+ in equal magnitude, resulting in “futile cycling” of NH_4^+ ions across the plasma membrane. This futile cycling has been shown to be energetically costly, slowing down cell growth, and playing an important part in the toxicity of NH_4^+ to terrestrial plant cells (Britto et al. 2001, Szczerba et al. 2008). Whether the same mechanism operates for aquatic photosynthetic organisms is unknown since no studies have been undertaken to characterize it. However, it is known that NH_4^+ toxicity does occur, and the concentrations at which it is exerted can vary by orders of magnitude depending on the species of aquatic plants or unicellular algae. Freshwater unicellular algae such as the chlorophyte *Chlorella vulgaris* isolated from wastewater settling ponds can tolerate NH_4^+ concentrations up to 3 mmol L^{-1} without exhibiting signs of toxicity or slowed growth (Berg et al. unpublished data, Perez-Garcia et al. 2011). Diatoms, including marine and freshwater species, also tolerate NH_4^+ concentrations in the mmol L^{-1} range (Antia et al. 1975, Lomas 2004, Hildebrand 2005, Pahl et al. 2012). In contrast, phytoplankton species with high affinities for NH_4^+ , primarily harmful algal bloom forming phytoplankton, can be susceptible to toxicity and slowed growth at NH_4^+ concentrations as low as 160-200 $\mu\text{mol L}^{-1}$ (Bates et al. 1993, Hillebrand and Sommer 1996, Berg et al. 2008).

2.3.1.3.3 NH_4^+ assimilation and amino acid synthesis: NH_4^+ is the only form of N that can be directly attached to C skeletons to produce amino acids. Other forms of N must first either be reduced or deaminated to NH_4^+ requiring energy (i.e. reductant). Following reduction or deamination, assimilation of NH_4^+ -N requires input of both energy, generated from the photosynthetic electron transport chain and from respiration of photosynthetically produced C, and C skeletons from the tricarboxylic acid (TCA) cycle (See Appendix A.2.2 for details; Syrett 1953, 1981, Elrifi et al. 1988, Guy et al. 1989). Copies of the enzymes required to assimilate NH_4^+ into amino acids are localized to the chloroplast, where NO_3^- reduced to NH_4^+ is assimilated, and to the cytosol where NH_4^+ produced by cellular process and direct NH_4^+ uptake is assimilated (Appendix A.2.3, Huppe and Turpin 1994, Mock et al. 2008, Brown et al. 2009, Hockin et al. 2012).

2.3.1.4. Expression of N transporter genes across phytoplankton taxa

In a comparison of NO_3^- transporter (*Nrt2*) expression across several phytoplankton taxa (two species of diatoms, one haptophyte and a chlorophyte) Song and Ward (2007) made two key discoveries. One was that the diatom species had 5-10 fold higher expression of *Nrt2* compared with the haptophyte and chlorophyte when grown on NO_3^- in the presence of NH_4^+ . In other words, NH_4^+ did not shut down NO_3^- transport completely in the diatoms as it did in the other algae. Second, they discovered that the diatoms, especially of the genus *Chaetoceros*, had much greater expression level of the *Nrt2* transporter under N starvation than did the non-diatom taxa. In contrast, the chlorophyte ceased *Nrt2* expression under N starvation. The significance of this finding is that once NO_3^- is re-supplied after a period of starvation, transport into the cell can proceed rapidly. In cells where expression is low or non-existent under starvation, up-regulation of gene expression, followed by protein synthesis, must take place before the transporters are translocated to the plasma membrane and uptake can commence. Therefore, high levels of transporter expression under starvation may be critical for competition for N under limiting conditions (Poulsen and Kroger 2005).

Just as expression of NO_3^- transport genes varies with phytoplankton taxa, so does expression of genes specific to other transport proteins. A surprising finding in the HAB pelagophyte *Aureococcus anophagefferens* is that the most highly expressed transporter gene in its genome is specific to purines, most likely guanine (Fig. 2.4). In this organism, growth on organic N suppresses expression of genes encoding transporters for inorganic N sources almost completely. In contrast, expression of genes encoding proteins associated with uptake of organic N sources is relatively high when grown on NO_3^- or NH_4^+ as the sole source of N. This pattern of expression suggests that this organism may change its strategy from using several sources of N at once in the presence of high external inorganic N concentrations, to concentrating its efforts on one N source at a time in the presence of high external organic N concentrations. This also highlights the risk of extrapolating from a few model organisms to the vast diversity of phytoplankton species, given the small number of organisms that have been studied in detail.

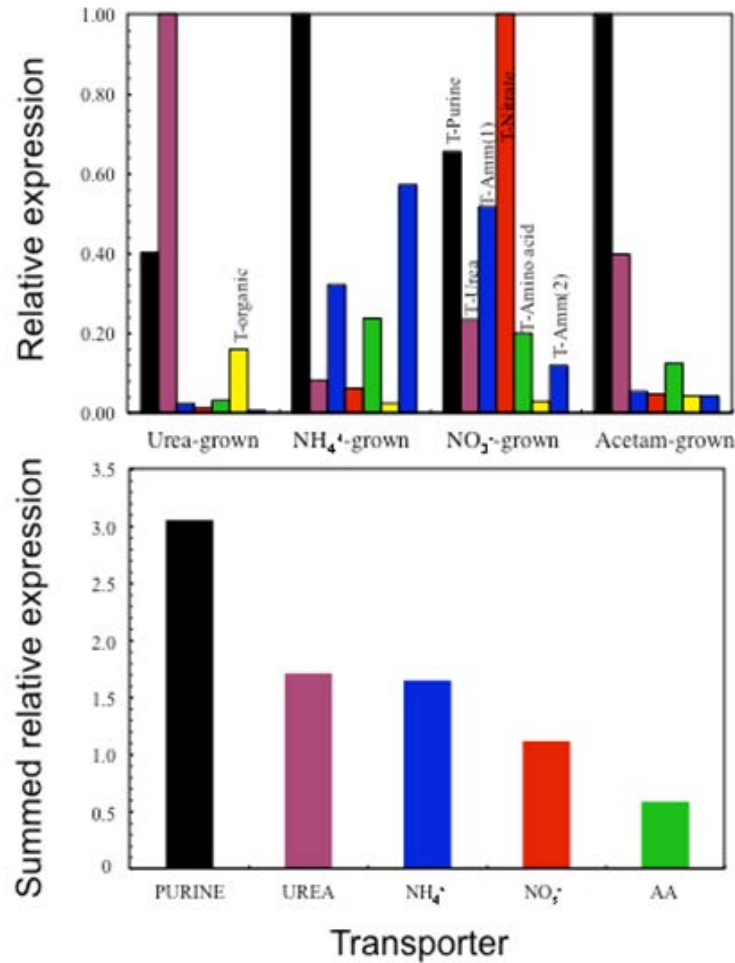


Figure 2.4. Top panel: relative expression (normalized to the most expressed transporter at each growth source) of N transporters in *A. anophagefferens* cultures grown on urea, NH₄⁺, NO₃⁻, and acetamide. Black bars represent mRNA abundance of the purine transporter URA; pink bars represent the urea transporter *DUR3*, blue bars represent NH₄⁺ transporters *AMT1* and *ABC*; red bars represent the NO₃⁻ transporter *Nrt2*, green bars represent a putative amino acid transporter AA, and yellow bars represent a putative DON transporter *NAR1.3*. Bottom panel: Summed relative expression for URA, *DUR3*, *AMT1&ABC*, and *Nrt2* across the four N growth sources in the top panel. Figure adapted from data in Berg et al. 2008.

In contrast with the eukaryotic phytoplankton discussed above, cyanobacteria appear to be NH₄⁺ specialists. For one, expression of the *AMT1* NH₄⁺ transporter gene in cyanobacteria is not regulated, meaning the transporter is always expressed regardless of the N status of the cell (Lindell and Post 2001, Lindell et al. 2005). In addition, it is one of the most highly expressed genes in cyanobacterial genomes (Berg et al. 2011, Berg et al. unpublished). In the marine cyanobacteria *Synechococcus* and *Prochlorococcus*, *AMT1* is expressed on par with, or at a greater level, respectively, than the gene encoding the C-fixation enzyme Rubisco (Berg et al. 2011). Considering the countless other critical processes happening within cells, it is noteworthy

that the protein responsible for NH_4^+ uptake is one of the most abundant proteins in cyanobacteria.

Although phytoplankton share the same genes encoding transport proteins for NH_4^+ , NO_3^- and urea, the expression of these genes can vary dramatically from organism to organism. This suggests that the surface area of a cell contains a mosaic of transporter proteins that is 1) unique to each cell and 2) is continuously changing in response to external nutrient concentrations.

2.3.2. Unialgal perspective on N source, irradiance, and temperature on growth in culture

2.3.2.1. Effect of assimilation of NH_4^+ versus NO_3^- on growth

Given that NO_3^- requires eight times the reductant compared with NH_4^+ to assimilate, one might expect that assimilating NO_3^- will lead to lower rates of phytoplankton growth. However, culture investigations clearly demonstrate that phytoplankton acclimated to growth on either NH_4^+ or NO_3^- have very similar or equivalent rates of growth (Fig. 2.5). Why does C fixation not appear to be affected by N source when NO_3^- requires more reductant to assimilate compared with NH_4^+ , or any other source of reduced N? The reason is that the reductant and energy demands of N assimilation, including assimilation of NO_3^- , are small in comparison to that of C metabolism and therefore growth is typically not affected by the source of N used by phytoplankton (Turpin 1991). Quite contrary, assimilation of N must keep pace with C fixation and growth. If a cell is assimilating only one source of N to satisfy its cellular N demand, the cell will tend to scale its uptake rate according to growth rate (once the uptake and assimilation pathways for the that particular N source are in place). Therefore, the rate of uptake of NO_3^- and NH_4^+ will be the same in two different cultures grown on NO_3^- and NH_4^+ , respectively, as the sole source of N under constant irradiance (Fig. 2.5).

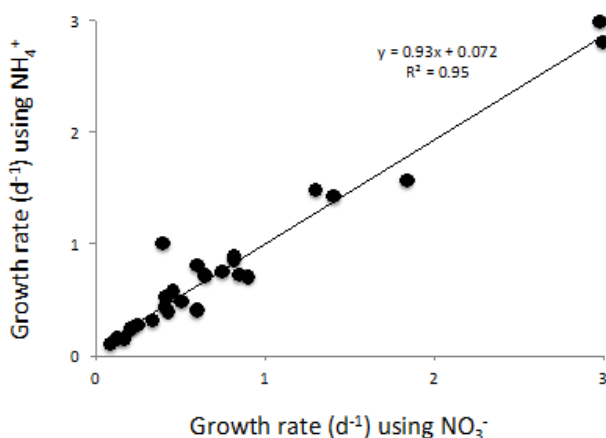


Figure 2.5. Growth rates of phytoplankton using NH_4^+ plotted as a function of growth rates using NO_3^- as the sole source of nitrogen. Data compiled from Ferguson et al. 1976, Dortch and Conway 1984, Levasseur et al. 1993, Berman and Chava 1999, Herndon and Cochlan 2007, Berg et al. 2008, Solomon and Glibert 2008, Sinclair et al. 2009, Strom and Bright 2009, Thessen et al. 2009, Solomon et al. 2010.

2.3.2.2. Effect of irradiance and N source on growth.

At non-limiting light intensities, it may be reasonable to expect no difference in growth rates with N source (NH_4^+ versus NO_3^-) as described above. Does this picture change as light intensities are decreased to the point where they may be limiting to growth? Examining a sub-set of the data used in Fig. 2.5, where the same cultures were grown at limiting ($7 \mu\text{mol m}^{-2} \text{s}^{-1}$) and non-limiting ($170 \mu\text{mol m}^{-2} \text{s}^{-1}$) light intensities, it is clear that even at the lowest light intensity there is a minimal effect of using NO_3^- versus NH_4^+ on the growth rate (Fig. 2.6a). One explanation for this may be that C metabolism and N metabolism scale to growth rate. Under this scenario, growth rate is lower at low light than at high light but the factor difference in the reductant need for C versus N metabolism remains the same, and just as large. Just how important is irradiance for growth? Plotting the data in Fig. 2.5 as a function of irradiance, we observe that below $200 \mu\text{moles photons m}^{-2} \text{s}^{-1}$ there is a 0.6 d^{-1} increase in growth rate with every $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ increase in irradiance (Fig. 2.6b). Above 200, this relationship breaks down as a consequence of photoinhibition (Fig. 2.6b). Given that changes in irradiance results in a doubling or more of growth rates over the irradiance range examined here, it is clear that irradiance exerts a far more important impact on the rate of growth than does N source (Fig 2.6a,b).

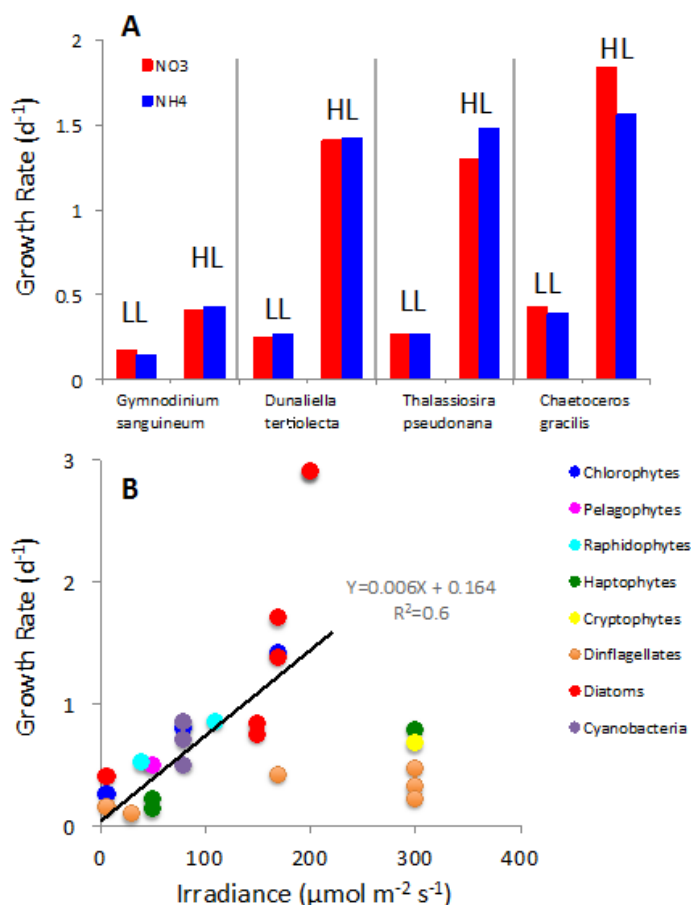


Figure 2.6. Growth rates of A) the dinoflagellate *Gymnodinium*, the chlorophyte *Dunaliella*, and the diatoms *Thalassiosira* and *Chaetoceros* using ammonium (blue bar) or nitrate (red bar) as the sole source of nitrogen at low light (LL; 7 μmol m⁻²s⁻¹) and high light (HL; 170 μmol m⁻²s⁻¹). Data from Levasseur et al. (1993) and B) Growth rates as a function of irradiance. Diatom outlier not included in regression line. Data from same sources as in Figure 2.5.

2.3.2.2. What matters most for achieving high growth rates?

Surprisingly, the answer appears to be nothing more than “being a diatom”. Recalling that Rubisco activity is the rate-limiting step for C fixation (section 2.2.2.1), it follows that phytoplankton with a high Rubisco expression level, and therefore enzyme production, will have the greatest rates of C fixation and growth. It turns out that among marine phytoplankton, diatoms exhibit the highest and *Prochlorococcus* the lowest, levels of Rubisco expression (Fig. 2.7a). Moreover, expression of diatom-specific Rubisco across a variety of field studies is significantly correlated with rates of C fixation (Fig. 2.7b). These recent molecular data indicate that diatoms have evolved the ability to express and produce Rubisco at very high levels compared with other phytoplankton taxa and suggest that any community dominated by diatoms will have higher rates of C fixation and growth compared with a community that is not dominated by diatoms. This is corroborated by unialgal culture investigations where growth rates achieved with diatoms are significantly greater than those achieved for other taxa (Fig. 2.8). Both across and within the eight major phytoplankton taxa shown in Fig. 2.8, the growth rates on NO₃⁻

and NH_4^+ were remarkably similar. The largest difference in growth rate was between species (and taxa), and not between N growth substrate.

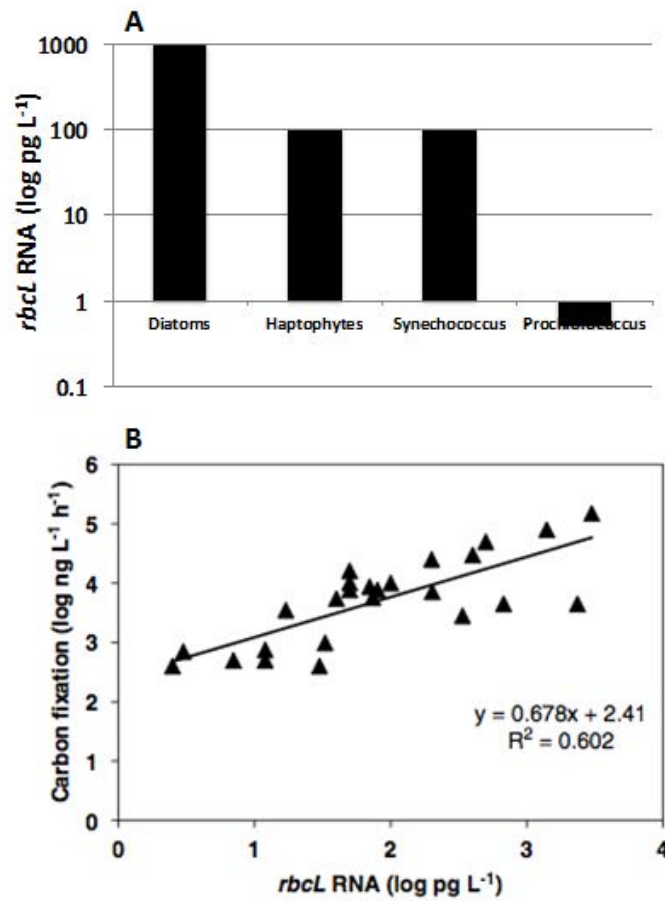


Figure 2.7. A) Rubisco expression as a function of phytoplankton taxa and B) carbon fixation as a function of diatom-specific Rubisco expression. Data are from John et al. 2007b.

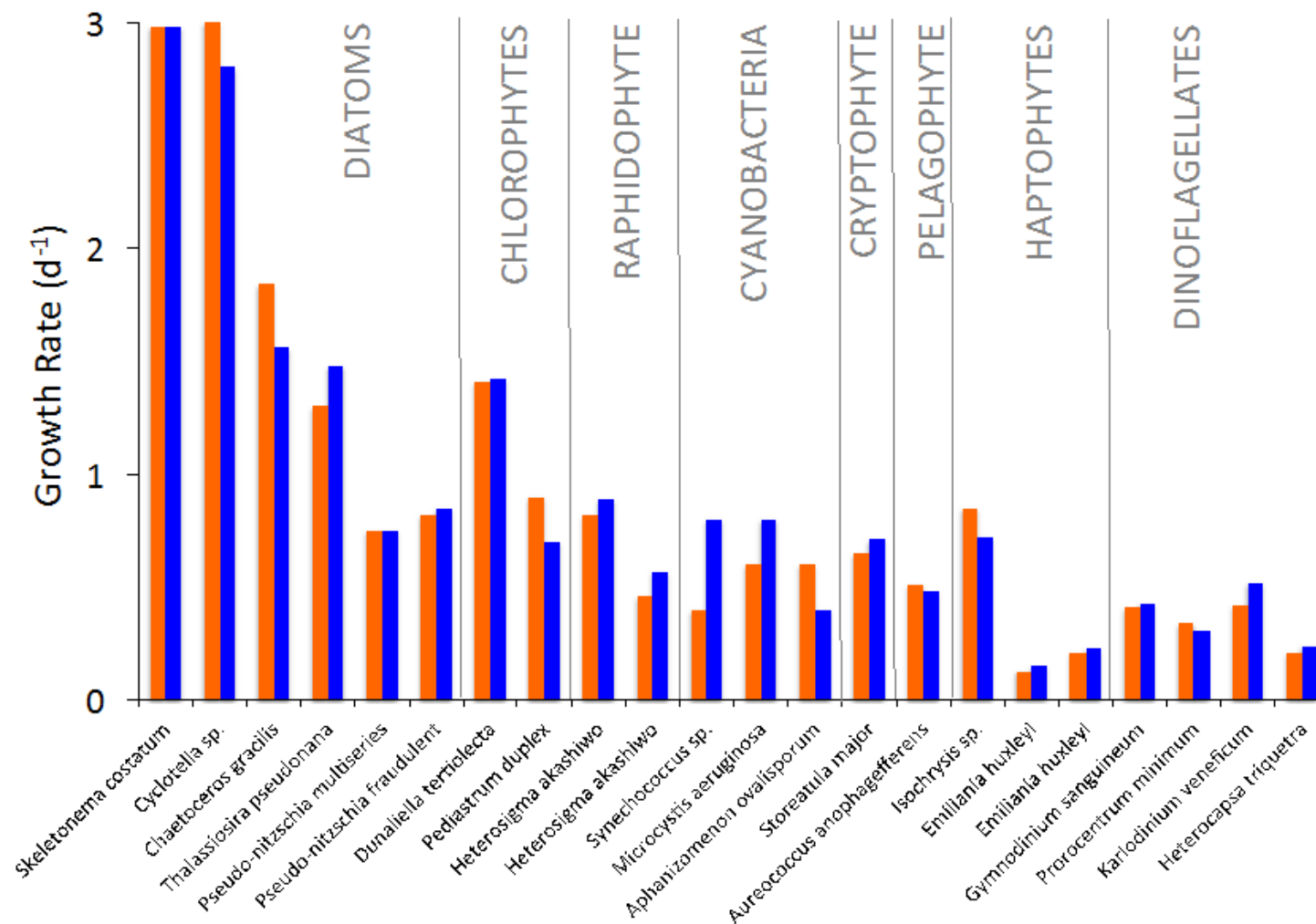


Figure 2.8. Growth rates of 8 major phytoplankton taxa. Red bars are cultures grown on nitrate and blue bars cultures grown on ammonium. Data sources the same as for Figure 2.5.

2.3.3. Phytoplankton N uptake under natural conditions: introducing choice into the equation

The results from the culture experiments discussed above reflect phytoplankton grown on a *single source of N* under N sufficient conditions. How does phytoplankton growth change when multiple sources of N are available to the phytoplankton simultaneously (i.e. they are given a choice of N substrate)? In addition, culture experiments consider growth of a *single species*. How does our picture change if several species are competing for the same, potentially limiting substrate? These questions are explored below, starting with how external nutrient concentrations relate to the rate of uptake of the nutrients into the cell. This relationship forms the basis for determining competitive interactions among phytoplankton under varying nutrient conditions, and is used to model population dynamics.

2.3.3.1. N uptake kinetics

The relationship between external nutrient concentration and phytoplankton nutrient uptake is often described by the Michaelis-Menten equation which takes the shape of a hyperbola (Fig. 2.9):

$$V=V_{\max} [S/(K_s+S)]$$

where V is the uptake velocity, V_{\max} is the maximum uptake velocity, S is the substrate concentration (i.e. concentration of N), and K_s is the half-saturation constant (see also Figure 3.1). The relationship between external nutrient concentration and phytoplankton growth is described by Monod equation (Monod 1942) which is identical in form to the Michaelis-Menten equation that describes the relationship between uptake and nutrient concentration:

$$\mu=\mu_{\max} [S/(K_{\mu}+S)]$$

where μ is the growth rate (d^{-1}), μ_{\max} is the maximum growth rate, S is the substrate concentration, and K_{μ} is the half-saturation constant. The affinity for a particular substrate typically refers to the slope of the hyperbola at low concentrations. When competing for a limiting resource, having a low K_s and V_{\max} (or K_{μ} and μ_{\max}) is considered advantageous (Eppley et al. 1969, Eppley and Renger 1974). Early investigators assumed that uptake was balanced with growth, therefore K_s could be used interchangeably with K_{μ} ; Eppley (1969) described a hybrid model where he employed K_s determined from nutrient uptake experiments with μ and μ_{\max} determined separately from growth experiments.

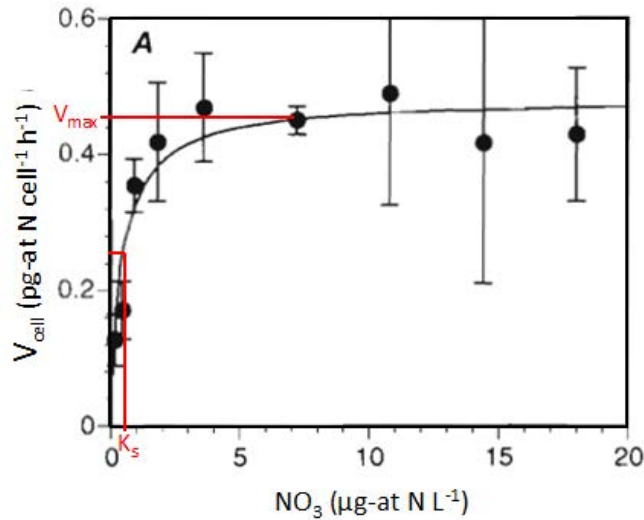


Figure 2.9. Uptake of NO_3^- (on a per cell basis) as a function of NO_3^- concentration. Figure from Kudela and Cochlan 2000.

However, Eppley and coworkers quickly determined that predictions of phytoplankton growth made based on kinetics of nutrient uptake overestimated growth 5-10 fold (Eppley et al. 1969, Eppley and Sournia 1971, Eppley and Renger 1974). The main reason for this is that K_s typically reflects the type of transporter used and external nutrient concentrations (Collos et al. 2005). Over a narrow concentration range, transport/uptake of a nutrient may follow Michaelis-Menten saturable kinetics (or other kinetic models), whereas over broader concentration ranges transport often cannot be fit to a single kinetic model due to induction of different transporters with increasing concentration. As a result, biphasic kinetics may occur. Additionally, constitutive expression of a transporter, as in the case of one or more of the AMT1 transporters, precludes uptake of NH_4^+ from fitting a kinetic model. For a more detailed discussion of uptake kinetics and various transporters employed in the uptake of N, P and DSi, see Appendix A.2.3. Key to note is that measurements of uptake kinetics on the order of minutes that produce non-saturable kinetics will produce saturable kinetics in the same cell after longer periods of time (on the order of hours). The transition between non-saturable and saturable kinetics with time is mediated by feedback between intracellular processes and transport into the cell (Thamatrakoln and Hildebrand 2008). These intracellular processes drive growth and reflect the length of daylight hours, irradiance, and temperature (Eppley and Sloan 1966, Eppley et al. 1971, 1972). Therefore, although short-term uptake kinetics may not always follow a simple hyperbola, growth kinetics frequently do (Thamatrakoln and Hildebrand 2008, Laws et al. 2011, 2013)

2.3.3.2. ^{15}N tracer technique and hierarchy of nitrogen uptake

Following up on the work by Eppley and coworkers, investigators used the ^{15}N tracer technique to determine kinetic parameters of NH_4^+ and NO_3^- utilization in natural, mixed phytoplankton communities (McCarthy et al. 1972, McCarthy and Goldman 1979, Wheeler et al. 1982).

Investigators soon noted that the rate of uptake of NH_4^+ was large, even at low ambient concentrations of NH_4^+ , the opposite of what would be expected based on the Michaelis-Menten relationship. Because phytoplankton maintained maximal uptake rates of NH_4^+ uptake at concentrations that were at the limit of detection, it was impossible to accurately determine the K_s and V_{\max} for NH_4^+ uptake (McCarthy and Goldman 1979).

In addition to finding high rates of NH_4^+ uptake at low concentrations, McCarthy et al. (1975, 1977) observed that NH_4^+ concentrations in excess of $0.5\text{--}2\ \mu\text{moles L}^{-1}$ almost completely suppressed NO_3^- uptake in natural mixed phytoplankton communities (Fig. 2.10, McCarthy et al. 1975, 1977), corroborating earlier culture investigations (section 2.3.2). In other words, in both culture and field investigations, phytoplankton exhibited a hierarchy of N uptake and assimilation within which NH_4^+ was preferred as a N source before NO_3^- and urea: when NH_4^+ was available, the uptake and assimilation of other N-forms were suppressed and only NH_4^+ was taken up.

Although these initial field measurements found that NH_4^+ in excess of $0.5\text{--}2\ \mu\text{moles L}^{-1}$ suppressed the uptake of other N forms, later field investigations found varying degrees of suppression of uptake of NO_3^- by NH_4^+ , and also by urea, potentially reflecting the diversity of mechanisms in individual phytoplankton cells (Dortch 1990).

The fact that NH_4^+ and urea were preferentially taken up relative to NO_3^- in natural, mixed populations led to efforts to characterize 1) the diversity of N uptake hierarchy among phytoplankton species; and 2) the time it took for phytoplankton to switch sources (from NO_3^- to NH_4^+). Whereas some studies indicated that, after NH_4^+ , urea was preferred over NO_3^- , others found that NO_3^- was preferred over urea (Williams and Hodson 1977, Horrigan and McCarthy 1982, Lund 1987). Still others demonstrated that not only did NH_4^+ suppress NO_3^- uptake, but NO_3^- could also to a lesser extent suppress NH_4^+ uptake and sometimes urea uptake (Dortch and Conway 1984, Lund 1987, Dortch 1990). The time it took for suppression to become evident ranged from immediately to half an hour (Williams and Hodson 1977, Horrigan and McCarthy 1982, Lund 1987). Whereas most of these culture investigations focused on one N source at a time, Lund (1987) investigated the uptake of N when the diatom *Skeletonema costatum* was presented with multiple sources of N simultaneously. In this case, the degree of suppression varied as a function of both the number of N sources available at one time and whether they were reduced or oxidized forms.

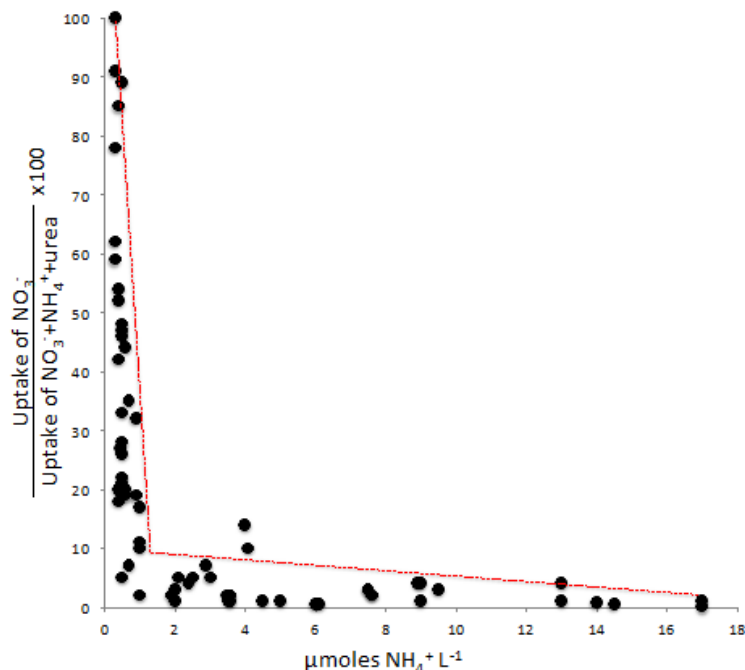


Figure 2.10. Percent NO_3^- uptake as a function of ambient NH_4^+ concentration in natural phytoplankton assemblages. Adapted from McCarthy et al. 1975

The take-home messages from these culture experiments can be summarized as follows:

- 1) Whereas uptake of most N substrates became suppressed when another substrate was added to the culture, NH_4^+ uptake tended to be the least suppressed by others, therefore NH_4^+ was considered “preferred”. Phytoplankton species tested differed in their exact order of preference for various N substrates after NH_4^+ . More recently, a number of investigators have found that diatoms are less likely to completely suppress NO_3^- uptake in the presence of NH_4^+ , even at high concentrations (Yin et al. 1998, Lomas and Glibert 1999, Song and Ward 2007) compared with non-diatoms (He et al. 2004, Song and Ward 2007).
- 2) The time it took to switch from one source to another, i.e. from NO_3^- to NH_4^+ , varied from instantaneous to half an hour.
- 3) Growth rates were not affected by switching N sources, or as a result of growth on more than one source of N in culture (Dortch and Conway 1984, Lund 1987).

The terms “preference” and “inhibition” were used historically to describe responses for which the molecular mechanisms were not clear. More recently these terms have been supplanted by “induction” and “repression”, which reflect the turning on and off, respectively, of the genes coding for N assimilation and transport proteins. For example, in studies of the green alga *Dunaliella tertiolecta*, transcript abundance of NR was minimal when cells were grown on NH_4^+ or urea, or without N (Figure 2.11). NR transcript was most abundant when cells were grown on

NO_3^- or nitrite (NO_2^-). When grown both on NO_3^- and NH_4^+ , NR transcript was similar to when cells are grown on NH_4^+ only. When grown on both NO_3^- and urea, NR transcript abundance was similar to when cells were grown on NO_3^- only. These results tell us that, in the phytoplankton species tested, NO_3^- was needed for induction of NR whereas NH_4^+ repressed NR. Urea neither induced nor repressed NR, i.e., it had no effect. Therefore, NR abundance was minimal when the cells were grown on urea, not because urea was suppressing NR transcription, but, because there was no NO_3^- (or NO_2^-) present to induce it (Fig. 2.11).

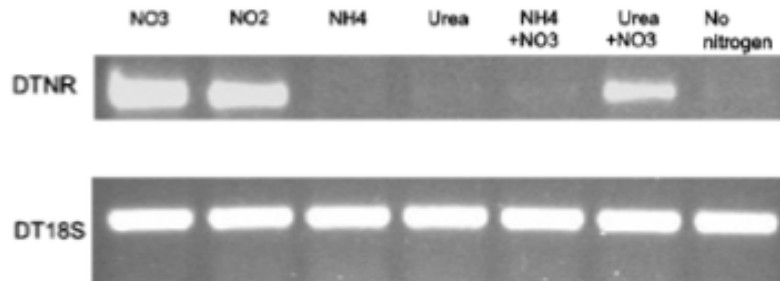


Figure 2.11. Nitrate Reductase (NR) transcript abundance in *D. tertiolecta* grown on NO_3^- , NO_2^- , NH_4^+ , urea, both NH_4^+ and NO_3^- , both urea and NO_3^- , and without N (top row) and 18S transcript abundance under the same N growth conditions (bottom row). DTNR and DT18S represent primers used to transcribe mRNA. From Song and Ward 2004.

2.3.3.3. Total N uptake by phytoplankton cells

As noted above, NH_4^+ is preferred in most phytoplankton followed in varying order by other N sources. While this preference hierarchy appears to suggest that one source is taken up at a time, uptake data demonstrate otherwise. Even during near-monospecific phytoplankton blooms, multiple forms of nitrogen are taken up simultaneously (Fig. 2.12).

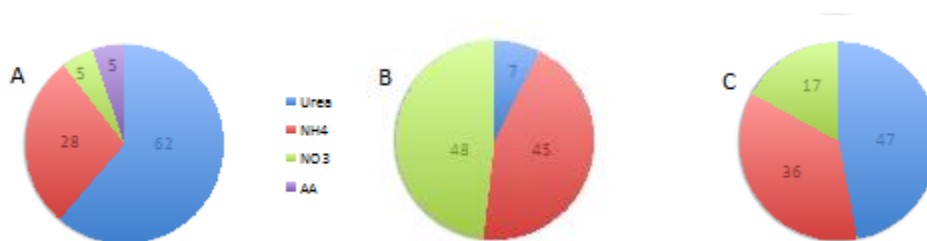


Figure 2.12. Percent contribution (number in each slice) of urea (blue), NH_4^+ (red), NO_3^- (green) and amino acids (AA, purple) to total N uptake during near-monospecific blooms (>90% community composition) of phytoplankton. A) The Brown Tide former *Aureococcus anophagefferens* (Berg et al. 1997), B) The spring bloom diatom *Thalassiosira baltica* (Berg et al. 2001), and C) the Red Tide dinoflagellate *Lingulodinium polyedrum* (Kudela and Cochlan 2000).

What seems to vary among different phytoplankton is the contribution of the various sources of N to the total N demand of the cell (Fig. 2.12). This varies according to cell type, as noted in the section on expression of transporters, as well as external N concentration. For example, early in spring, diatom N demand may be met mostly by NH_4^+ until it's depleted at which time diatoms will begin to support a sizeable proportion of their total N demand with uptake of NO_3^- . However, NH_4^+ continues to be taken up as it becomes available through remineralization (Fig. 2.12b). Taking up both NH_4^+ and NO_3^- simultaneously enables diatoms to grow at near-maximal rates. Because diatoms grow faster than any other taxonomic group, and rates of total N uptake scale to growth rate, the rate of either NH_4^+ or NO_3^- uptake by diatoms will outpace any other taxonomic group so long as nutrients are plentiful. Since NH_4^+ pool sizes are generally smaller than NO_3^- and become depleted more rapidly, greater NO_3^- uptake rates at a certain point in the bloom may simply reflect greater availability of NO_3^- in the water column at that time. Once NO_3^- is depleted, diatom growth becomes diffusion-limited as nutrient concentrations do not permit full doublings of their biomass and mortality becomes relatively more important in determining net growth of the population.

Under conditions of inorganic N limitation, smaller phytoplankton tend to dominate community composition because they are less affected by diffusion limitation (Sunda and Hardison 1997). These species may outcompete diatoms for inorganic N as well as dissolved organic nitrogen (DON) substrates that become progressively more abundant as inorganic sources of N are depleted (Berman and Bronk 2003). This scenario hinges on concentrations of bioavailable DON substrates being too low for diatoms to be competitive, or diatoms not being able to efficiently access components of the DON pool due to either a lack of necessary hydrolytic enzymes or poor efficiency of those enzymes relative to other members of the plankton community (including heterotrophic bacteria). Both may be true. In the latter scenario, both smaller and larger phytoplankton species that are able to meet more of their total N demand with DON substrates can double unrestrained to dominate community composition (Fig. 2.12 a, c). HAB species tend to fall into this category (LaRoche et al. 1997, Berg et al. 1997, Kudela and Cochlan 2000, Anderson et al. 2008, Gobler et al. 2011).

2.3.3.4. Nitrogen uptake and phytoplankton succession

As concentrations of N substrates change from non-limiting to limiting, phytoplankton community composition changes as well. When N is limiting, the ability of a cell to either 1) cover more surface area with proteins to capture the limiting nutrient or 2) tap into alternative N source comes into play and can impact growth rates. Investigations have used the tracer ^{15}N to examine how uptake of various N sources varies as a function of phytoplankton community composition. Results suggest that cyanobacteria, cryptophytes and dinoflagellates tend to be positively correlated with the uptake of NH_4^+ or urea, whereas diatoms tend to be negatively correlated with the same substrates (Fig. 2.13). In fact, when examining relationships between percent community composition and percent uptake of a specific N substrate, only diatoms are

positively correlated with uptake of NO_3^- (Landry et al. 1997, Berg et al. 2001, 2003, Heil et al. 2007, Glibert and Berg 2009). These observations do not suggest that phytoplankton are only associated with a single source of N, but rather that the proportions of the various N sources taken up differs among the various community members. For example, diatoms do not solely utilize NO_3^- , but, compared with cyanobacteria, NO_3^- may comprise a larger fraction of their total N uptake. However, the proportion of N uptake does not only reflect genetic capabilities of the dominant phytoplankton group, *it also reflects the availability of nutrients*. Even if NH_4^+ tends to be “preferred” it may not be available in sufficient quantities that phytoplankton can “choose” it. For example, Panel A in Fig. 2.13 can be interpreted as diatoms preferring to take up a greater proportion of NO_3^- , or that mainly NO_3^- is available in sufficiently high concentration. It could be a combination of both as diatoms may have evolved to take advantage of NO_3^- accumulating in the water column over the winter season.

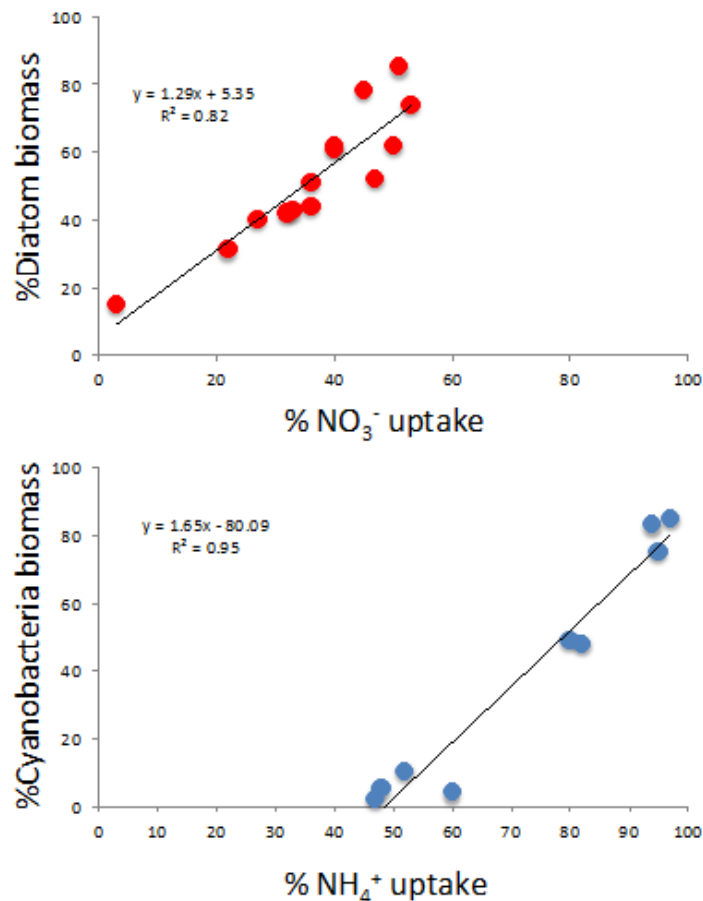


Figure 2.13. Percent diatom community composition as a function percent NO_3^- uptake (top panel). Percent cyanobacteria community composition as a function of percent NH_4^+ uptake. Figure from Glibert and Berg 2009.

In summary, when phytoplankton grow on only one source of N, their entire N demand is met by that source. As long as the molecular machinery to assimilate the source is in place, uptake of the particular source will not affect growth rates – growth rate is determined by C fixation which in turn is controlled by temperature, irradiance and level of Rubisco expression. Under natural conditions, several sources of N are available simultaneously and the proportions in which phytoplankton take these up are determined by 1) their concentrations (and the interaction between phytoplankton size and concentration in respect to how easily they become diffusion limited) and 2) phytoplankton's intrinsic regulation of uptake and assimilation of each source. When nutrients and light are plentiful, species-specific regulation of uptake and assimilation matters little and intrinsic growth rates determine the outcome of population dynamics. As light becomes limiting, growth is down-regulated but C and N metabolism are still coupled resulting in very little impact on N preferences. As nutrients become limiting, phytoplankton regulatory mechanisms and ability to assimilate “alternative” N sources may become more important in influencing competition and community composition.

2.3.3.5. Light-Nitrogen Interactive Effects

As noted in Section 2.3.2.2, investigations into varying irradiance and N source have demonstrated that there is no interactive effect when phytoplankton are grown on a single source of N. Whether this source is NO_3^- or NH_4^+ , growth rates are similarly low at low light and similarly high at high light. The question is what happens when multiple sources of N are available? In other words when phytoplankton have a choice, will uptake of one source dominate over the other at low and at high light? Indeed, field studies appear to indicate that NO_3^- uptake is more light-dependent than NH_4^+ uptake. This is supported by two lines of evidence; one is that it takes a greater light level to reach maximal uptake velocities for NO_3^- than for NH_4^+ (Slawyk 1979, Kanda et al. 1989, Muggli and Smith 1993, Cabrita et al. 1999, Maguer et al. 2011) and the other is that uptake rates in the dark are lower for NO_3^- than NH_4^+ , suggesting that NO_3^- uptake is more dependent on light (Cochlan et al. 1991, Kudela et al. 1997, Clark et al. 2002). However, caution must be exercised when interpreting field data as phytoplankton community composition tends to differ between stations where differences in light dependence of N uptake are observed; in some cases this difference composition may preclude a simple explanation of irradiance effects on N uptake and phytoplankton N status (Cochlan et al. 1991).

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3. Research on NH_4^+ inhibition of primary production in Suisun Bay

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3.1. Introduction

Observations made since the early 2000's have noted declining abundances of important, pelagic members of the northern San Francisco Estuary (SFE) foodweb, a phenomenon referred to as the Pelagic Organism Decline (POD). In response, numerous investigations have been launched, aimed at identifying the underlying causes of the POD. The POD conceptual model recognizes that multiple factors may act in concert to degrade habitat and contribute to declines in fish abundance (Baxter et al., 2010; NRC 2012), including: changes in flow regime, physical alternations to habitat, land use changes, invasive species, contaminants, and nutrients.

This section synthesizes observations from several recent investigations that explored the hypothesis that anthropogenically-elevated ammonium (NH_4^+) inhibits phytoplankton production in the northern SFE, and thereby contributes to habitat impairment by limiting food supply (Wilkerson et al. 2006, Dugdale et al. 2007, Dugdale et al. 2012, Parker et. al 2012a, Parker et al., 2012b). The section begins with an overall description of the conceptual model that has evolved from studies conducted by researchers from the San Francisco State University Romberg Tiburon Center (RTC) over the past 10 years. Next, the RTC studies whose observations form the basis for the conceptual model are discussed in more detail, including how their findings compare to the conceptual model of N utilization and phytoplankton growth that emerges from the broader scientific literature (Chapter 2). The section closes with a summary and important questions remaining to be addressed.

3.2. Background: Chl-a levels in Suisun Bay

Extremely low phytoplankton biomass, which is the major source of energy for Suisun Bay's pelagic food web, is considered to be among the main factors contributing to fish declines. In the 1970s and early 1980s, phytoplankton biomass in Suisun Bay was much higher than present levels. Prior to 1987, chl-a concentrations commonly reached $20\text{--}30\ \mu\text{g L}^{-1}$ (Figure 3.1), but exhibited a marked decrease beginning around 1987, coincident with the well-documented invasion of the Asian overbite clam, *Potamocorbula amurensis* (Jassby 2008). Chl-a levels have rarely reached $10\ \mu\text{g L}^{-1}$ in the subsequent 25 years. Figure 3.2 (panels A, C, E) illustrates the seasonality in chl-a concentrations as well as changes over time. Chl-a concentrations were commonly in the range of $10\text{--}20\ \mu\text{g L}^{-1}$ during May–September prior to 1987. Not all of that biomass was necessarily produced within Suisun Bay: efflux of phytoplankton produced within in the Delta to Suisun Bay has historically been an important source of phytoplankton biomass (Jassby et al., 2002). Analysis of monthly trends over the entire record of 1975–2011 shows, as expected and as reported elsewhere (e.g., Jassby 2008), that chl-a concentrations exhibited statistically significant decreases over more than half the year (Fig. 3.2B,D, and F).

The current persistently-low levels of phytoplankton biomass are commonly attributed to several factors. High suspended sediment concentrations and the associated low light levels limit phytoplankton growth rates. Strong tidal mixing limits the duration of any vertical stratification,

which, combined with low light levels, limits growth rates in deep sections of Suisun Bay. In addition, residence times as short as a few days during high flow periods can flush phytoplankton out of Suisun Bay at a rate faster than they can grow, limiting the accumulation of new biomass. Grazing by *Potamocorbula* has been considered to be a major factor causing the low biomass in Suisun Bay. Recent estimates by Kimmerer and Thompson (in press) suggest that *Potamocorbula* filter the Suisun water column at rates faster than phytoplankton can grow, and can readily explain the persistently low phytoplankton biomass in Suisun Bay. More recently, several studies have hypothesized that elevated NH_4^+ concentrations in Suisun Bay cause slowed phytoplankton growth and thereby limit phytoplankton biomass (Dugdale et al. 2007, Dugdale et al. 2012, Parker et al. 2012a, Parker et al., 2012b), and these studies are the focus of this report section.

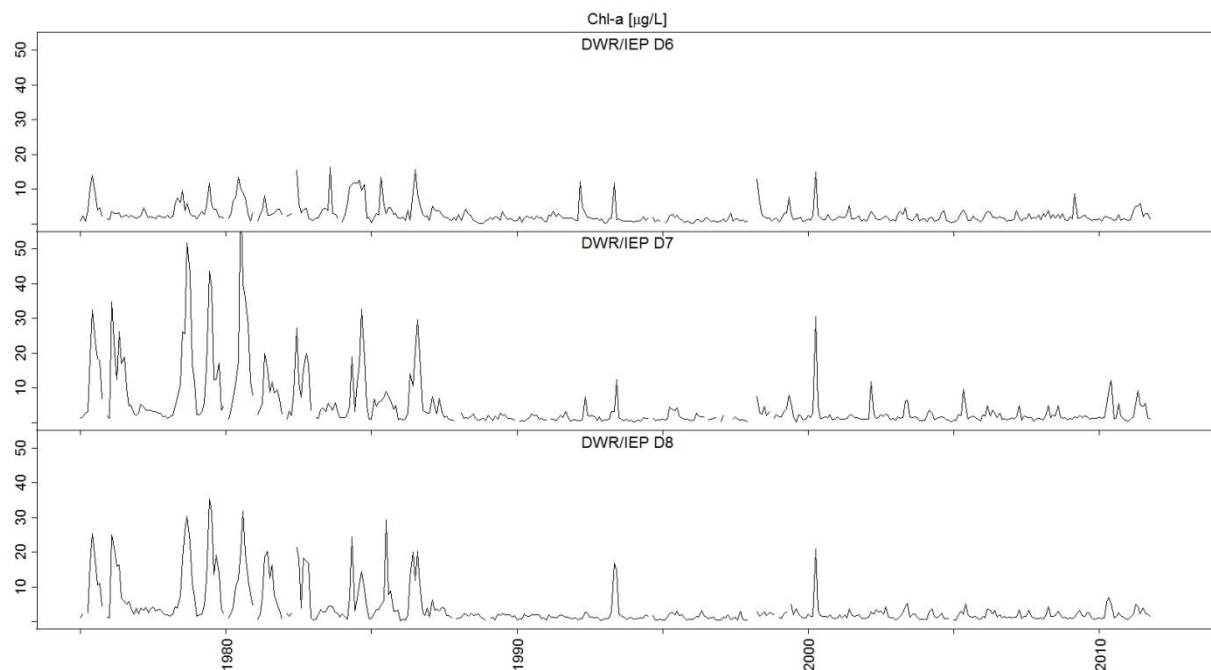


Figure 3.1. Time-series of available chlorophyll-a data (in $\mu\text{g L}^{-1}$) at DWR/IEP stations D6, D7 and D8 in Suisun Bay. Station D7 is located in relatively shallow Grizzly Bay, and D6 and D8 are located within the deep channel southwest and southeast, respectively, of D7.

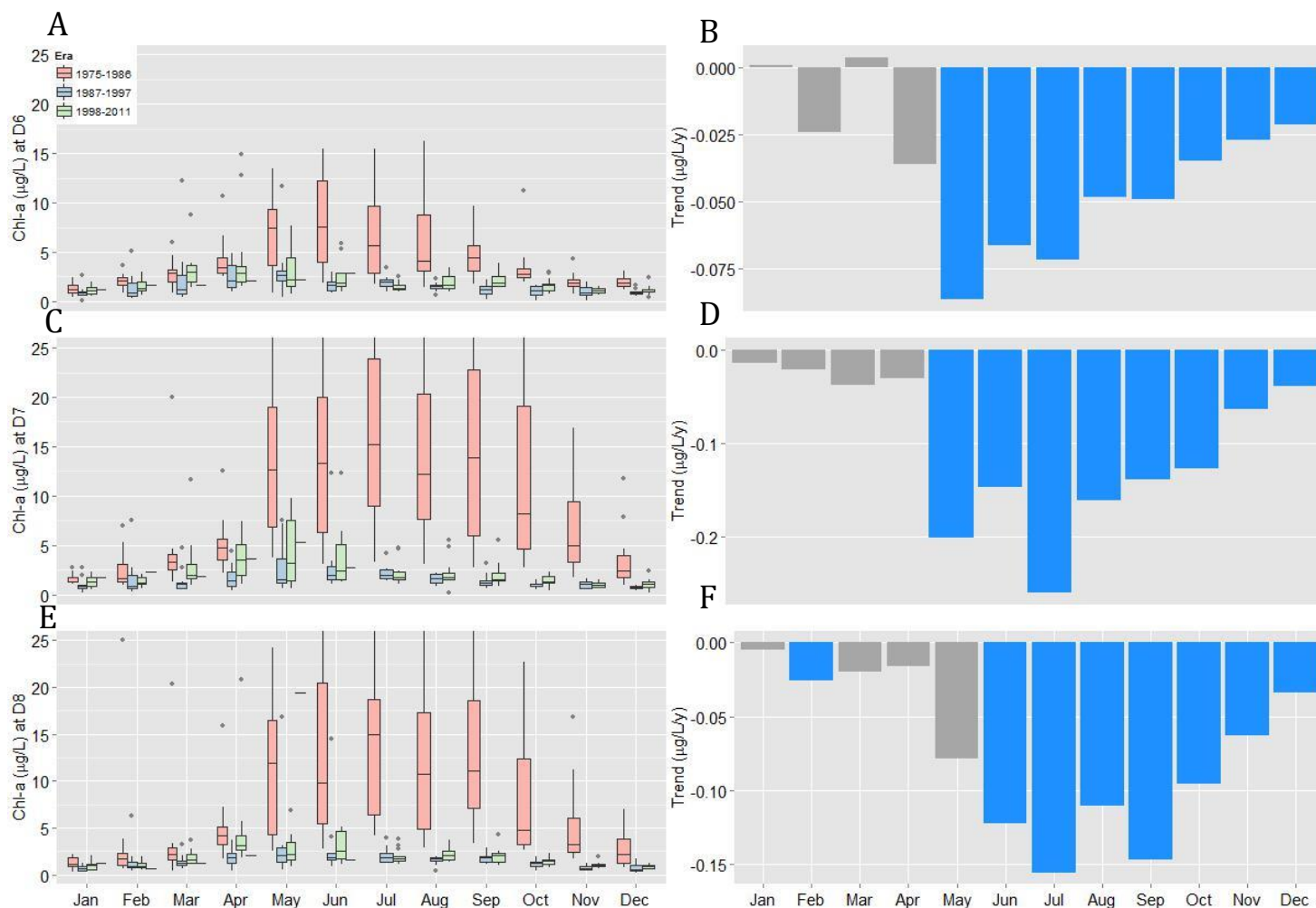


Figure 3.2 Chlorophyll-a concentrations at DWR/IEP stations D6, D7 and D8 in Suisun Bay. Station D7 is located in relatively shallow Grizzly Bay, and D6 and D8 are located within the deep channel southwest and southeast, respectively, of D7. Monthly data were first combined into three eras (1975-1986, 1987-1997 and 1998-2011), and then averaged by month within each era (panels a, c and e). The boxes extend to the 25th and 75th percentile, the whiskers extend to the highest (or lowest) value within additional 1.5x the interquartile range from the 75th (or 25th) percentiles, and any outliers are shown with dots. Long-term trends were calculated by the Theil slope (see description in Section 6.2.3) (panels b, d and f). Blue bars indicate statistically significant trends with $p < 0.05$ as determined by the Kendall Tau test. Concentrations are presented in panels (a, c and e) and trends are reported in panels (b, d and e).

3.3. Conceptual model: the ammonium paradox

Dugdale et al. (2012) propose that increased ammonium NH_4^+ loads to the northern San Francisco Estuary, including Suisun Bay, have resulted in reduced primary production, which they refer to as an “ammonium paradox”. The conceptual model for the ecological impacts of the NH_4^+ inhibition hypothesis is built around three main points:

- P.1** The presence of NH_4^+ at elevated levels ($>1\text{--}4\ \mu\text{mol L}^{-1}$) inhibits the uptake of nitrate by phytoplankton.
- P.2** The rate of NO_3^- uptake (when NH_4^+ is absent or less than $1\text{--}4\ \mu\text{M}$) is greater than the rate of NH_4^+ uptake. Thus, when NO_3^- uptake is suppressed, and only NH_4^+ is being taken up by phytoplankton, the overall rate of N uptake is lower.
- P.3** The lower rate of N uptake resulting from this mechanism translates into lower rates of primary production.

[Note: The conceptual model described below, including the three main points above, was developed by this report's authors through reading key studies and through discussions with RTC researchers. It may differ from how RTC researchers would describe certain aspects.]

When NH_4^+ levels are relatively high in Suisun Bay, the NH_4^+ -inhibition conceptual model argues that phytoplankton grow slowly and are flushed out of Suisun Bay before they can sufficiently draw down NH_4^+ to allow faster growth on NO_3^- . When conditions are such that NH_4^+ levels are lower during spring (e.g., due to high enough flows that NH_4^+ is present at more dilute concentrations), the phytoplankton community is able to draw down NH_4^+ to low enough levels that NO_3^- can be accessed, and blooms occur. When conditions in Suisun Bay are interpreted through this conceptual model lens, the NH_4^+ levels in Suisun Bay, which frequently exceed $1\text{--}4\ \mu\text{mol L}^{-1}$ (Section 6), cause NH_4^+ -inhibition of primary production that contributes to the current rarity of spring phytoplankton blooms in Suisun Bay. RTC researchers argue that the elevated NH_4^+ levels, and the associated lack of phytoplankton blooms, in turn contribute to food-limited conditions in Suisun Bay and deleterious bottom-up impacts on estuarine fish populations.

Dugdale et al. (2012) suggest that spring phytoplankton blooms in Suisun Bay follow a predictable sequence, which they describe as follows:

“In early spring, phytoplankton nitrogen demand in Suisun Bay is satisfied by ammonium, but with low biomass-specific and depth-integrated ammonium uptake rates due to high turbidity and poor irradiance (Parker et al., 2012b). Nitrate uptake is low or near zero during this period due to ammonium inhibition. With improved irradiance conditions (via increased water transparency, water column stability or seasonal increase in irradiance), phytoplankton ammonium uptake rates and biomass increase, causing water column ammonium concentrations to decrease. Once ammonium decreases to $< 4\ \mu\text{mol L}^{-1}$, phytoplankton nitrate uptake is enabled. With continued phytoplankton growth, ammonium concentration is further reduced to $\leq 1\ \mu\text{mol L}^{-1}$ and biomass-specific nitrate uptake rates accelerate resulting in a rapidly developing bloom nourished by nitrate. However, if residence time is too low to allow the phytoplankton to assimilate the inflowing ammonium, as may happen with high river flow conditions or if there is very elevated ammonium inflow, the production processes are only ammonium-based. Nitrate is

unused and exported from the ecosystem (i.e. to the Pacific Ocean). Reduced primary production is a counter-intuitive result of elevated ammonium: the ammonium paradox.”

The RTC studies acknowledge that other factors play a role in limiting primary production rates and decreasing biomass accumulation, including light limitation, benthic grazing by filter-feeding clams, and flushing. Dugdale et al (2007) notes that “Low annual primary production in SFB is due primarily to turbid conditions...”. Dugdale et al (2012) argue, however, that during spring, clam grazing rates can exert only a minor influence because clam biomass is at seasonally-low levels. Thus, NH_4^+ inhibition of primary production is considered to be an additional mechanism that contributes to lower levels of phytoplankton biomass, and one that has the potential to ‘tip the scales’ away from a bloom occurring when conditions might otherwise favor a bloom.

The RTC studies also note that some other factor appears to be acting in Suisun Bay, beyond NH_4^+ inhibition, to cause lower rates of primary production (Dugdale et al., 2007; Parker et al., 2012a). Efforts have been under way to characterize potential toxins in Suisun Bay through toxicity identification evaluations (TIE), although the results of this work have thus far have been inconclusive (J Miller et al., in preparation).

The NH_4^+ inhibition conceptual framework was developed through observations in multiple studies over the past 10 years in which RTC researchers used field observations (spatial and temporal variations in nutrient and chl-a concentrations); stable isotope tracer (^{15}N , ^{13}C) addition experiments to measure uptake rates of $^{15}\text{NH}_4^+$, $^{15}\text{NO}_3^-$, and $^{13}\text{CO}_2$; and enclosure experiments in which NO_3^- , NH_4^+ , chl-a and tracer uptake rates were measured in mixed plankton communities over time.

The NH_4^+ -inhibition conceptual model can be visualized most straightforwardly by considering the biomass specific uptake rates of NO_3^- , NH_4^+ , and C (with carbon uptake being a direct measure of primary production rate). In the discussion below, these are abbreviated as V_{NO_3} , V_{NH_4} , and V_{C} , respectively. The sum of all dissolved inorganic nitrogen (DIN) is abbreviated here as V_{DIN} . While the description below and the reference to experimental observation of these rates in Section 3.3 are somewhat detailed, the concepts are critical to understanding and evaluating the NH_4^+ -inhibition conceptual model. V_{NO_3} , with units of h^{-1} , is quantified by measuring the amount of isotopically-labeled¹ NO_3^- taken up by phytoplankton over the course of an incubation experiment. In essence, V_{NO_3} represents the rate at which the overall

¹ For N uptake studies, solutions containing dissolved NO_3^- or NH_4^+ having artificially high proportions of the minor stable isotope, ^{15}N , are spiked into the sample at low enough levels that they ideally do not influence reaction rates in the sample. For C uptake measurements, enriched stable $\text{H}^{13}\text{CO}_3^-$ or radio-labeled $\text{H}^{14}\text{CO}_3^-$ are used. For example, in natural samples, ^{14}N is the major isotope (99.632%) and ^{15}N is the minor isotope (0.368%), while in enriched isotope solutions ^{15}N abundance is much higher (e.g., 98%). (^{14}C is added in much lower proportions because its lower natural abundance and ease of detection). The amounts of the minor stable (or radioactive) isotope that accumulates as particulate organic N or C are then measured and used to quantify the uptake rate constant.

phytoplankton community takes up NO_3^- per unit mass of phytoplankton, and is considered diagnostic of the “average” physiological or biochemical state of the phytoplankton in terms of their ability or need to take up NO_3^- . Thus, changes in V_{NO_3} as a function of space or time signal a change in either the phytoplankton community’s need for NO_3^- (e.g., more or less light causing changes in primary production rates) or their ability to take up NO_3^- (e.g., inhibition of NO_3^- by relatively elevated ambient NH_4^+). V_{NH_4} is calculated in an analogous way. V_c represents the rate at which inorganic C is taken up by the overall phytoplankton community per unit mass of phytoplankton, and is considered to be a direct measure of the rate of primary production. These approaches for measuring N and C uptake or assimilation rates are prone to some amount of uncertainty or bias; nonetheless they are well-established and widely-used techniques in oceanographic and limnologic studies.

In laboratory experiments, when a pure culture of phytoplankton is grown under constant light with varying levels of NO_3^- (and NO_3^- as the only N source), V_{NO_3} is generally considered to vary as shown in Figure 3.3A, i.e., Michaelis-Menten uptake kinetics (see also discussion in Section 2.3.3). V_{NO_3} increases almost linearly as a function of NO_3^- concentration at low NO_3^- concentrations, and asymptotically approaches $V_{\text{NO}_3, \text{MAX}}$ as NO_3^- increases. V_{NH_4} responds in a similar manner to increasing concentrations of NH_4^+ (Figure 3.3B). The illustrations in Figure 3.3 represent the net measurable response of numerous biochemical processes, and are simplifications of actual cell physiology. Nonetheless, NO_3^- and NH_4^+ uptake by a wide range of phytoplankton species and under a broad array of environmentally-relevant conditions can be characterized or modeled reasonably well using this construct (see Section 2 for further discussion), although there are notable exceptions (see Section 2.3.3).

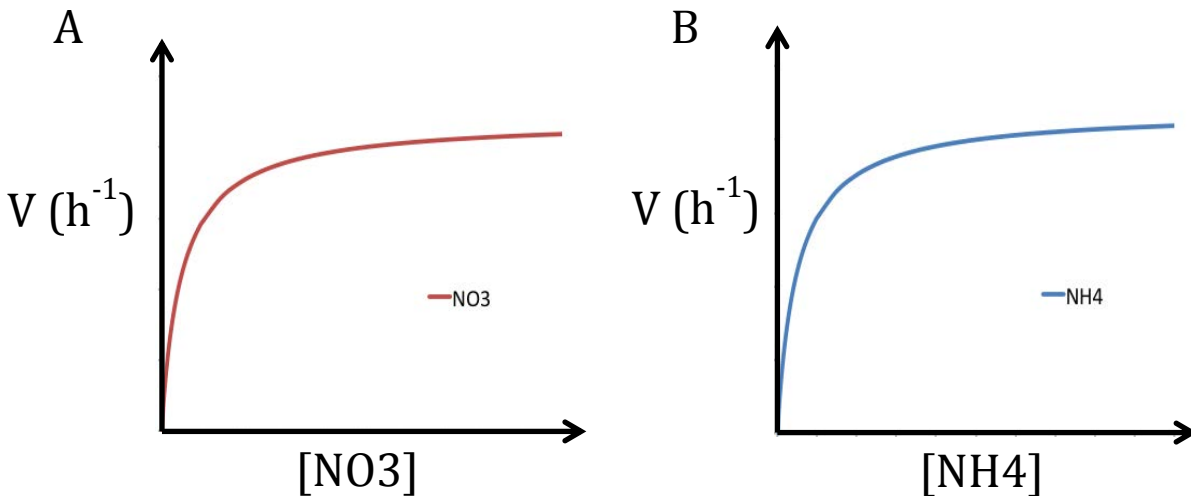


Figure 3.3. Conceptualization of the uptake kinetics of A. NO_3^- and B. NH_4^+ , under constant light conditions (classic Michaelis-Menten uptake kinetics)

When phytoplankton are supplied with both NH_4^+ and NO_3^- simultaneously, P.1 of the NH_4^+ -conceptual model says that the relationship should look quite different. When NH_4^+ concentrations exceed $1\text{--}4\ \mu\text{mol L}^{-1}$, P.1 argues that V_{NO_3} should be inhibited, and phytoplankton will preferentially take up NH_4^+ . This is presented schematically for 3 scenarios in Figure 3.4, with varying levels of NH_4^+ but constant levels of NO_3^- ; as NH_4^+ concentrations increase, V_{NO_3} is illustrated as decreasing. At the same time, V_{NH_4} increases with increasing NH_4^+ concentration (see three examples in Figure 3.4).

P2 of the NH_4^+ -inhibition conceptual model argues that NH_4^+ uptake is slower than NO_3^- uptake. Two illustrations of how this could be the case are presented in Figure 3.4A and Figure 3.4B. Figure 3.4A illustrates how V_{NO_3} , V_{NH_4} , and V_{DIN} would look under scenario that maximum NO_3^- uptake rate ($V_{\text{NO}_3,\text{MAX}}$) is greater than the maximum NH_4^+ uptake rate ($V_{\text{NH}_4,\text{MAX}}$). As NH_4^+ concentrations increase, V_{DIN} (i.e., the total rate at which DIN is taken up by phytoplankton) would decrease. Figure 3.4B illustrates the case when $V_{\text{NO}_3,\text{MAX}}$ and $V_{\text{NH}_4,\text{MAX}}$ are equal, but when phytoplankton exhibit low efficiency for using NH_4^+ at relatively low concentrations (i.e., the half-saturation constant, K_{NH_4} , is large). When NH_4^+ concentrations are $1\text{--}4\ \mu\text{mol L}^{-1}$ and greater, V_{DIN} would be less than V_{NO_3} when NH_4^+ levels are extremely low. Figure 3.4C illustrates the case in which $V_{\text{NO}_3,\text{MAX}}$ and $V_{\text{NH}_4,\text{MAX}}$ are equal, and phytoplankton are efficient at using NH_4^+ at low levels (i.e., K_{NH_4} is small)..

The final point, P.3, argues that if P.1 and P.2 are true, the production rate of new phytoplankton biomass will also decrease. It stands to reason that if some factor causes a prolonged decrease in the rate at which phytoplankton can take up DIN – and the DIN uptake rate is the factor limiting growth – the rate of primary production will decrease. Under these conditions, an experimental or field observation which demonstrates that V_{DIN} is lower when NH_4^+ is elevated would be consistent with the hypothesis that primary production rates are also lower. However this is an indirect measure of production. A direct measurement consistent with this effect would involve, e.g., measuring V_C and demonstrating that lower V_C co-occurs in space or time with elevated NH_4^+ and decreased V_{DIN} .

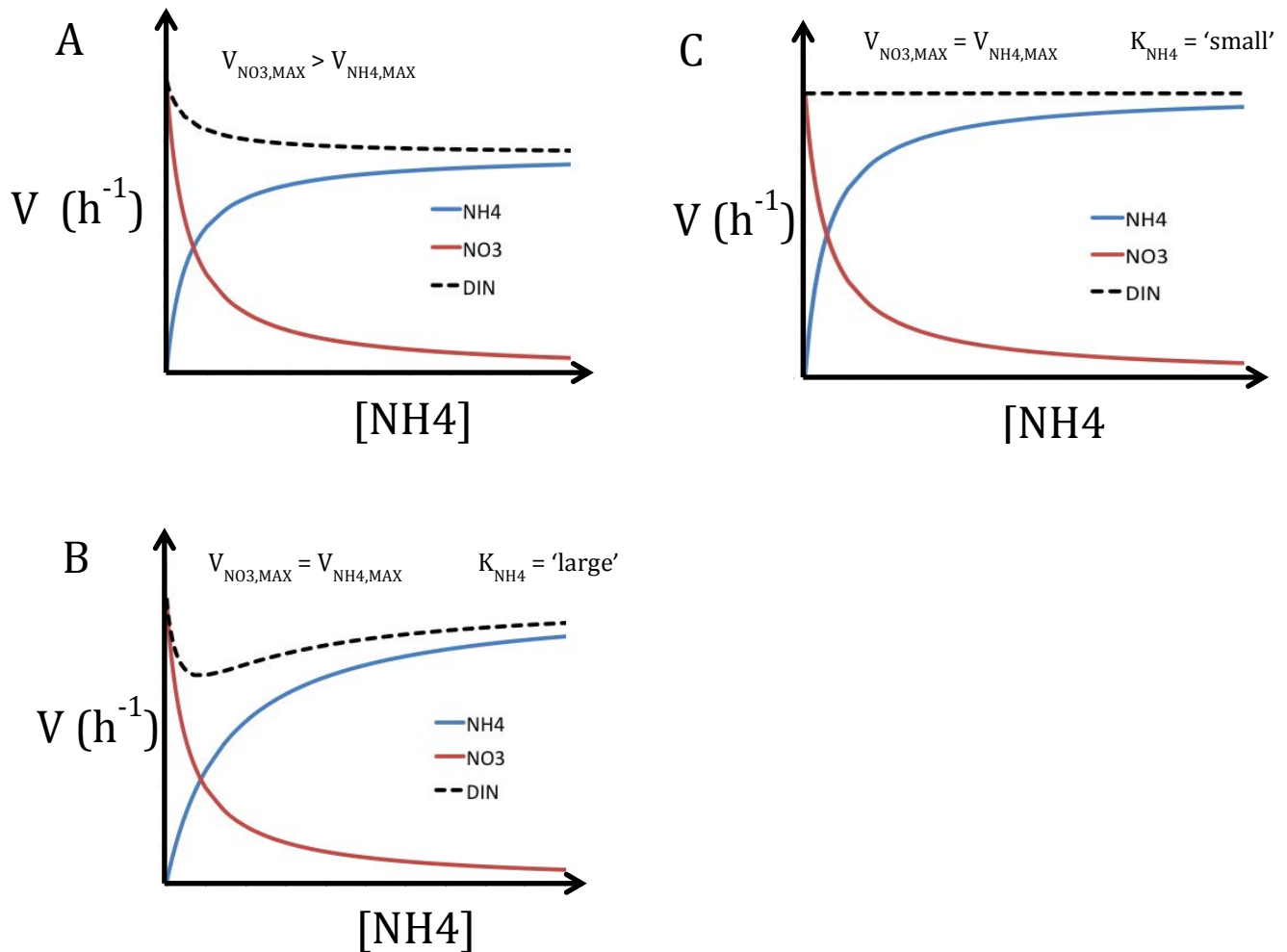


Figure 3.4. Conceptualization of DIN uptake kinetics in the presence of both NH_4^+ (variable) and NO_3^- (constant) under three scenarios: A. $V_{\text{NO}_3, \text{MAX}} > V_{\text{NH}_4, \text{MAX}}$; B. $V_{\text{NO}_3, \text{MAX}} = V_{\text{NH}_4, \text{MAX}}$ but relatively inefficient NH_4^+ uptake at low NH_4^+ ; C. $V_{\text{NO}_3, \text{MAX}} = V_{\text{NH}_4, \text{MAX}}$ and efficient NH_4^+ uptake at low NH_4^+

3.4. State of the science

3.4.1. Field observations in Suisun, San Pablo, and Central Bays: 2000-2003

Dugdale et al. (2007) present a combination of field data and N uptake measurements from Central, San Pablo, and Suisun Bay from November 1999 through May 2003, collected at monthly or greater frequency. The core hypothesis proposed is that ammonium concentrations above a $4 \mu\text{mol L}^{-1}$ threshold inhibit uptake of nitrate by phytoplankton (Wilkerson 2006; Dugdale 2007) resulting in lower primary production rates in Suisun Bay. Dugdale et al. (2007) state: “The substantial inventory of nitrate (NO_3^-) in San Francisco Bay (SFB) is unavailable to the resident phytoplankton most of the year due to the presence of ammonium at inhibitory concentrations that prevents NO_3^- uptake,” and argues that high biological productivity in Suisun Bay depends on the availability of nitrate to phytoplankton.

Dugdale et al. (2007) and Wilkerson et al. (2006) observed that bloom levels of chlorophyll were evident only when NO_3^- uptake by phytoplankton occurred, and that NO_3^- uptake only occurred when NH_4^+ concentrations dropped below $4 \mu\text{mol L}^{-1}$. In Suisun Bay, a bloom (chl-a $\sim 30 \mu\text{g L}^{-1}$) was only observed in April 2000. Suisun chl-a levels were also increasing in May 2003, but the field program did not continue into June. Three blooms of modest magnitude ($8\text{--}17 \mu\text{g chl-a L}^{-1}$) were observed in both San Pablo Bay and Central Bay over this time period. In all cases, the blooms coincided with relatively low ($<4 \mu\text{mol L}^{-1}$) NH_4^+ concentrations. Increases in NO_3^- transport rates (i.e., $V_{\text{NO}_3} \times [\text{particulate nitrogen}]$) generally coincided in time with elevated chl-a during these bloom periods. Primary production rate measurements (e.g., ^{13}C or ^{14}C uptake rates) were not performed by Dugdale et al. (2007) or Wilkerson et al. (2006), but were inferred from changes in chl-a concentration or variations in N uptake rates.

In all three sub-embayments, there was a clear relationship between ambient NH_4^+ concentration and V_{NO_3} (Figure 3.5A). The authors note that V_{NO_3} began to increase when NH_4^+ decreased below $4 \mu\text{mol L}^{-1}$; V_{NO_3} increased rapidly as ammonium approached concentrations of $\sim 1 \mu\text{mol L}^{-1}$ and lower. The observed exponentially-increasing NO_3^- transport rate when NH_4^+ decreased below $4 \mu\text{mol L}^{-1}$, and the nearly uninhibited uptake when NO_3^- was below $1 \mu\text{mol L}^{-1}$, strongly support P.1 of the NH_4^+ -inhibition conceptual model. As discussed in Section 2, the concept of NH_4^+ preference, or NH_4^+ inhibition of NO_3^- uptake, is well-supported in the phytoplankton literature (e.g., Dortch et al., 1990), and the results of Dugdale et al. (2007) are consistent with those findings.

While V_{NO_3} decreased sharply as NH_4^+ increased, V_{NH_4} , actually increased with increasing NH_4^+ in San Pablo and Central Bays (Figure 3.5B). This increase is consistent with classical Michaelis-Menten-like kinetics of phytoplankton nutrient uptake (e.g., Eppley et al., 1967), in which V_{NH_4} is proportional to NH_4^+ concentration at non-saturating levels, until some $V_{\text{NH}_4,\text{MAX}}$ is reached.

The highest observed values for V_{NH_4} , which occurred at the highest observed NH_4^+ concentrations (Figure 3.5B) were comparable to those for V_{NO_3} at low NH_4^+ concentrations (Figure 3.5A). Interestingly, this suggests that the phytoplankton community was able to utilize NH_4 at similar rates as NO_3^- at the extreme ends of the observed NH_4 levels. The overall rate of DIN uptake, $V_{\text{DIN}} = V_{\text{NO}_3} + V_{\text{NH}_4}$, is ultimately the amount of N being taken up by phytoplankton (assuming uptake of organic N is negligible). To a first approximation, $V_{\text{NO}_3,\text{MAX}}$ and $V_{\text{NH}_4,\text{MAX}}$ do not appear substantially different; i.e., it is not obvious that the conceptualized illustration of P.1 as presented in Figure 3.4A is consistent with this set field observations. From Figures 3.5A and 3.5B, the relationship between V_{DIN} and NH_4^+ concentration at intermediate NH_4^+ concentrations is unclear. Does V_{DIN} vary as a function of NH_4^+ , or is it more or less constant? Understanding this point is critical to evaluating P.2 of the NH_4^+ -inhibition conceptual model.

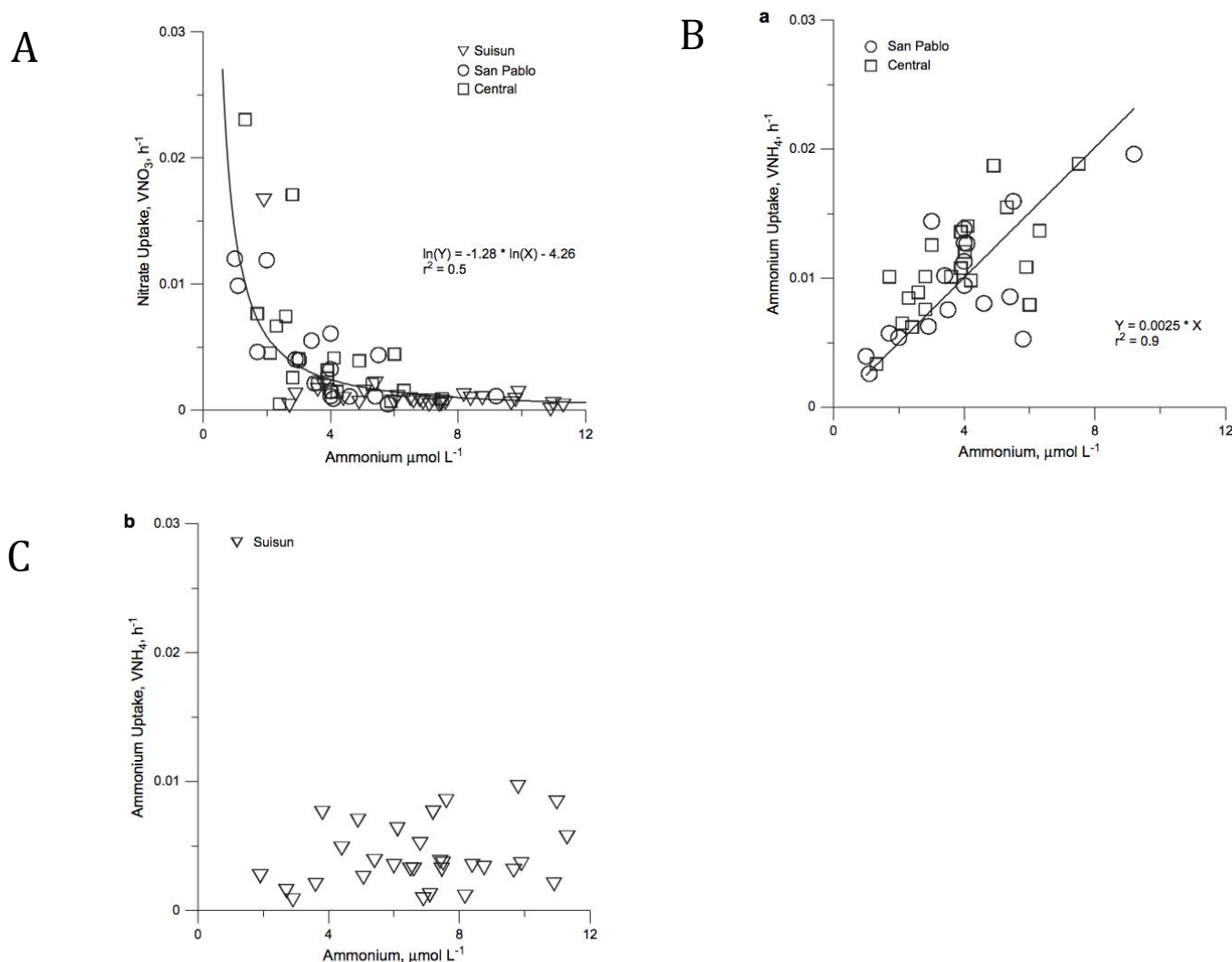


Fig. 4. Trace V_{NH_4} , h^{-1} versus NH_4 concentration, $\mu mol L^{-1}$ for the spring bloom periods in (a) San Pablo and Central Bays, (b) Suisun Bay.

Figure 3.5. From Dugdale et al. (2007) A. V_{NO_3} versus NH_4^+ concentration in Suisun, San Pablo, and Central Bays. B. V_{NH_4} vs. NH_4^+ in San Pablo and Central Bays. C. V_{NH_4} vs. NH_4^+ in Suisun Bay.

The relationship between V_{NH_4} and NH_4 concentration in Suisun Bay differed considerably from that observed in San Pablo Bay and Central Bay. Across the range of observed NH_4^+ concentrations in Suisun, V_{NH_4} remained low and relatively constant (although with considerable variability), and was not correlated with NH_4^+ concentration (Figure 3.5C). V_{NO_3} (Figure 3.5A) was also low in Suisun, even at the lowest NH_4^+ concentrations, although there were limited data in this concentration range because of generally higher NH_4^+ concentrations in Suisun. Thus, although V_{NH_4} and V_{NO_3} were both low, some other factor appears to be playing a major role in regulating N uptake rates, beyond an effect that may be exerted by NH_4^+ . Dugdale et al (2007) note that the “relationship for V_{NH_4} versus NH_4^+ for Suisun Bay shows no obvious pattern, which cannot be explained at present...”. This observation of unexplained low N uptake rates has led to

the so-called “bad Suisun” interpretation, and has been subsequently observed (Parker et al 2012a), as described below.

A time-series of N uptake rates in San Pablo was also presented (Figure 3.6). In the San Pablo time series, the highest measured rate of N uptake was actually a V_{NH_4} around April 1, when NH_4^+ was approximately $10 \mu\text{mol L}^{-1}$. V_{NO_3} was low, and did increase when NH_4^+ concentrations decreased. However, $V_{\text{DIN}} = V_{\text{NO}_3} + V_{\text{NH}_4}$ was actually greater around April 1 compared to subsequent months, arguing that, although NH_4^+ concentrations decreased and V_{NO_3} increased, the overall rate of N uptake did not increase when NH_4^+ was low. A time series in Central Bay was qualitatively similar to the San Pablo Bay time series (not shown; see Dugdale et al., 2007 Figure 6). The time series for V_{NO_3} , V_{NH_4} , and V_{DIN} in San Pablo and Central Bays are not necessarily consistent with P.2 of the NH_4^+ -inhibition conceptual model; on the contrary they might be interpreted as suggesting that NH_4^+ and NO_3^- are actually utilized comparably well by the phytoplankton community. Since environmental conditions play a strong role in shaping the physiological state of phytoplankton, the variation in the total size of the summed bars in Figure 3.6 complicates these interpretations. (e.g., phytoplankton would up-regulate growth and N uptake if light levels increased, or down-regulate at lower light levels, and changes in light levels could be caused by periods of stratification).

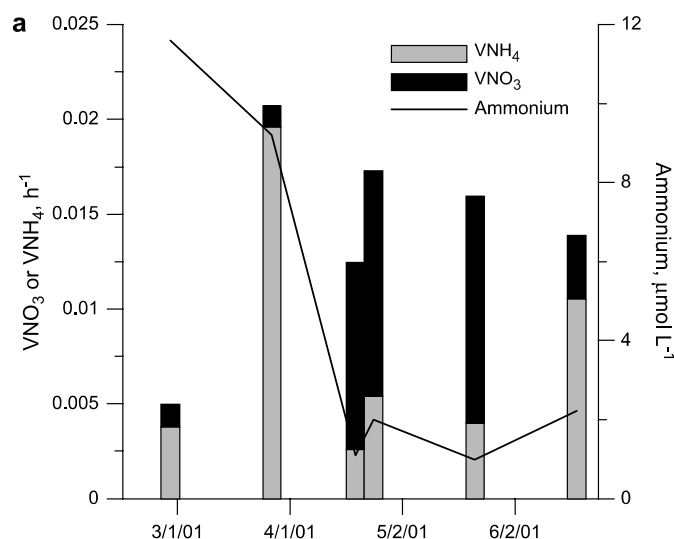


Figure 3.6. From Dugdale et al. 2007. Time series of uptake rates and NH_4^+ concentrations in San Pablo Bay (field investigation). Chl-a concentrations (not shown here) increased steadily from 2 mg/L at end of February to 12 mg/L toward the end of April. Chl-a levels decreased linearly to 1 mg/L by late June. (chl data not shown)

3.4.2. Enclosure experiments

Dugdale et al. (2007) also performed enclosure experiments, using Central Bay water to which they added ammonium at different concentrations. Incubations were carried out over 4 days at constant temperature under 50% of incident light. In spring 1999 incubations, when NH_4^+ concentrations decreased below a few micromolar, V_{NO_3} increased substantially, consistent with

field observations and consistent with an inhibition by or preference for NH_4^+ (see Figure 7, Dugdale et al. 2007). In addition, in some incubations, maximum observed values for V_{NO_3} exceeded maximum values for V_{NH_4} , consistent with P.2 of the NH_4^+ -inhibition conceptual model. Primary production rates were not measured, but changes in chlorophyll were monitored. While chlorophyll levels did increase more rapidly once NH_4^+ was low, it is difficult to infer whether or not (biomass specific) primary production rates increased, since V_C was not measured. Differences in starting chlorophyll concentrations further complicate the interpretations. A second set of enclosures experiments were carried out using Central Bay water in spring 2003, with NH_4^+ additions of 0, 5, 10, 20, and 30 $\mu\text{mol L}^{-1}$. These incubations demonstrate clearly that V_{NO_3} remained low until NH_4 concentrations decreased to below $\sim 4 \mu\text{mol L}^{-1}$, consistent with P.1 of the NH_4^+ inhibition conceptual model (see Figure 8 of Dugdale et al., 2007). No data were presented for how V_{NH_4} varied with NH_4^+ concentrations, which would be valuable information for interpreting how $V_{\text{DIN}} = V_{\text{NO}_3} + V_{\text{NH}_4}$ varied as a function of NH_4^+ concentration.

Parker et al (2012a) carried out enclosure experiments to test the hypothesis 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” compared to NH_4^+ uptake. Enclosure experiments were conducted during March, July, and September 2005, with samples collected from Suisun, Central, and San Pablo Bays. The enclosure experiments were similar to those conducted by Dugdale et al. (2007), carried out at approximately 50% incident light over 96 hours. In addition to measuring V_{NO_3} and V_{NH_4} , a main difference in the study design was that Parker et al. (2012a) also measured C uptake (a direct measure of primary production) by spiking samples with ^{13}C -enriched inorganic carbon and quantifying the amount converted into new phytoplankton biomass. Chlorophyll-a was also size-fractionated over the course of the experiments, allowing Parker et al (2012a) to partition increases in chlorophyll among larger-celled species ($>5 \mu\text{m}$, e.g., diatoms) or smaller-celled species ($<5 \mu\text{m}$).

The Parker et al (2012a) enclosure experiments from San Pablo and Central Bay behaved similarly to each other with respect to their uptake of N and C (Figure 3.7). The presence of NH_4^+ above $\sim 1 \mu\text{mol L}^{-1}$ resulted in suppressed V_{NO_3} (Figure 3.7A and 3.7C). This result is consistent with the P.1 of the NH_4^+ -inhibition conceptual model, with field and enclosure results of Dugdale et al. (2007), and with the broader literature that NH_4^+ inhibition of (or preference over) nitrate uptake is a common phenomenon among marine and estuarine phytoplankton (Section 2). V_{NH_4} was approximately 2-fold greater than V_{NO_3} at the beginning of incubations (Figure 3.7C and 3.7D). Both V_{NH_4} and V_{NO_3} increased substantially over the first 24 hours, although NH_4^+ was still present at $\sim 3 \mu\text{mol L}^{-1}$, and V_{NH_4} remained 2-3 times greater than V_{NO_3} . By 36 hours, nearly all NH_4^+ had been consumed, and V_{NO_3} increased to $0.05\text{--}0.06 \text{ h}^{-1}$, which was approximately 2-fold greater than the maximum values for V_{NH_4} measured during the experiments. These observations are consistent with P.2 of the NH_4^+ -inhibition conceptual model that maximum NO_3^- uptake rates – once NH_4^+ has decreased below $\sim 1 \mu\text{mol L}^{-1}$ – are

greater than maximum NH_4^+ uptake rates, and that overall inorganic N uptake (V_{DIN}) is greater when NH_4^+ concentrations are low. Parker et al. (2012a) suggest that the mechanism underlying the higher rate of NO_3^- uptake relates to the concept of acceleration of uptake - "shift-up" - such that the maximal NO_3^- uptake ($V_{\text{NO}_3, \text{MAX}}$) is variable and proportional to the NO_3^- concentration (Zimmerman et al., 1987; Wilkerson et al., 1987; Dugdale et al., 1990).

Although both V_{NH_4} and V_{NO_3} increased substantially over the first 24 hours, V_C remained relatively constant over this time period. However, V_C increased substantially between 24 and 36 hours, coincident with the sharp increase in V_{NO_3} after NH_4^+ levels decreased to near-zero values. These observations are consistent with P.3 of the NH_4^+ -inhibition conceptual model that primary production is greater when NH_4 levels are low and phytoplankton begin utilizing NO_3^- for growth. Plots for July and September incubations are not presented but maximum V_{NH_4} and V_{NO_3} presented in table form suggest that the results were similar across the different experiments. Across all incubations, chl-a increases occurred mostly (72-100%) in $>5 \mu\text{m}$ size fraction, suggesting that much of the new production was due to larger-celled phytoplankton, such as diatoms.

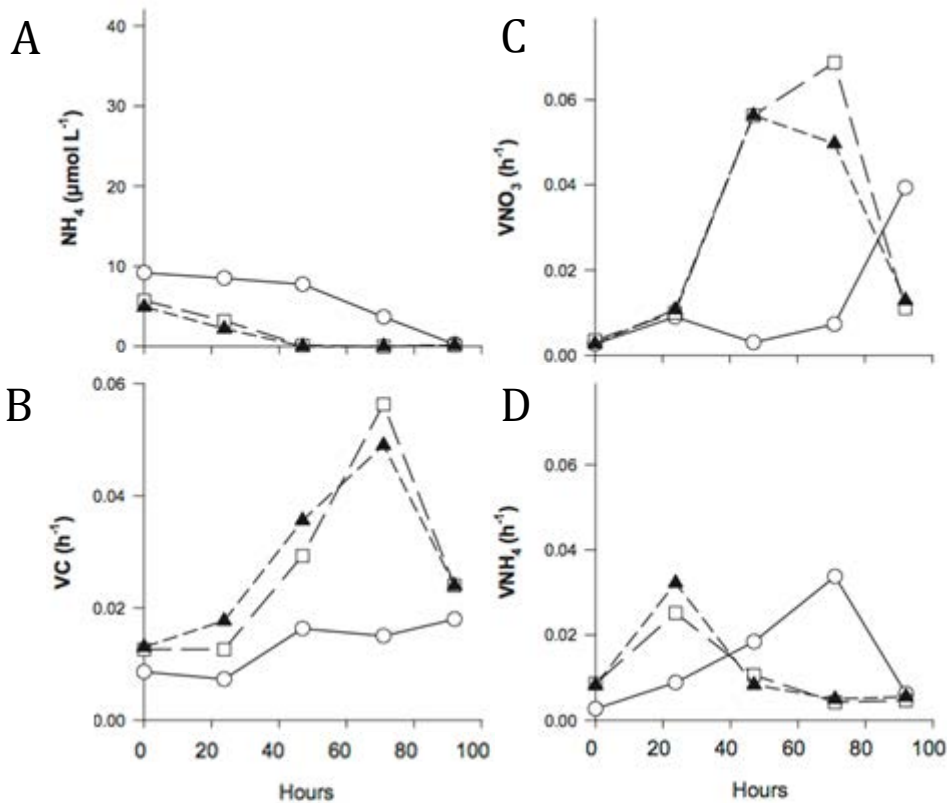


Figure 3.7. From Parker et al. 2012a. Time series of NH_4^+ and NO_3^- concentrations and specific uptake during March enclosure experiments conducted in Suisun Bay (open circles), San Pablo Bay (open squares) and Central Bay (closed triangles). A. $[\text{NH}_4^+]$, B. specific C uptake, V_C , C. specific NO_3^- uptake, V_{NO_3} , D. specific NH_4^+ uptake rate, V_{NH_4} .

Enclosure experiments from Suisun Bay behaved differently than those from San Pablo Bay and Central Bay (Figure 3.7). Initial values ($t = 0$) of V_{NH_4} were lower in Suisun than the other embayments, and gradually increased over the experiment, eventually (after 72 h) equaling the maximum V_{NH_4} values observed in the other sub-embayments (which occurred after only 24 h; Figure 3.7D). After 72 h, NH_4^+ decreased to approximately zero, and V_{NO_3} was higher at 96 h than the maximum V_{NH_4} observed at 72 h. V_c increased modestly beginning at 48 h, but did not show a pronounced increase between 72 and 96 hr to correspond with the increase in V_{NO_3} . V_{NH_4} was ~3-fold lower at $t = 0$ and $t = 24$ h than V_{NH_4} in the San Pablo and Central Bay enclosures, and ~40% less than their maximum even after 48 h. Although the starting NH_4^+ levels were greater in Suisun enclosures than those from the other sub-embayments, this difference cannot explain the marked differences in behavior between the Suisun and other enclosure experiments. Dugdale et al (2007) observed that V_{NH_4} actually increased linearly with increasing NH_4^+ concentration up to $\sim 10 \mu\text{mol L}^{-1}$ in Central and San Pablo Bays, so elevated NH_4^+ in Suisun cannot be readily invoked as the cause for suppressed V_{NH_4} . In fact, the suppressed V_{NH_4} in Suisun enclosures in Parker et al (2012) are qualitatively consistent with the low V_{NH_4} measurements in Suisun observed by Dugdale et al. (2007) (Figure 3.5C). Parker et al. (2012) explain the behavior in Suisun Bay as: “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).”

The hypothesis of another toxicant in Suisun Bay has been proposed elsewhere (Baxter et al. 2010), and alluded to in Dugdale et al. (2007), and is a plausible explanation given the agriculture- and wastewater-derived anthropogenic contaminants loaded to the system. However, the fact that V_{NH_4} and V_c gradually increase over the incubation would require that bioavailable levels of the toxic substance(s) decreased over the course of the experiments.

Parker et al. (2012a) also quantified maximum uptake rates for NH_4^+ and NO_3^- using natural phytoplankton assemblages from Central Bay, incubated at 50% incident light. The maximum uptake rate for NO_3^- , $V_{\text{NO}_3, \text{MAX}}$, was 0.044 h^{-1} while $V_{\text{NH}_4, \text{MAX}}$ was 0.033 h^{-1} (Figure 3.8A). This finding is consistent with P.2 of the NH_4^+ -inhibition conceptual model that phytoplankton in SFE can take up NO_3^- more rapidly than NH_4^+ . While it seems plausible, based on a visual inspection of the data, that the $V_{\text{NO}_3, \text{MAX}}$ and $V_{\text{NH}_4, \text{MAX}}$ are consistently different, there is currently limited data, and it is not stated whether the values are significantly different. Furthermore, the near-saturation of uptake rate shown in Figure 3.8A (i.e., V_{NH_4} reaching a relatively constant value) at NH_4^+ concentrations of $\sim 3\text{-}4 \mu\text{mol L}^{-1}$, differs from the results presented in Dugdale et al. (2007) (Figure 3.3B), where V_{NH_4} increased linearly with NH_4^+ concentrations up to $8\text{-}10 \mu\text{mol L}^{-1}$.

While a graph for V_{NO_3} vs. NH_4^+ was presented for the entire set of incubations in Parker et al (2012a) (Figure 3.8B), no similar plot was presented for V_{NH_4} vs. NH_4^+ . If, however, the V_{NH_4} vs. NH_4^+ relationship in Figure 3.8A was developed under the same experimental conditions as the V_{NO_3} vs. NH_4^+ results presented in Figure 3.8B, and the curve from Figure 3.8A is superposed on Figure 3.8B, it appears that $V_{\text{DIN}} = V_{\text{NH}_4} + V_{\text{NO}_3}$ at NH_4^+ concentrations above 2-3 $\mu\text{mol L}^{-1}$ are comparable to or greater than all but several of the V_{NO_3} values at low NH_4^+ . A similar observation was made above regarding the Dugdale et al. (2007) findings (comparing Figure 3.5A and B). Thus, the published experimental results do not yet allow us to rigorously assess how V_{DIN} varies as a function of NH_4^+ concentration or quantitatively test P.2 of the conceptual model (i.e., distinguish between the idealizations in Figure 3.4A,B, or C). Parker et al. (2012) note that few studies exist showing faster phytoplankton growth on NO_3^- than NH_4^+ . The literature review in Section 2 is consistent with that assessment.

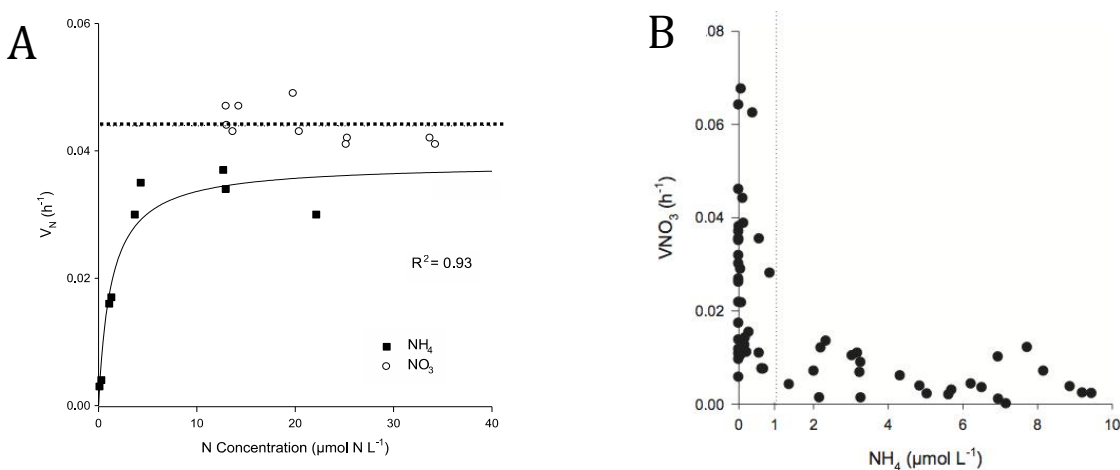


Figure 3.8. A. Michaelis-Menten kinetic curves for NO_3^- (open circles) and NH_4^+ (closed squares) in central San Francisco Bay in April 2005. Data for V_{NH_4} vs. $[\text{NH}_4^+]$ were fit to a hyperbolic function. Dotted line at 0.044 h^{-1} is average V_{NO_3} . B. Biomass-specific NO_3^- uptake versus NH_4^+ concentration. Results from enclosure experiments conducted in March, July and September ($n = 120$).

Parker et al (2012a) make two broad comments on the overall potential impact of elevated NH_4^+ in the northern SFE that deserve some discussion. First, “An ammonium based system will likely exhibit a primary production of <20% of that where NO_3^- is fully used.” The experimental support for this statement is a comparison of dissolved inorganic carbon utilization in Suisun incubations compared to those from Central and San Pablo Bays. Given that earlier in this paper the low productivity in Suisun Bay enclosures was attributed to a factor other than NH_4^+ (i.e., pesticides or other toxic compounds), it is not obvious that the results from these experiments can be used to support this point. Second, the authors make a related point: “...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”. The initial part of this statement is qualitatively the

same as the above “<20%” statement. The latter point (NH_4^+ toxicity to phytoplankton) is not necessarily well-supported by data from this study (i.e., Figure 3.8; V_{NH_4} increases or remains constant over NH_4^+ concentrations ranging up to $\sim 10 \mu\text{mol L}^{-1}$); is not well-supported by data reported in Dugdale et al. (2007) (Figure 3.5; V_{NH_4} increased with increasing NH_4^+ up to 8-10 $\mu\text{mol L}^{-1}$); and is not consistent with the broader literature on NH_4^+ toxicity to phytoplankton (Section 2) when ambient NH_4^+ concentrations in SFE are considered

Finally, there remains the possibility that experimental artifacts could explain some of the observations in Figure 3.7. Water samples were collected from relatively low-light conditions, and the physiological state of the phytoplankton in those samples would have been optimized for growth at those light levels. The incubations were carried out at 50% natural light, which represents a 2-3-fold increase in irradiance relative to *in situ* conditions. Phytoplankton are not able to instantaneously upregulate to grow at higher rates light levels; this can take 10s of hours to days. Thus, in the early stages of the enclosure experiments (≤ 24 hr), some portion of the low V_C and low $V_{\text{DIN}} = V_{\text{NH}_4} + V_{\text{NO}_3}$ could be an experimental design artifact related to phytoplankton populations not yet having fully adjusted to growth at higher light levels. Some of the acceleration of V_C and V_{NO_3} after 24 hr could conceivably be related to an overall increase in growth due to phytoplankton finally acclimating, as opposed to all of the increase in V_C being related to a shift in the N source utilized. Given that Suisun Bay typically has substantially higher turbidity than the other locations studied (resulting in up to 2-fold less light), some of the difference between Suisun enclosures and the other enclosures could be related to additional time being required for Suisun organisms to acclimate to higher light intensities.

3.4.3. Transect observations: Sacramento River through Suisun and San Pablo Bays

Parker et al. (2012b) presents observations from transects along the Sacramento River and through Suisun and San Pablo Bays carried out in March and April 2009. Water quality measurements and N and C uptake measurements were performed at 21 stations extending from the I-80 crossing of the Sacramento River (~ 30 km upstream of the SRWTP input) into San Pablo Bay (Figure 3.9). Parker et al. (2012b) note sharp declines in NO_3^- uptake and C uptake rates downstream of SRWTP that co-occur with sharp increases in NH_4^+ concentrations. The authors conclude that the high NH_4^+ levels along the Sacramento River and through Suisun Bay prevented phytoplankton from accessing the large NO_3^- pool, and limited primary production rates, and that this inhibition is among the factors that presently limits large spring phytoplankton blooms from occurring in Suisun Bay.

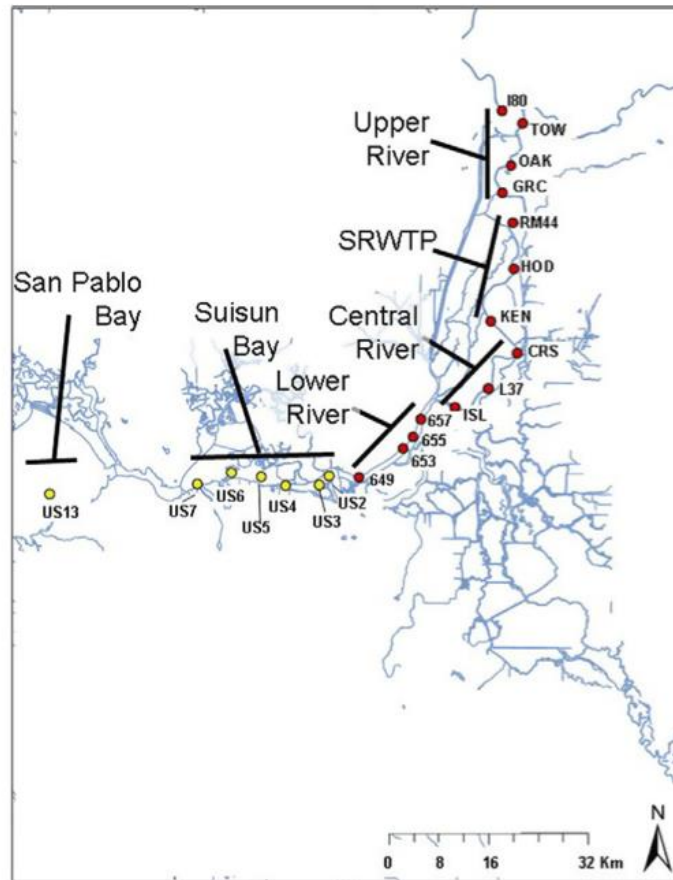


Fig. 1. Study region of the Sacramento River and San Francisco Estuary, CA showing sampling stations and river and Northern estuary transect regions.

Figure 3.9. From Parker et al. 2012b

Upstream of SRWTP, the majority of inorganic nitrogen was present as NO_3^- , with higher concentrations in March ($\sim 15 \mu\text{mol L}^{-1}$) than April ($\sim 2 \mu\text{mol L}^{-1}$), likely due to larger contributions from agricultural runoff in March (Figure 3.10). During both months NH_4^+ concentrations increased from low levels ($< 1 \mu\text{mol L}^{-1}$) upstream of SRWTP to $30\text{--}40 \mu\text{mol L}^{-1}$ immediately downstream of SRWTP. NH_4^+ concentrations decreased by a factor of 2 over the subsequent 50–70 km (travel time $\sim 4\text{--}6$ days), due primarily to nitrification, and, as expected, was accompanied by increases in NO_3^- . NH_4^+ concentrations continued to decrease as water traveled through Suisun and San Pablo Bays, likely due to additional nitrification or NH_4^+ uptake by phytoplankton, in addition to tidal mixing with saltier lower- NH_4^+ waters.

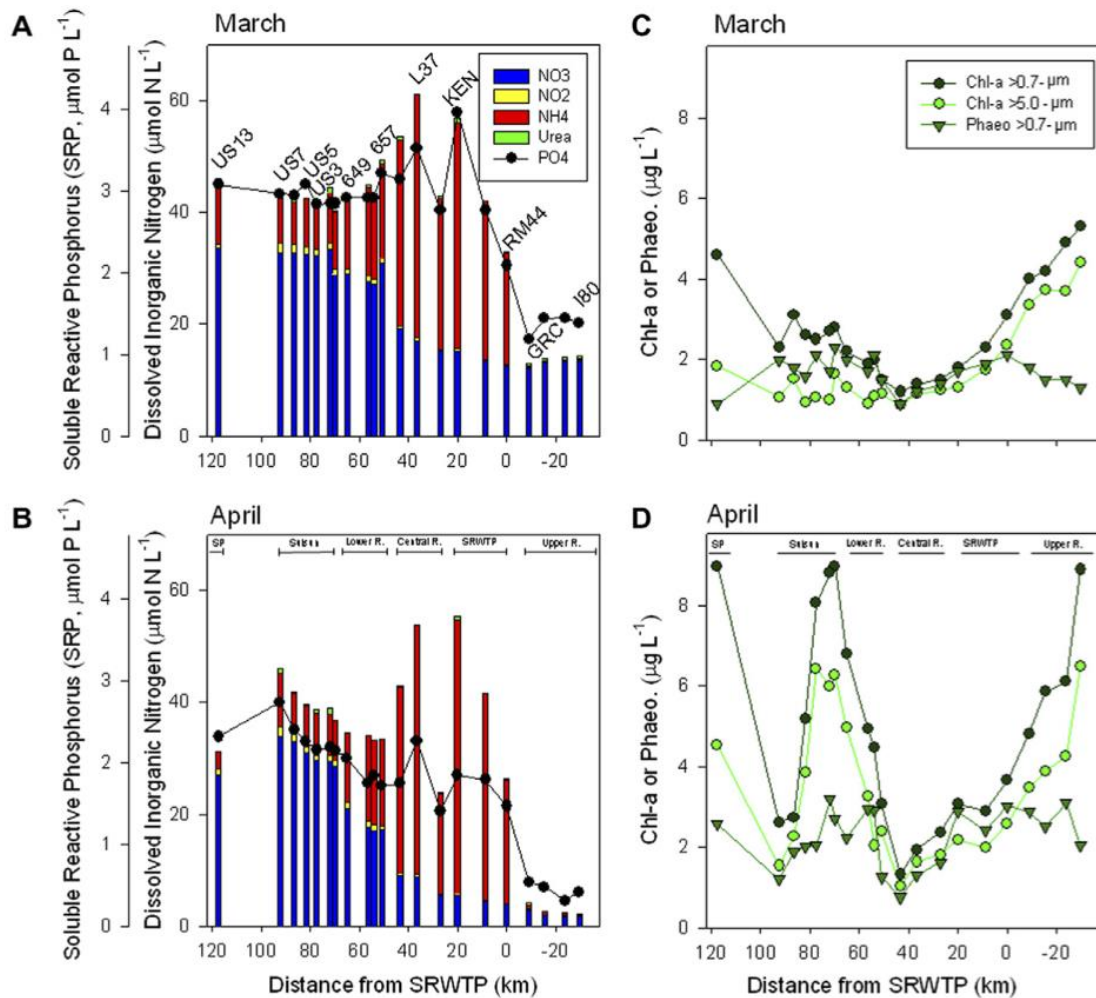


Fig. 4. Inorganic nutrient concentrations measured in the Sacramento River and Northern SFE in (A) March and (B) April 2009 (NO₃; blue, NO₂; yellow, NH₄; red, urea-N; green, SRP; black). Concentrations of chlorophyll-a in cells >0.7-μm diameter (closed circle) and >5.0-μm (open circles) and phaeophytin >0.7-μm (inverted triangles) during (C) March and (D) April 2009.

Figure 3.10. From Parker et al. 2012b

Introduction of treated effluent from SRTWP had a substantial influence on the form of N taken up by phytoplankton. Upstream of the SRWTP, V_{NO_3} was relatively high and V_{NH_4} was low (Figure 3.11). The relative magnitudes of V_{NO_3} and V_{NH_4} shifted sharply downstream of SRWTP. V_{NO_3} decreased by more than one order of magnitude, and V_{NH_4} increased by approximately one order of magnitude. This sharp decline in V_{NO_3} is consistent with P.1 of the NH_4^+ -inhibition conceptual model that at elevated NH_4^+ levels NO_3^- uptake is either inhibited or that NH_4^+ uptake is preferred. In March 2009, $V_{\text{DIN}} = V_{\text{NH}_4} + V_{\text{NO}_3}$ measured at stations upstream of SRWTP were larger than all $V_{\text{DIN}} = V_{\text{NH}_4} + V_{\text{NO}_3}$ measurements at riverine stations downstream of SRWTP, and those in Suisun Bay. A similar pattern of sharp increase of V_{NH_4} and decrease of V_{NO_3} also occurred downstream of SRWTP in April 2009. However, over the first 40 km downstream of SRWTP, V_{NH_4} was comparable to, and sometimes exceeded, V_{NO_3} upstream of SRTWP. Although there was substantial variation, V_{NH_4} tended to decrease with distance

downstream from SRWTP over the 100 km in March and April (April peak in Suisun discussed below), approaching minimum values in Suisun Bay, before sharply increasing in San Pablo Bay.

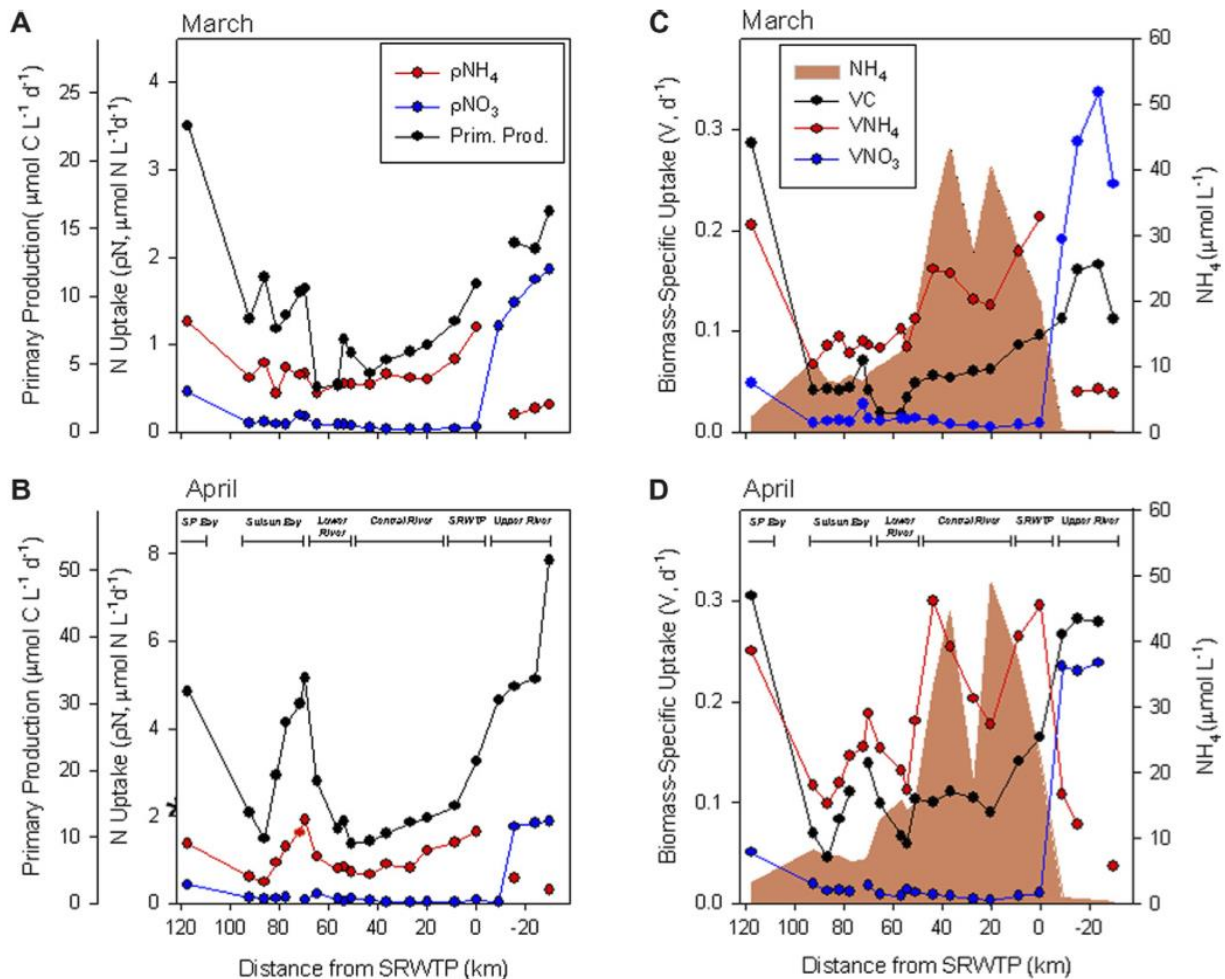


Fig. 5. Primary production and phytoplankton nitrogen uptake in the Sacramento River and Northern SFE during (A) March and (B) April 2009. Biomass-specific carbon uptake and phytoplankton nitrogen uptake and NH_4 concentrations (shaded area) during (C) March and (D) April 2009. Y-axes for phytoplankton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

Figure 3.11. From Parker et al. 2012b

V_C values were either fairly constant or showed modest variation upstream of SRWTP, and decreased consistently but gradually downstream of SRWTP in March, and more sharply in April (Figure 3.11C and 3.11D). The decreases in V_C , coinciding with high NH_4^+ concentrations and uptake of primarily NH_4^+ , are consistent with P.3 of the NH_4 -inhibition conceptual model that the phytoplankton community grows more slowly when primarily utilizing primarily NH_4^+ .

During March and April, phytoplankton biomass (as measured by chl-a) decreased from relative maximum levels at I-80 to minimum values approximately 40 km downstream of SRWTP

(Figure 3.10). In both cases, a substantial portion of the chl-a decrease occurred upstream of SRWTP (30-50%). Some of the chl-a decrease along the river stretch upstream of SRWTP could have resulted from a deepening of the water column (depth ~ 1.5 m at I-80 and > 6 m near SRWTP), lower resulting light availability and productivity, and gradual loss of phytoplankton (e.g., cell death, settling). Unlike chl-a, V_c did not exhibit a pronounced decrease upstream of SRWTP (Figure 3.11), consistent with the notion that light limitation played a role in the lower chl-a, as opposed to other potential explanations (e.g., an unknown contaminant)

During the April 2009 field campaign, a substantial peak in chl-a was observed 50-80 km downstream of SRWTP, peaking in the western half of Suisun Bay. The peak in chl-a co-occurred with peaks in V_{NH_4} and V_c , both of which increased by a factor of 2, but neither climbed back to their higher values upstream of SRWTP. The location of the peaks in chl-a, V_{NH_4} , and V_c is interesting in that light penetration was 2-3 times lower in these areas due to higher turbidity. It is possible that stratification of the water column may have been occurring in this area, allowing phytoplankton in the surface waters greater access to light; salinity stratification was evident at one station in Suisun.

Although the observed decreases in V_c coinciding with shifts to primarily NH_4^+ utilization downstream of SRWTP are consistent with P.3 of the NH_4^+ -inhibition conceptual model, multiple factors varied along this stretch of river and through Suisun and San Pablo Bays, and uncertainties remain about their potential contribution to the observed changes. One factor that is difficult to tease out with the existing data is the potential role that spatial variations in light attenuation may have played. Parker et al. (2012b) address this point to a certain degree by, for example, noting that the photic zone extended over 70-100% of the water column over the river stretch of 10-50 km downstream of SRTWP in April (travel time ~ 3 d), and that there was no increase in chl-a or V_c along this stretch of river. However, there was still substantially more light available to phytoplankton at the furthest upstream station (I-80 crossing, -30 km), where light attenuation was similar to the 10-50 km stretch but the water column was a factor of 4 shallower. Similarly, the light penetration increased by almost a factor of 2 between Suisun Bay and San Pablo Bay in April, and this increase in light availability may account for some of the increases in V_c , $V_{DIN} = V_{NH_4} + V_{NO_3}$, and chl-a between the last Suisun station and the San Pablo station. In addition, the potential for another primary production-inhibiting contaminant, introduced by treated wastewater effluent along with NH_4^+ and that inhibits primary production (discussed in Dugdale et al. 2007, above; and in Parker et al. 2010; Section 3.3.4) remains a possibility. However, in order for productivity to have increased between 50-80 km in April, the concentration or bioavailability of that contaminant must have decreased.

Some inconsistencies, or open questions, with respect to the NH_4^+ -inhibition conceptual model also emerge in Parker et al. (2012b) and require additional investigation. For example, in April 2009 the largest rates of N uptake along the river were actually V_{NH_4} , with V_{NH_4} downstream of SRWTP being comparable to or exceeding V_{NO_3} upstream of SRWTP (V_{DIN} values appear comparable), which does not support P.1 of the NH_4^+ -inhibition conceptual model, that uptake

of V_{NO_3} is greater than V_{NH_4} . Furthermore, the highest values for V_c in March and April were observed at the San Pablo Bay site, where the vast majority of inorganic N uptake was NH_4^+ . Some of these observations may be related to variations in light availability (e.g., due to differences in suspended sediment concentrations, or due to stratification), which could have influenced the physiological state of the cells and thus the rates at which they took up C or N.

Parker et al. (2012b) also suggest that at elevated NH_4^+ levels (e.g., above $20 \mu\text{mol L}^{-1}$) V_{NH_4} also began to decrease. Although a statistically significant negative correlation between V_{NH_4} and NH_4^+ concentration was found for a subset of samples, the number of data were limited, and a more targeted investigation may be needed to confirm this mechanism and the concentrations at which it occurs.

3.4.4. Ammonium addition experiments using either NH_4Cl salt or treated wastewater effluent

In a report to the State Water Resources Control Board, Parker et al. (2010) describe a series of experiments designed to investigate the direct impact of ammonium in wastewater on phytoplankton production and nitrogen uptake (Parker et al. 2009a). The first series of additions were “clean”, i.e. the source of the added ammonium was an ammonium-based salt, ammonium chloride (NH_4Cl). In a parallel series of experiments, ammonium was added as dilutions of SRWTP wastewater effluent containing specific concentrations of ammonium that matched those of the “clean” experiments. In both series, the ammonium was added to Sacramento River water collected at the Garcia Bend monitoring station, which is located just above the SRWTP outfall.

Results from the “clean” additions demonstrated that strong inhibition of nitrate NO_3^- uptake occurred at NH_4^+ concentrations above $1 \mu\text{mol L}^{-1}$. In one set of experiments, V_{NH_4} appeared to decrease at higher NH_4^+ levels ($50 \mu\text{mol L}^{-1}$). In a second experiment, NH_4^+ uptake followed classical Michaelis-Menten uptake kinetics, and no decrease in V_{NH_4} was observed in experiments with NH_4^+ concentrations up to $100 \mu\text{mol L}^{-1}$. Although inhibition of NO_3^- uptake was observed, no change in primary production rates (i.e. V_c) were discernible in the “clean” experiments.

In the experiments carried out using treated wastewater effluent, V_{NO_3} was also suppressed as the effluent proportion and NH_4^+ concentration increased, consistent with results from the clean experiments. However, in the effluent addition experiments, V_{NH_4} also decreased when effluent was added to levels at which $\text{NH}_4^+ > 8 \mu\text{mol L}^{-1}$. Furthermore, in contrast to the “clean” additions, there was a discernable decrease in V_c with increasing effluent proportions (again, when $\text{NH}_4^+ > 8 \mu\text{mol L}^{-1}$). Parker et al. (2010) concluded that whereas the inhibition of nitrate uptake by ammonium held for both types of spiking experiments, only effluent spiking reduced carbon and ammonium uptake at ammonium concentrations above $8 \mu\text{mol L}^{-1}$ (Parker et al. 2009a).

Dr. Parker and collaborators submitted a proposal to the IEP in August 2012 to carry out further experiments using treated wastewater effluent (A Parker, pers. comm.). That proposal was not funded, but further experimentation using treated wastewater effluent remains a goal.

3.4.5. Presence/absence of blooms: Spring 2010, Spring 2011, and pre-1987 observations

Researchers from RTC performed field studies in Suisun Bay in Spring 2010 and 2011 to investigate nutrient uptake rates and phytoplankton growth rates in Suisun Bay. Their sampling program included 6-7 stations within Suisun Bay that were sampled on a weekly basis over the course of ~2 months. The results presented in Figures 3.12-3.13 are for stations situated along an east-west transect in the deep channel of Suisun Bay, extending from near Chipps Island (DWR-D4) to the eastern edge of the Carquinez Straits (USGS 8). Measurements were also made at a shallow station in Grizzly Bay that coincided with the DWR/IEP station D7. Those data are not included in the contour plots because DWR/IEP D7 does not lie along the east-west transect; however, conditions at that site are described in the text below and in Figures A.3.1 and A.3.2.

Contour plots of NH_4^+ concentrations during weekly sampling during Spring 2010 illustrate two 3-4 week periods during which relatively low NH_4^+ concentrations were observed ($< 4 \mu\text{mol L}^{-1}$) over 20-30 km segments (Figure 3.12). Those low NH_4^+ areas approximately coincided with areas of elevated chl-a ($10\text{-}20+ \mu\text{g chl-a L}^{-1}$). NH_4^+ concentrations at D7 were also low ($< 2 \mu\text{mol L}^{-1}$) during most of April May 2010, and chl-a concentrations there ranged from $10\text{-}30 \mu\text{g L}^{-1}$ (see Fig. A.3.1).

The RTC data for 2011 have not yet been published in a peer-reviewed paper, but RTC shared that data for this report. NH_4^+ concentrations were less than $4 \mu\text{mol L}^{-1}$ for most of April-May 2011 along the east-west channel transect (Fig. 3.13). Chl-a levels remained low throughout the entire time period along this transect (Fig. 3.13). At D7, NH_4^+ concentrations were $\leq 2 \mu\text{mol L}^{-1}$ over this time period (Fig A3.2) and two short-lived (<1 week) and modest chl-a peaks were observed there in mid and late May (15 and $10 \mu\text{g L}^{-1}$, respectively), but otherwise chl-a levels remained low.

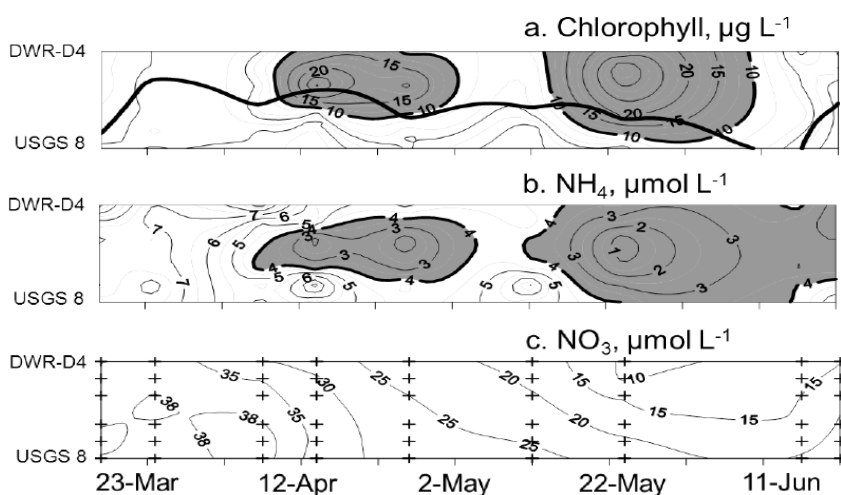


Figure 3.12 NH_4^+ contour plots of data collected by SFSU-RTC during Spring 2010 in Suisun Bay. Data were collected on 9 days at 7 stations along a roughly linear transect through Suisun Bay between DWR/IEP-D4 and USGS-8. (Data from samples collected at the same location as DWR/IEP D7 are not included here – see Fig. A.3.1) Figure from Dugdale et al (2012).

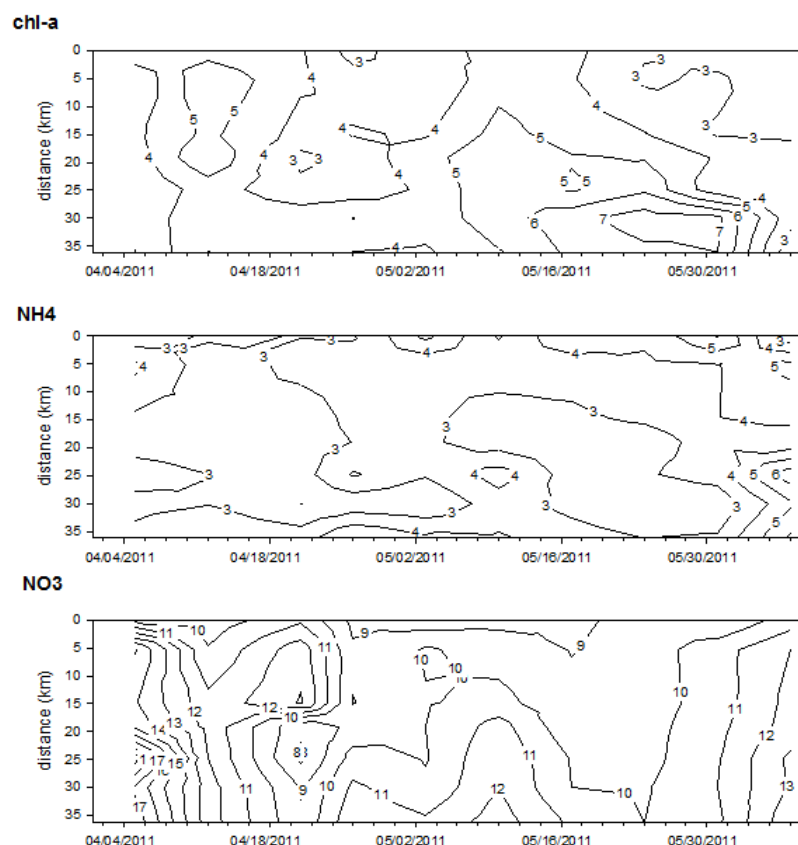


Figure 3.13. Contour plots of NH_4^+ ($\mu\text{mol L}^{-1}$), NO_3^- ($\mu\text{mol L}^{-1}$), and chlorophyll-a ($\mu\text{g L}^{-1}$) concentrations collected by SFSU-RTC on 9 dates during Spring 2011 in Suisun Bay. Data were collected at 7 stations along a roughly linear transect through Suisun Bay between DWR/IEP-D4 and USGS-8. (DWR/IEP D7 not included here – see Fig A.3.2)

Spring phytoplankton biomass (as measured by chl-a concentration) differed considerably between Spring 2010 and Spring 2011. However, both sets of observations can be considered consistent with the NH_4^+ -inhibition conceptual model. In April and May 2010, hydraulic residence times in Suisun Bay were 5-35 days. Dugdale et al. (2012) argued that the flow rates from the Delta were in the appropriate range in Spring 2010 to allow a phytoplankton bloom to occur: flows were low enough (i.e., residence times were sufficiently long) that phytoplankton grew faster than they were flushed from the system (and thus biomass accumulated, i.e., a bloom occurred); and flows were sufficiently high to dilute NH_4^+ down to concentrations that allowed phytoplankton to eventually access NO_3^- and grow more rapidly. Suisun Bay experienced higher flows in Spring 2011 than Spring 2010, and the resulting residence times were shorter (<6 days). Thus, despite the low NH_4^+ concentrations, phytoplankton were flushed from Suisun Bay a faster than they grew, preventing a bloom from occurring..

While the Spring 2010 and Spring 2011 observations can be viewed as consistent with the NH_4^+ -inhibition conceptual model, there are other reasonable explanations for the observed changes that do not require invoking the NH_4^+ -inhibition conceptual model. The spatial and temporal trends in Spring 2010 are also consistent with classical understanding of nutrient utilization and bloom development. As noted earlier, P.1 of the NH_4^+ -inhibition conceptual model – that phytoplankton will tend to utilize NH_4^+ before NO_3^- – is well-established in the scientific literature. Therefore, the argument can be made that phytoplankton first utilized NH_4^+ , and then moved to NO_3^- , and their biomass increased during this time to levels at which substantial increases in chl-a became detectable. Using a ‘rule-of-thumb’ for the amount of dissolved inorganic nitrogen ($\text{DIN} = \text{NH}_4^+ + \text{NO}_3^-$) required to produce new biomass in terms of chl-a ($1 \mu\text{g L}^{-1} \text{ chl-a} : 1 \mu\text{mole L}^{-1} \text{ DIN}$), a 5-10 $\mu\text{g L}^{-1}$ increase in chl-a requires 5-10 $\mu\text{mol L}^{-1}$ of DIN. If one considers an increase in chl-a of 5-10 $\mu\text{g L}^{-1}$ above baseline as constituting a bloom, bloom formation would require a 5-10 $\mu\text{mol L}^{-1}$ decrease in either NH_4^+ or NO_3^- levels. Since NH_4^+ tends to be utilized first by phytoplankton, a coherent bloom may only become evident once 5-10 $\mu\text{mol L}^{-1}$ of NH_4^+ has been utilized, which happens to be an amount that is comparable to typical NH_4^+ levels in February-March (5-10 $\mu\text{mol L}^{-1}$; see Section 6). It stands to reason that elevated chl-a concentrations are only observed once NH_4^+ concentrations are low because NH_4^+ was utilized first to build the biomass that is actually detected as the bloom – not because NH_4^+ inhibited growth.

We also examined data from Suisun Bay prior to 1987 (Fig. 3.14), when substantial blooms were a common occurrence, to see if observations during this period offered correlative evidence to help distinguish between the traditional and NH_4^+ -inhibition conceptual models. Prior to 1987, chl-a concentrations in the range of 10-30 $\mu\text{g L}^{-1}$ were frequently observed for multiple months each year, primarily during spring, summer, and fall months (Fig 3.1 and Figure 3.2). The years 1975-1986 correspond to a period before the invasive clam, *Potamocorbula*, became established in Suisun Bay, and when NH_4^+ concentrations in Suisun Bay were still elevated but before substantial increases in NH_4^+ loads and concentrations in the 1990s and 2000s (see Section 6 for

further discussion). While blooms did tend to occur after NH_4^+ concentrations decreased to their lowest values (typically below $4 \mu\text{mol L}^{-1}$), NO_3^- concentrations also decreased in the lead-up to blooms. Both NH_4^+ and NO_3^- uptake could have been contributing to the early stages of bloom development, with a bloom only becoming evident after sufficient growth occurred that consumed a sizable portion of DIN of either form. Therefore, as was the case for Spring 2010, visual inspection of the does not allow one to distinguish between the competing hypotheses.

In summary, as far as can be determined through correlations among field observations, system behavior with respect to phytoplankton blooms and NH_4^+ concentrations in Spring 2010, Spring 2011, and the period pre-1987 do not allow one to distinguish between the traditional conceptual model of bloom development and the NH_4^+ -inhibition conceptual model. The key question remains whether phytoplankton growth rates were different due to the form of N utilized at different stages in bloom development, and whether such a difference influences the likelihood that a bloom will occur (i.e., P.3 of the NH_4^+ -inhibition conceptual model). In addition to experiments that test the mechanism(s) and quantify C and N uptake rates under environmentally-relevant conditions, modeling will be needed. Through modeling, it will be possible to test the relative importance of processes that may play a role in phytoplankton biomass accumulation: including light limitation, grazing by clams, flushing rates, and the hypothesized role of NH_4^+ -inhibition.

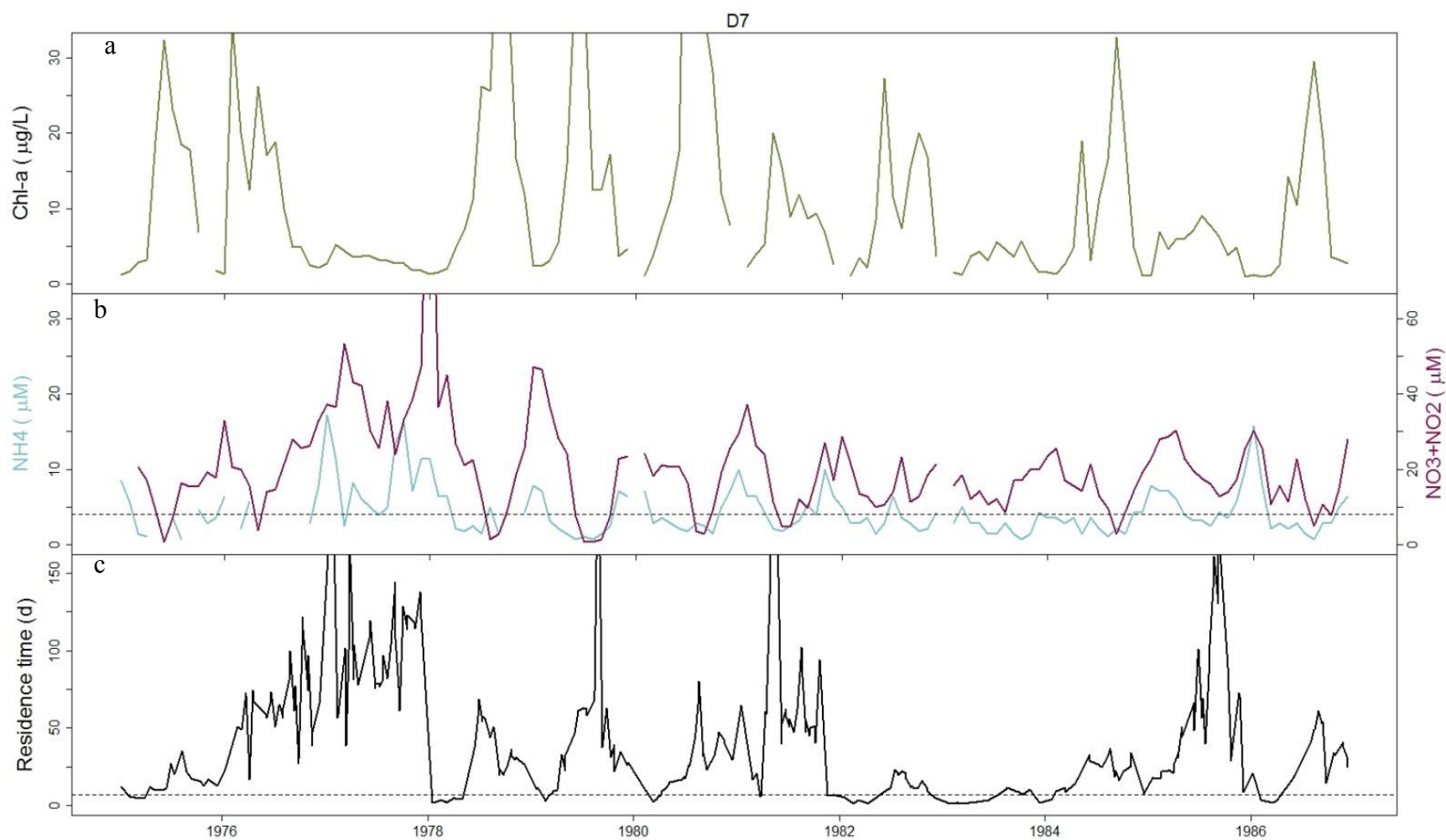


Figure 3.14. Chl-a concentrations (panel a), NH₄⁺ and NO₃⁻ concentrations (panel b) and residence time (panel c) in Suisun Bay for the period 1975-1986. Water quality data is presented for DWR D7 which is located in Grizzly Bay. Residence time was calculated by dividing the volume of Suisun Bay (6.54e11 L) by tidally-corrected flows exiting the Delta (Source for flow data was DAYFLOW).

3.5. Summary

The NH_4^+ -inhibition hypothesis was developed through multiple studies by researchers at San Francisco State University Romberg Tiburon Center over the past decade (e.g., Wilkerson et al., 2006; Dugdale et al., 2007; Parker et al., 2012a, 2012b; Dugdale et al., 2012). The conceptual model for the NH_4^+ -inhibition hypothesis is built around three main points:

- P.1** The presence of NH_4^+ at elevated levels ($>1\text{-}4\ \mu\text{mol L}^{-1}$) inhibits the uptake of nitrate by phytoplankton
- P.2** The rate of NO_3^- uptake (when NH_4^+ is absent or less than $1\text{-}4\ \mu\text{M}$) is greater than the rate of NH_4^+ uptake. Thus, when NO_3^- uptake is suppressed, and only NH_4^+ is being taken up by phytoplankton, the overall rate of N uptake is lower
- P.3** The lower rate of N uptake resulting from this mechanism translates into lower rates of primary production.

There is strong support in the scientific literature for P.1, with numerous studies demonstrating either that multiple species of phytoplankton exhibit a strong preference for NH_4^+ or that NO_3^- uptake is actively inhibited by elevated NH_4^+ concentrations. RTC studies offer convincing support for P.1, with NO_3^- uptake by phytoplankton strongly inhibited when NH_4^+ concentrations exceeds $1\text{-}4\ \mu\text{mol L}^{-1}$.

P.2 is not well-supported by the broader scientific literature on N uptake rates by phytoplankton (see Section 2). Few well-controlled studies have actually investigated N uptake rates during experiments in which both NO_3^- and NH_4^+ were available over a range of concentrations. Thus, there remains a critical gap in the literature on this topic. While there are limited studies that explicitly compare NO_3^- vs. NH_4^+ uptake kinetics, the more broadly accepted concept among phytoplankton ecologists and modelers is that, when nutrients are abundant, the cells access whichever N source is most readily available, and that uptake rates of NO_3^- and NH_4^+ are similar. The RTC studies provide some support for P.2 through enclosure experiments carried out with Bay water and using ambient phytoplankton community assemblages (Parker et al., 2012a), and with one set of uptake kinetic experiments using ambient community assemblages. However, RTC studies also yield some experimental evidence that suggests NH_4^+ uptake rates may be comparable to or even greater than NO_3^- uptake rates under certain conditions. In addition, , uncertainty remains about whether experimental artifacts or other reasonable explanations could explain some of the observations used as evidence in support of P.2. While P.2 remains a plausible hypothesis, additional research is needed to more rigorously establish the NO_3^- and NH_4^+ kinetics under a range of conditions (temperature, light levels), including experiments carried out with mono-cultures of phytoplankton species or taxa commonly present in Suisun Bay, and San Francisco Bay and the Delta more generally.

P3 is not well supported by the broader scientific literature. As with P2, the more broadly accepted concept is that most phytoplankton taxa grow equally well when using NH_4^+ or NO_3^- as their nitrogen source (see Section 2 for further discussion). Multiple studies have found similar growth rates (rates of carbon fixation) across a range of taxa when using NH_4^+ or NO_3^- . While the rate of growth varies with different levels of light, experiments in which monocultures of phytoplankton were grown under different light regimes and different N sources found that growth rate was not strongly dependent on whether NO_3^- or NH_4^+ was provided (see Section 2). As with P.2, few studies have done growth experiments in which phytoplankton have the choice between NH_4^+ and NO_3^- , so there also remains a critical gap in the literature on this related topic. RTC field and enclosure experiments provide some evidence that is consistent with the hypothesis that primary production rates (using rates of C uptake) are slower at high NH_4^+ levels, and that growth rates increase when NH_4^+ is depleted and phytoplankton begin utilizing NO_3^- (Parker et al., 2012a, 2012b). In other studies, primary production rates are inferred from changes in chl-a or assumed to be proportional to the N uptake rate, both of which are prone to considerable uncertainty (due to variations in C:chl-a and C:N). In addition, in some components of RTC studies, experimental artifacts (e.g., acclimation time to light conditions in enclosures) or competing explanations have not been sufficiently ruled out, including the potential role of other contaminants, either co-occurring in treated wastewater effluent, or other sources such as agricultural runoff. Even if P.2 and P.3 are occurring, N uptake and primary production in Suisun Bay appear to behave differently compared to the conceptual model, which was developed largely based on observations in San Pablo and Central Bay (Dugdale et al., 2007; Parker et al., 2012). Dugdale et al. (2007) and Parker et al (2012a) acknowledge the potential role of other factors, such as other contaminants. However, their conclusions about Suisun Bay do not sufficiently address this nuance, or the extent to which the NH_4^+ -based explanations can be readily applied in Suisun Bay. Finally, NH_4^+ levels are present at comparable levels in South San Francisco Bay, and examples of NH_4^+ inhibition of primary production rates have not been documented there.

Similar to P.2, P.3 remains a plausible hypothesis. Inhibition of primary production by elevated NH_4^+ has been proposed as one possible mechanism to explain lower production rates elsewhere (e.g., Delaware Bay; Yoshihama and Sharp, 2006). The RTC studies have tackled the issue with field observations and experimental studies using ambient phytoplankton assemblages, as opposed to pure culture experiments. Their field studies and simulation of field conditions through enclosure experiments with Bay water and ambient phytoplankton communities provide an important perspective on net effects at the field scale. However, the complexity introduced by field conditions or simulated-field conditions, when multiple underlying factors are changing over space or time (e.g., phytoplankton community composition, grazing, acclimation to experimental light conditions, increases or decrease in light attenuation as a function of space in field studies, stratification) can make it difficult to directly evaluate the role of the NH_4^+ inhibition mechanism. Additional research is needed to:

- Determine whether statistically significant differences in primary production rates occur due to the N form utilized. Effort should be directed toward establishing NO_3^- and NH_4^+ uptake kinetics and phytoplankton growth kinetics under a range of conditions (e.g., varying temperature and light levels, varying proportions of NO_3^- and NH_4^+), including experiments carried out with mono-cultures of phytoplankton species or taxa commonly present in Suisun Bay, and San Francisco Bay and the Delta more generally.
- Determine the ecological significance of this mechanism at the ecosystem scale, including understanding the mechanisms and the conditions under which differences in growth rates will occur, and the magnitude of the effect.
- Rule out competing explanations and experimental artifacts in field observations and enclosure experiments.

Some of these research needs are the focus of on-going or proposed studies by RTC researchers, their collaborators, and other research groups. Those studies have not been discussed for this report; therefore, this review may need to be revisited as that data becomes available.

Independent of whether the set of processes laid out in the NH_4^+ -inhibition conceptual model occur as proposed, their potential importance at the ecosystem scale has not been adequately investigated. Other factors are known to play important if not dominant roles in limiting primary production rates (e.g., light limitation) or biomass accumulation (clam grazing, residence time) in Suisun Bay. The RTC studies acknowledge the roles of light limitation and clam grazing; they point out that NH_4^+ inhibition of primary production is an additional factor that limits production when conditions might otherwise allow for blooms to occur. However, this important point sometimes gets lost when the NH_4^+ -inhibition conceptual model is discussed in the context of its management implications. A quantitative analysis of the ecosystem-scale importance of the NH_4^+ -inhibition conceptual model is feasible now, using relatively basic biogeochemical models and existing data, and using parameterizations of the proposed mechanisms. Such modeling efforts would have benefits far beyond testing the NH_4^+ hypothesis, in that they will simultaneously provide a tool for quantitatively synthesizing existing nutrient and phytoplankton data in Suisun Bay and other embayments, identifying data and monitoring needs, and informing the broader modeling strategy for the Bay.

Finally, and as noted in Section 1, changes in the form of nitrogen available to phytoplankton – e.g., NH_4^+ vs. NO_3^- , and changes in N:P – have been hypothesized to influence phytoplankton assemblages in Suisun Bay and the Delta (e.g. Glibert et al., 2011, Glibert et al, 2012), selecting for populations that poorly support food requirements at higher trophic levels, or have direct toxicity (i.e., harmful algal blooms). This is an important topic, but is beyond the scope of this report, and will be addressed in a subsequent report.

3.6. References

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4. Summary of studies exploring ammonium toxicity to copepods and other aquatic invertebrates

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4.1. Introduction

Changes in quality and abundance of food for pelagic fishes has been identified as one potential factor contributing to the recent Pelagic Organism Decline (POD) in the Delta and Suisun Bay. Zooplankton abundance and size have decreased over the last four decades, and these declines in food availability may be exerting bottom-up pressure on the food web (Baxter et al., 2010), since zooplankton are the primary prey for Delta smelt and other pelagic fishes whose decline lie at the center of the POD. High grazing rates by invasive benthos, low food abundance (i.e., low phytoplankton biomass), and direct toxicity of contaminants have been hypothesized to be acting in concert to keep zooplankton populations low. NH_4^+ has been hypothesized to be among the potential contaminants that may adversely affect copepod populations.

In this section, we summarize the results and proposed toxicity mechanisms from a recent study (Teh et al. 2011) that focused on the effects of ammonia/ammonium on a common and ecologically important copepod species found in the northern San Francisco Estuary. This section is intended only as a brief summary, not a thorough review of the study's design or its interpretations, and is included in this report because of its relevance to the ammonium debate. The summary is also included to provide context for the comparison in Section 6 of ambient NH_4^+ concentrations in Suisun Bay with toxicity thresholds identified by Teh et al. (2011).

4.2. NH_4^+ toxicity on *Pseudodiaptomus forbesi*

Teh et al. (2011) explored the acute and chronic effects of ammonia/ammonium on *Pseudodiaptomus forbesi*. The overall results are summarized in Table 1. *P. forbesi* is of particular interest, because studies examining gut contents of larval fish captured in the Delta-Suisun system found that, during most times of the year, *P. forbesi* was the dominant food source for all fish that have shown declining populations (delta smelt, longfin smelt, striped bass and threadfin shad; as noted in Teh et al., 2011). As such, factors that adversely affect *P. forbesi* abundance have the potential to substantially affect Delta-Suisun food webs.

Teh et al. (2011) reported that at a fixed concentration of total ammonia nitrogen (TAN; $\text{TAN} = \text{NH}_3 + \text{NH}_4^+$) of 5ppm survival of *P. forbesi* decreased to 30% as pH increased to 8.6. Teh et al (2011) hypothesized that decreasing survival resulted from the increasing fraction of TAN that was present as NH_3 , the TAN form that is known to be toxic to fish and other aquatic species. NH_3 is expelled from many aquatic organisms by passive diffusion, and higher external NH_3 concentrations reduces this efflux, allowing toxic levels of NH_3 to accumulate internally (Armstrong 1978). These results from Teh et al. (2011) agree with previous studies that have found decreasing NH_3 LC50 as pH increases (Sullivan and Ritacco, 1985; Wang et al, 2008).

Teh et al (2011) also observed decreased survival of *P. forbesi* as pH decreased. Under experimental conditions of pH = 7.8 and TAN = 8 ppm, survival was 36%, whereas survival was nearly 0% when pH = 7.4 and TAN = 8 ppm.

Table 4.1. Subset of Teh et al (2011) results for typical Suisun Bay pH values.

Study Goal	Results	Notes
6-day LC on juvenile <i>P. forbesi</i> at pH 7.8, 20°C	LC ₅ = 3.374 mg/L TAN LC ₁₀ = 3.834 mg/L TAN LC ₅₀ = 6.014 mg/L TAN	No toxicity observed after 4 days
4-day LC on juvenile <i>P. forbesi</i> at pH 7.4, 20°C	LC ₅ = 1.703 mg/L TAN LC ₁₀ = 1.924 mg/L TAN LC ₅₀ = 2.960 mg/L TAN	<i>P. forbesi</i> are more sensitive at lower pH
4-day LC on nauplii (larval) <i>P. forbesi</i> at pH 7.8, 20°C	LC ₅ = 0.591 mg/L TAN LC ₁₀ = 0.731 mg/L TAN LC ₅₀ = 1.547 mg/L TAN	Nauplii <i>P. forbesi</i> are more sensitive than juvenile <i>P. forbesi</i>
Chronic effects over 31-day life cycle	LOEL = 0.36 mg/L	NOEL is unknown, but is < 0.36 mg/L
Reproductive fitness of gravid female	# offspring at 0 mg/L TAN = 7.6 # offspring at 0.38 mg/L TAN = 5.5 # offspring at 0.38 mg/L TAN = 5.4	

In a chronic toxicity 31-day life cycle test, Teh et al. (2011) observed that gravid females either produced significantly lower numbers of nauplii or survival of nauplii and juveniles to adulthood was significantly lower when they were exposed to NH_4^+ at levels as low as 0.36 mg N L⁻¹ (26 $\mu\text{mol L}^{-1}$). Since 26 $\mu\text{mol L}^{-1}$ was also the lowest dose used in the study, 26 $\mu\text{mol L}^{-1}$ was the lowest observed effect level (LOEL), and a no observed effect level (NOEL) was not established.

4.3. Other ammonium toxicity studies

A limited literature search found that few studies have explored direct ammonium toxicity to copepods. However, there are multiple studies of NH_4^+ toxicity to other aquatic invertebrates, such as arthropods and crustaceans. Several studies have documented that at certain pH values, TAN is a better predictor than NH_3 of acute toxicity, and have suggested that toxicity may be exerted by both NH_3 and NH_4^+ on a variety of aquatic invertebrates (Armstrong 1978, Erickson 1985, Borgmann 1993, Kater 2006). Other studies have demonstrated that NH_4^+ toxicity decreases as cation concentrations increase in the test water (Borgmann 1993, Ankley

1995, Borgmann 1996). It should be noted that these studies reported acute effects, not chronic effects, and even the lowest reported LC50 (approximately 100 μM for *Hyaella azteca*, Borgmann 1996) is an order of magnitude above typical ambient ammonium concentrations in Suisun Bay.

None of the above studies involved copepods. Studies that have involved copepods (species of the genus *Arcatia*, another common copepod species in Suisun Bay) have either evaluated the toxicity of the unionized form (Sullivan and Ritacco, 1985) or did not specify pH, so the partitioning of TAN between ionized and unionized forms is unknown (Buttoni 1994). Buttoni observed an LC₅₀ on adult females of 0.91 mg/L TAN, and survival of eggs produced by females exposed to 0.12 mg/L TAN was lower by nearly a factor of 2 after 9 days than those of females exposed to 0 mg/L TAN. However, no pH was reported.

4.4. Mechanism of NH_4^+ toxicity

The exact process of NH_4^+ toxicity has not been well studied in copepods, but there have been some efforts to characterize this mechanism in other crustaceans. Armstrong et al (1978) proposed that NH_4^+ may interfere with the normal functioning of Na^+/K^+ pumps embedded in the membranes of gill epithelium cells of the larval prawn *Macrobrachium rosenbergii*. In a normally functioning pump, Na^+ is actively transported into the cell and K^+ or waste N in the form of NH_4^+ (which can substitute for K^+) are transported out of the cell. In crustaceans, more than half of waste nitrogen is expelled as NH_4^+ (Regnault 1986), which is different from fish and other aquatic organisms that excrete N primarily in the form of NH_3 . Teh et al. (2011) hypothesize that higher NH_4^+ concentrations on the exterior of the cell may limit the net export of waste NH_4^+ from the organism by decreasing the concentration gradient (Teh et al. 2011). This model has also been adopted to explain observed effects in crustacean species, such as changes in Na^+ influx by membrane bound pumps of the Chinese crab *Erocheir sinensis* in the presence of elevated ammonium concentrations (Pequeux and Gilles, 1981).

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5. Synthesis of information on zooplankton of the upper San Francisco Estuary

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5.1. Introduction

The foodweb of the northern San Francisco Estuary has suffered a long-term decline in productivity at nearly all trophic levels. These include phytoplankton (Alpine and Cloern 1992, Jassby 2008), zooplankton including rotifers, cladocera, and some copepods (Kimmerer and Orsi 1996, Winder and Jassby 2010), mysids (Orsi and Mecum 1996), and many fish including delta and longfin smelt, striped bass, and northern anchovy (Kimmerer 2002, 2006, Sommer et al. 2007, Thomson et al. 2010). Although the general decline has occurred over several decades, two particular events are noteworthy. The first was the sharp decline of many species around 1987, when the introduced overbite clam *Corbula amurensis* became abundant (Alpine and Cloern 1992, Orsi and Mecum 1996, Kimmerer and Orsi 1996). The second was the Pelagic Organism Decline of several fish species which occurred around 2002 (Sommer et al. 2007, Thomson et al. 2010). The decline in copepod biomass and changes in copepod species composition have been identified as potentially contributing to this decline in pelagic fishes (Baxter et al. 2010).

There are several complementary or competing hypotheses about limits on productivity and long-term declines in the system, each of which has significant ramifications for the actions that would most effectively restore estuarine productivity and recover listed species of fish, as well as for water-project operations. There has been a long tradition of attributing problems in the estuary to impacts from the water projects, although the actual magnitude and importance of those effects has been difficult to pin down (e.g., Stevens et al. 1985, Kimmerer et al. 2001, Jassby et al. 2002, Kimmerer 2008, 2011, Miller 2011). Species introductions have clearly had an effect, including that of *C. amurensis* and those of several copepod species to be discussed below. The potential role of nutrient loading in limiting phytoplankton production through inhibition of nitrate uptake (Wilkerson et al. 2006, Dugdale et al. 2007) has not been resolved, and has proved difficult to resolve because of the influence of clams and the severe light limitation throughout most of the northern estuary (Kimmerer et al. 2012).

This section presents a synthesis of the ecology of zooplankton in the upper San Francisco Estuary. The geographic focus is mostly Suisun Bay and the Low-Salinity Zone (LSZ), although information from other regions is brought into the discussion where needed. The taxonomic focus is mainly copepods, because of their dominance of the mesozooplankton (~0.2 – 2mm length) and their importance in the diets of fish, and because we have more information about them than other groups such as rotifers and cladocera.

This section is to be part of a larger synthesis report on research and monitoring data related to changes in the low-salinity environment of the estuary and the mechanisms that may underlie these changes. While numerous factors potentially contribute to ecosystem declines in the upper estuary (Baxter et al. 2010), this particular report focuses on the potential role of nutrients, and specifically ammonium, in causing changes in the estuarine foodweb. More broadly we are interested in understanding the roles of various potential causes of change in the system and how they interact, and providing background information to inform potential management actions that are under consideration to mitigate some of the potential causes of decline.

A main goal of this report is to put these changes in a historical and ecosystem context, to serve as a foundation from which to consider the impact of various stressors on copepods. This section first identifies sources of information about zooplankton, then provides an overview of key species and a history of species introduction that have played a role in shaping current community composition. Next, life-history descriptions are presented for copepods in general, and for key species of the SFE. This information provides important background for interpreting seasonal, temporal, and spatial variations in copepod abundance and composition in Suisun Bay, for interpreting results of past studies, and for designing future studies of population dynamics, ecotoxicology, and abundance of copepods. Factors including both natural processes and anthropogenic pressures that influence copepod abundance are then discussed, including an overview of hypothesized pathways through which nutrients could exert pressure on copepod abundance, biomass, and community composition. The section closes with an overview of research and monitoring needs.

5.2. Sources of Information

Information for this report comes from monitoring data, published papers, and unpublished experimental and field data (see Appendix 5.1). The earliest examinations of zooplankton in the SFE reported that the most abundant taxon was *Paralabidocera* (which does not occur in temperate waters, so this probably refers to *Epilabidocera*) followed by *Calanus* (Esterly 1924, Aplin 1967). Neither study provided details of abundance. Both species are large (>2 mm), so their high relative abundance implies that both studies had used large-mesh nets. Aplin (1967) used a plankton net with an aperture of ~ 0.8mm. By contrast, all of the studies included in this analysis used mesh sizes of 150 μm or smaller (Table 5.1). In all of the more recent studies *Acartia* spp. vastly outnumbered other copepods, reinforcing the importance of using a suitably fine-mesh net for plankton studies even in estuaries (Turner 2004).

The principal source of monitoring data is the Interagency Ecological Program (IEP) zooplankton monitoring program (Orsi and Mecum 1986; Table 5.1). This program has been sampling the estuary since 1972 with relatively few changes in sampling design or methods, and a consistently high level of expertise in discovering and then identifying new species. For example, the species description of the copepod *Oithona davisae* was published based on specimens from the SFE (Ferrari and Orsi 1984), even though the species is native to Japan which has a strong tradition of high-quality marine science and taxonomy.

IEP monitoring does have a few drawbacks: 1) It does not sample in Central or South San Francisco Bays and until 1998 did not sample routinely in San Pablo Bay; 2) Many taxonomic groups are not identified to species, although most of the copepods are; and 3) Until 2008 the pump sampler used to collect small (45 – 150 μm) organisms such as copepod nauplii took a very small sample so that a single individual represented about 500 m^{-3} in the estuary, with the result that much of the data give only crude estimates of abundance unless large numbers of samples are aggregated.

Table 5.1. Attributes of sampling programs. The number of stations is the total number sampled in at least 10% of the surveys for which data are available. Data used from IEP surveys are from Suisun and San Pablo Bays and the western Delta (about half of the data). NS, not stated

* 846 all stations; 439 samples in the region of this study

Attribute	Painter 1966	Caskey 1976	IEP	Ambler 1985	Kimmerer unpubl.
Years	1963	1972-1974	1972-present	1978-1981	Sept 1997- Jan 1999
Regions	San Pablo Bay – W. Delta	South Bay - western Delta	San Pablo Bay - Delta	South Bay - western Delta	South Bay - San Pablo Bay
Stations	12	13	41	32	30
Total Samples	383	172	19,984	846 (439)*	422
Sampling method	Horizontal tow with net	Oblique tow with ½-meter net	Oblique net tow, vertically integrated pump sample	Pump @ nominally 3 depths	Vert. (channel) or surface (shoal) tow, ½-meter net
Mesh, µm	150	140	150 (net), 43 (pump)	64 or 80	150
Sample Volume, m ³	NS, 5-10	NS, probably > 10	Net mean 7, pump 1.5-1.8 L	1.5	Vertical: median 2.6; Surface 35
Processing	NS	Entire sample for large orgs., remainder subsampled.	Net sample subsampled; entire pump sample	Some subsampled; averaged over depths	Subsampled
Taxonomic details			<i>Acartia</i> spp. not distinguished	<i>Acartia hudsonica</i> as <i>A. clausi</i>	<i>Acartia</i> spp. distinguished in 109 samples
Data avail.	No	No	Yes	Yes	Yes

Additional monitoring data are available from some of the fish surveys, notably the 20-mm survey for young delta smelt (Dege and Brown 2004). The USGS conducted a study of zooplankton abundance throughout the estuary in 1978-1981 (Ambler et al. 1985), and there have been a few other short-term studies since, mostly focused on the saltier parts of the estuary (Bollens et al. 2011, Kimmerer unpublished).

Monitoring data tell us a lot about the long-term trends in distribution and abundance but relatively little about the processes that underlie these patterns. Mechanistic studies have been

done on zooplankton only in the last ~20 years, and the pace of discovery has increased in recent years. Now modeling is beginning to provide useful insights to complement these other approaches, and overall there is now a small but active and well-linked community of scientists engaged in understanding these organisms.

5.3. The key species: native and introduced

The class Copepoda comprises about ten orders, of which four are common in the San Francisco Estuary. Three (Calanoida, Cyclopoida, and Harpacticoida) have abundant representatives throughout the estuary, but most of the harpacticoids are benthic and represented in the water column only by juvenile stages which have not been identified to species. The pelagic harpacticoid *Euterpina acutifrons* is abundant in saline waters, as is one common Siphonostomatoid (*Corycaeus anglicus*). Because of these distributions the remaining discussion concerns the calanoids and cyclopoids.

In most estuaries the copepod fauna is depauperate compared with the fauna of the adjacent ocean. This is true in the SFE, but introductions have raised species diversity and transformed the species composition of the upper estuary (Fig. 5.1, Table 5.2, see Winder and Jassby 2010). The majority of dominant copepod species – both in terms of abundance (organisms L⁻¹) and biomass (µg C L⁻¹) – in the northern SFE are introduced species. The current levels of abundance are much higher than they were before the introduction of the small cyclopoid copepods *Limnithona sinensis* in freshwater in 1979 and *L. tetraspina* in brackish water in 1993 (Fig. 5.1). However, biomass has declined slightly because these small copepods are about 10% of the mass of the other common copepods in the region.

All of the copepod introductions came ultimately from Asia, and the species assemblage of the upper estuary has been referred to as an "eastern Asian fauna" (Orsi and Ohtsuka 1999). Ballast water is a likely vector for most of the introductions, although *Pseudodiaptomus marinus* may have come with the transport of shellfish for aquaculture, as apparently happened in Hawaii and several small estuaries in California (Jones 1964, Fleminger and Kramer 1988, Kimmerer 1993, Orsi and Walter 1991).

The introductions came over a limited number of years, with no introductions during the first 6 or latest 18 years of the sampling program (1972-present). The invasion-heavy period is roughly the time period when shipping traffic from Asia was high and regulations requiring ballast treatment (e.g., exchange at sea) were not yet in place (Carlton et al 1990, Choi et al. 2005).

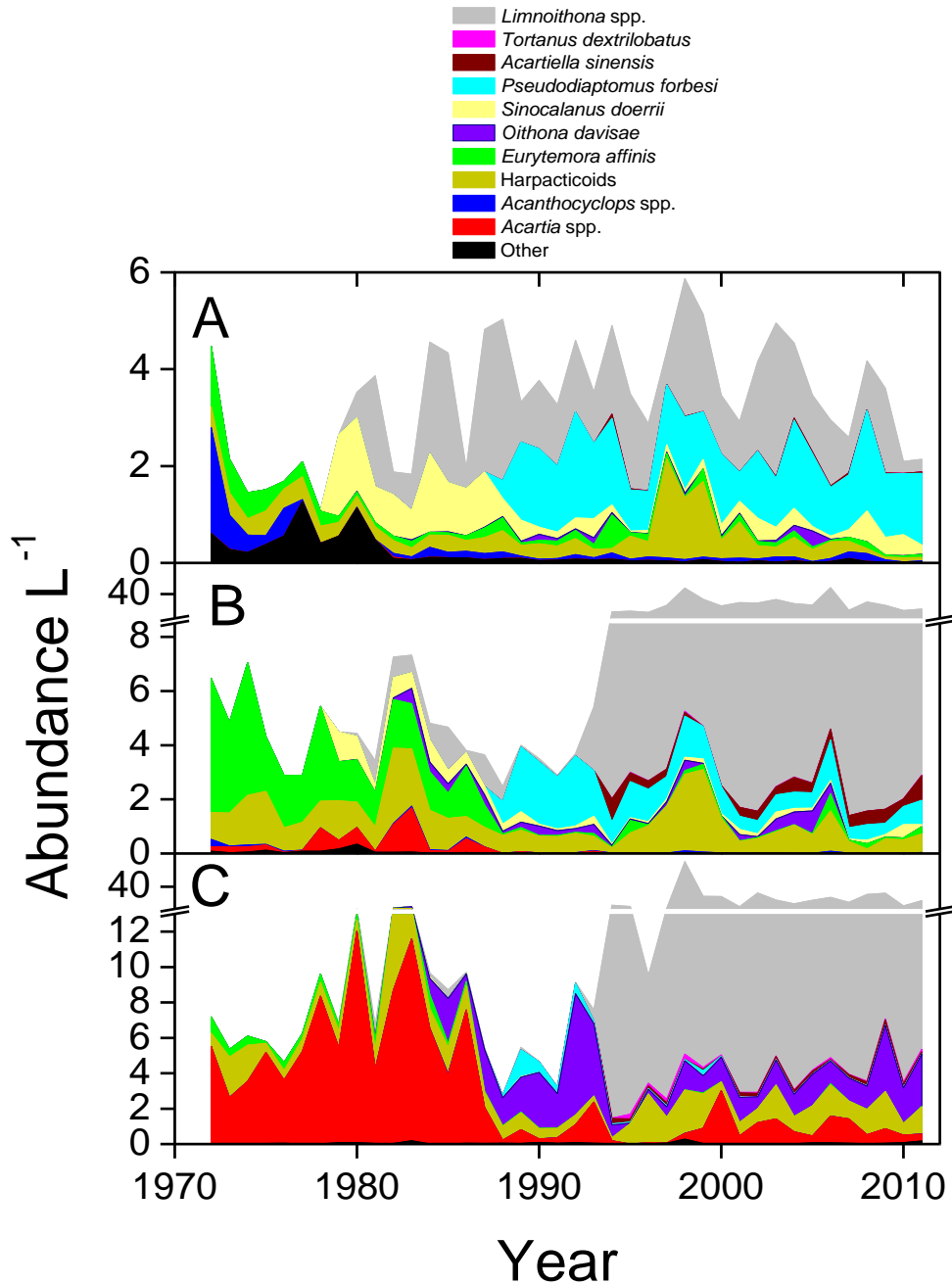


Figure 5.1. Cumulative abundance of adult copepods in three salinity ranges: A, <0.5, B, Low-Salinity Zone at 0.5 - 6, and C, >6. Immature stages have been excluded because nauplii have not been consistently identified to species, and copepodites only in some years. Copepod species are ordered vertically by approximate time of introduction.

Thus, this pattern could be seen as series of more or less random events during a period of vulnerability. However, the introductions of *Pseudodiaptomus forbesi*, *Limnoithona tetraspina*, and *Acartiella sinensis* were probably facilitated by the intensive grazing pressure due to the invasive clam *Corbula amurensis* (see life history discussion below).

Table 5.2. Copepod and mysid introductions to the San Francisco Estuary in approximate order of introduction. Salinity is the reported value where the species was taken, not necessarily where it is most abundant.

Species	Date of first capture	Location	Salinity	Likely source	Reference
<i>Eurytemora affinis</i>	1879?	Upper estuary	<5	Eastern U.S.	Lee 2000
<i>Deltamysis holmquistae</i>	August 1977	Not stated	Not stated	Not stated	Bowman and Orsi 1992
<i>Sinocalanus doerrii</i>	May 1978	Confluence	3.4	Asia	Orsi et al. 1983
<i>Limnoithona sinensis</i>	August 1979	Stockton	~0	China	Ferrari and Orsi 1984
<i>Oithona davisae</i>	October 1979 or before 1963	Suisun Bay	>12	Japan	Ferrari and Orsi 1984
<i>Pseudodiaptomus marinus</i>	October 1986	Suisun Bay	6-8	Japan	Orsi and Walter 1991
<i>Pseudodiaptomus forbesi</i>	October 1987	San Joaquin R.	~0	China	Orsi and Walter 1991
<i>Acanthomysis aspera</i>	August 1992	Suisun Bay	Not stated	Korea, Japan	Modlin and Orsi 1997
<i>Hyperacanthomysis longirostris</i>	July 1993	Suisun Bay	Not stated	China, Korea?	Modlin and Orsi 1997
<i>Tortanus dextrilobatus</i>	August 1993	Suisun Bay	3.6	China	Orsi and Ohtsuka 1999
<i>Limnoithona tetraspina</i>	September 1993	Suisun Bay	1-3.8	China	Orsi and Ohtsuka 1999
<i>Acartiella sinensis</i>	October 1993	Suisun Slough	2.8-4.6	China	Orsi and Ohtsuka 1999
<i>Acanthomysis hwanhaiensis</i>	September 1997	San Pablo Bay	10-30	Korea	Modlin and Orsi 2000

An alternative explanation for the apparently non-random temporal pattern of introductions related the introductions to drought exacerbated by water withdrawals from the watershed (Winder et al. 2011). There are several problems with this interpretation. The analysis of Winder et al. used a flow variable averaged over 3 years up to the year of introduction, but these copepods go through their entire life cycles in under 2-4 weeks in summer (Gould and Kimmerer 2010, Kimmerer and Gould 2010, L. Sullivan, SFSU, unpubl.). Many species in the estuary with longer life cycles (e.g., clams, Thompson 2005; fish, Sommer et al. 1997, Kimmerer 2002) respond within a year to interannual changes in freshwater flow. Thus, this averaging period is far too long, and a shorter averaging period results in no pattern. Furthermore, there is no conceivable mechanism by which copepods would respond to drought, and the abundance patterns of the copepods now in the estuary or abundant in the past do not do so (e.g., Kimmerer 2002, Kimmerer et al. 2009).

It is helpful to distinguish introduction events as a class of drivers of change from the continuing dominance of non-native species in some estuarine habitats. Introduction events can cause a step change in the ecosystem that is usually viewed as catastrophic. Although some introduced

species overshoot in abundance and then settle down to some background level (e.g., mitten crabs, Rudnick et al. 2003), the introduced zooplankton seem to have become established and then remained so until another introduction caused a readjustment. Once established the introduced species play some species-specific role in the foodweb, and there is no evidence (at least for zooplankton) that introduced species as a group are more or less suitable in those roles, particularly as prey for fish. Thus, a general category of "introduced species" is not helpful in explaining changes or low productivity, for which it is necessary to examine the characteristics of each species.

5.4. Life histories

Copepods are probably the most abundant animals on earth and occupy a key place in pelagic foodwebs. They are important consumers of organic particles in the 5-100 μm range, which includes most of the biomass of phytoplankton and microzooplankton. They are also the main food for early life stages of most fish, and through much of the lives of some fish such as delta smelt (Nobriga 2002, Feyrer et al. 2003, S.Slater CDFG pers. comm.).

Pelagic copepods have a conservative life history consisting of an egg, six nauplius (larval) stages, five copepodite (juvenile) stages, and the adult stage. Adults are sexually dimorphic, and the last one or two copepodite stages have some dimorphic features. Males hunt for females and, if successful, grasp the females and transfer a sac called a spermatophore to initiate fertilization. Reproduction is by broadcast spawning, i.e., releasing single eggs or groups of eggs into the water, or by carrying one or two clutches of eggs in egg sacs until they hatch. The eggs develop over one to a few days depending on temperature. Development time through the post-hatching life stages is species-specific and similarly temperature-dependent to that of the eggs, but is often lengthened by food limitation.

Adult copepods and copepodites all have six pairs of appendages used to detect and consume food, and four or five pairs of paddle-like swimming legs ("copepod" is from the Greek κουπί-πόδι, "paddle-foot"). Similar morphology of these appendages among species within a genus usually means generally similar feeding mode and swimming behavior. The feeding appendages can have sensory apparatus to detect chemical compounds (analogous to a sense of smell) and hydrodynamic disturbance, which may be used in feeding, mate finding, and detection of predator attacks. The fifth swimming leg of adults is used in mating and is usually sexually dimorphic, and therefore a good character for distinguishing species.

Use of Habitat Planktonic animals live in a moving frame of reference and are not tied to any geographic location, but rather to a range of salinity and other water properties, and are influenced by spatial patterns of food supply and predation. In considering the habitat of estuarine zooplankton it is helpful to consider a particular salinity range rather than a geographic region. This range can be linked to X2, the distance up the axis of the estuary measured from the Golden Gate to a salinity of 2 (Jassby et al. 1995). X2 is inversely related to freshwater flow and is a measure of the physical response of the estuary to freshwater flow, but is also a handy gauge

of the position of any salinity range and therefore where a particular species is likely to be most abundant.

The dynamic aspect of copepod populations is illustrated by comparing the relationships between X2 and abundance of *Eurytemora affinis* from the IEP monitoring program at two fixed stations (Fig. 5.2A and 5.2C) and at the station defined by salinity closest to 3.5 (Fig. 5.2B). Either of the fixed stations gives an incorrect picture of the relationship of abundance to flow, which is actually negligible when examined in the copepod's salinity-based frame of reference (Fig. 5.2B).

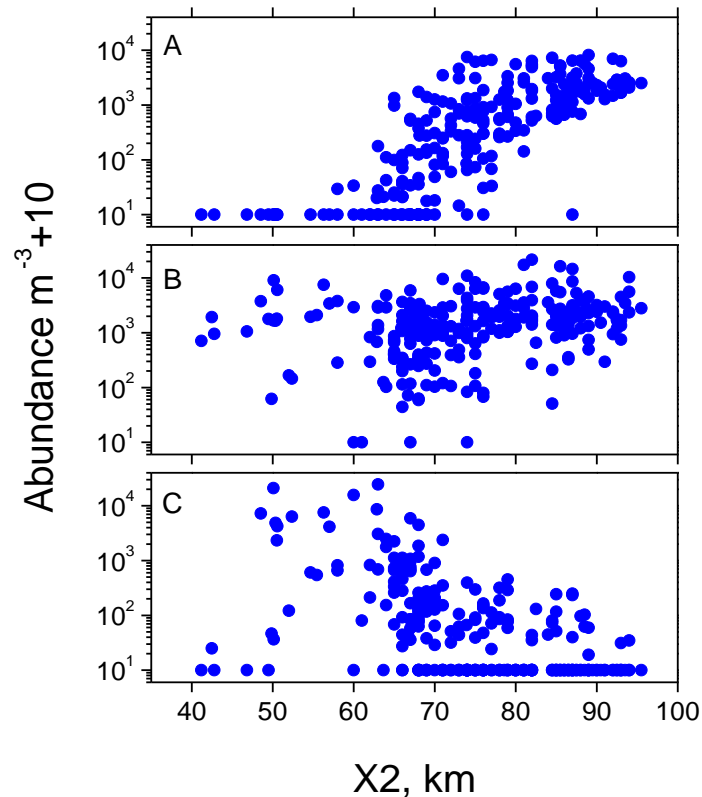


Figure 5.2. Abundance of *Eurytemora affinis* during 1972-1986 vs. X2. Data from: A, station NZ062 on the lower Sacramento River; B, the station in each survey with salinity closest to 3.5, the approximate salinity where the abundance of *E. affinis* was highest; C, station NZ020 in western Suisun Bay.

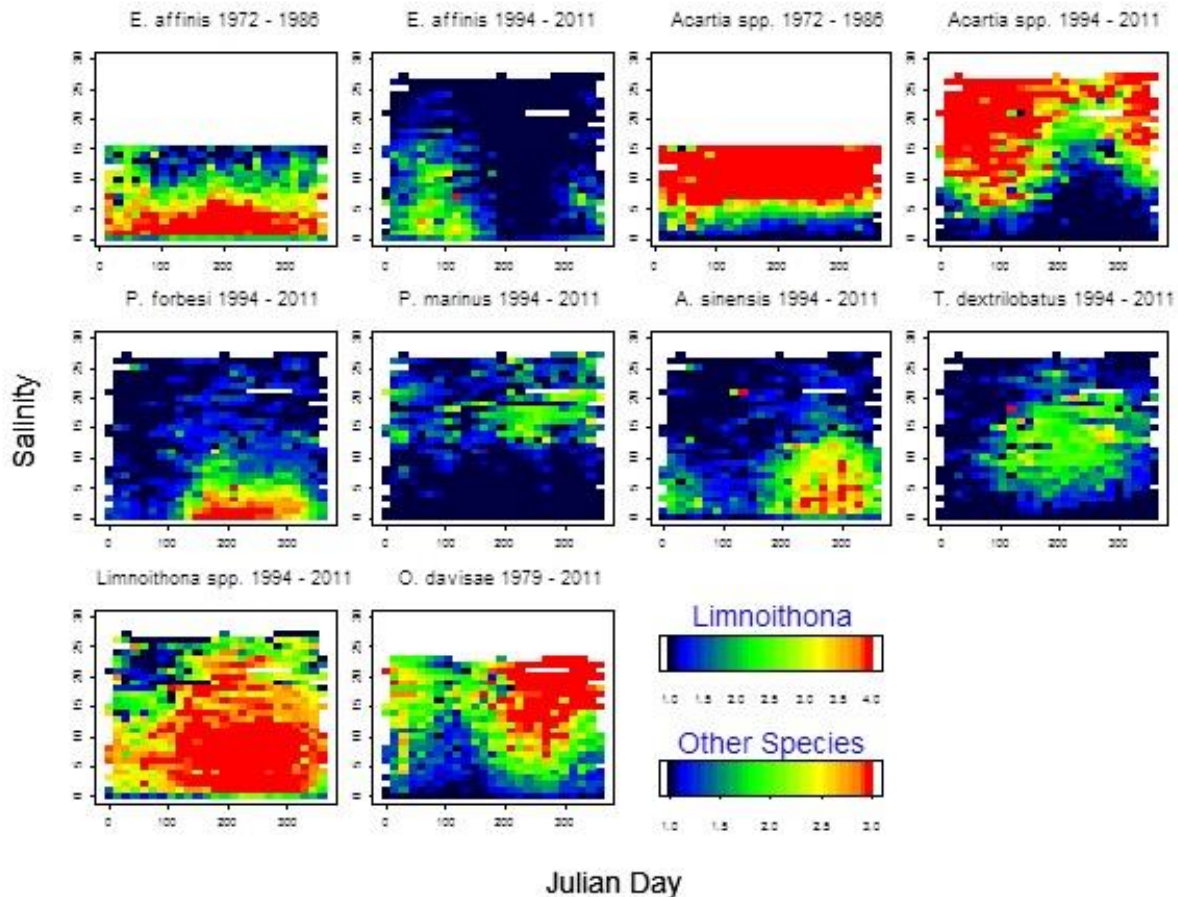


Figure 5.3 Image plots of \log_{10} (abundance + 10) of common zooplankton species by Julian day and salinity. Note that the lowest salinity band (0-1) includes large areas of freshwater containing few copepods. The highest salinity bands are cut off for early years because those salinities were not sampled consistently before ~1994. Note that the upper limit for *Limnoithona* is 10-fold higher than that for other species.

Figure 5.1 is plotted by salinity range because zooplankton generally are arranged in the estuary according to salinity (Fig. 5.3). This arrangement is only partly a result of salinity tolerance, because many zooplankton species can tolerate a wide range of salinity. For example, members of the global species complex *Eurytemora affinis* are most often found in low-salinity regions of estuaries (Lee 2000) but experiments have invariably shown good survival, reproduction, or growth across a wide range of salinity (Roddie et al. 1984, Nagaraj 1992, Kimmel and Bradley 2001). Rather, this pattern likely arises through a combination of retention mechanisms (see below), spatially variable mortality, and salinity tolerance.

Responses to temperature usually take the form of seasonal cycles of abundance, which in the SFE almost universally result in high abundance in summer and low in winter. This pattern is predominant for some species of warm-temperate to subtropical origin; for example,

Pseudodiaptomus forbesi is very abundant in spring through autumn but rare in winter, and laboratory experiments show poor reproduction at temperature below ~16°C (L. Sullivan²).

Many planktonic organisms respond to light, avoiding surface waters by day, usually to avoid visual predators (Bollens and Frost 1991). *Eurytemora affinis* and *Pseudodiaptomus* spp. in many estuaries remain on or near the bottom by day, probably also for predator avoidance (Fancett and Kimmerer 1985, Vuorinen 1987), but in the upper SFE these species occur throughout the water column by day and night (Kimmerer et al. 1998). Turbidity may be high enough to make much of the water column too dark for visual planktivores to see their prey. Some copepods, notably *Eurytemora affinis*, are associated with estuarine turbidity maxima (e.g., Morgan et al. 1997), which may provide shelter from visual predators. However, turbidity maxima usually occur in the LSZ, so it is difficult to distinguish the relative importance of turbidity, salinity, and retention mechanisms for maintaining the abundance maximum.

Responses to other water quality variables are less well known. Interest in the effect of pH is growing because of concerns over ocean acidification, and there is some evidence of negative effects on copepods (e.g., Fitzer et al. 2012). However, the pH in an estuary is often highly variable because of variations in inputs by rivers and wastewater and variation due to diurnal cycles of primary production and respiration.

Movement of organisms through water depends on the Reynolds number (Re), the ratio of inertial forces to viscous forces on the organism. At $Re \gg 1$ inertial forces prevail and organisms move by accelerating water to overcome drag that becomes increasingly turbulent as Re becomes larger. At $Re \ll 1$ viscous forces prevail and organisms move by pulling themselves through the water. By virtue of their size (~1 mm for adults of most species in the SFE) copepods live on the boundary between the "viscous world" where interactions are mediated by the movement of the water and particles in it, and the "inertial world" where interactions are governed by speed of attack and escape (Naganuma 1996). Thus, copepods have sensory and feeding appendages with which to detect and feed on particles moving in a viscous medium (Yen 2000). Viscous drag is an important characteristic of the environment that makes the flow field laminar and inhibits mixing (Koehl and Strickler 1981). However, the calanoid copepods have escape mechanisms by which to accelerate very briefly to about 1000 body lengths/second (Kiørboe et al. 2010), putting them squarely in the inertial world and enabling them to avoid attacks by visual and suction predators. This is probably the fastest swimming speed of any aquatic animal for its size; by comparison, scombroid fishes (tunas, sailfish) have a maximum swimming speed of around 10 body lengths/second (Walters and Fierstin 1964).

Retention mechanisms Estuaries can be difficult places for planktonic organisms to live because of the seaward transport due to river flow and tidal mixing. All estuarine resident organisms must

² Names in parentheses refer to unpublished data collected by these researchers in my laboratory.

have mechanisms for overcoming these losses. Most small organisms (e.g., phytoplankton, bacteria, microzooplankton such as ciliates) have high enough growth rates to overcome these losses and maintain population abundance during some seasons. These rates are lowest in winter and insufficient to overcome winter flood flows, and small planktonic organisms are likely reseeded into the main body of the estuary following floods from peripheral habitats of longer residence time.

Copepods and other larger planktonic organisms often have behavioral mechanisms that favor retention within the estuary. These include tidally-timed vertical migration by which the organisms are higher in the water column on the flood than on the ebb (Kimmerer et al. 1998, 2002, Bennett et al. 2002). In the presence of sheared tidal currents this can result in a reduction of seaward transport or retention (Kimmerer et al. 1998). A bottom-oriented behavior can also retain plankton within the estuary, as happens with sinking sediment, because of cells of gravitational circulation in deeper parts of the estuary (i.e., in salinity-stratified water, the tidally-averaged velocity near the bottom can be landward while the surface velocity is seaward, Schoellhamer 1998, Monismith et al. 2002). Gravitational circulation is common only in deep waters of the SFE, and is uncommon in the shallow Suisun Bay where tidal currents cause strong vertical mixing that inhibits stratification.

The observed tidal migration of copepods and larval fish appeared to be insufficient to retain the organisms within Suisun Bay (Kimmerer et al. 1998). However, ongoing work with particle-tracking models shows that these observed behaviors as well as the bottom-orientation of mysids and shrimp can result in retention within the LSZ because of interactions between Suisun Bay and deeper regions such as Carquinez Strait (Kimmerer et al. in prep).

Feeding Copepods have several different methods for feeding, all of which allow for strong selection for certain food types. Feeding may involve detecting food particles through contact with feeding appendages. However, because viscous forces predominate at the scale of feeding appendages, chemical or vibration signals emanating from food particles can be detected some distance away from the appendages. Chemical signals propagate by (slow) molecular diffusion, but if the copepod produces a feeding current or the food particle is swimming the resulting shear can stretch the chemical signal quickly and allow detection from a considerable distance.

Some copepod genera set up a feeding current by beating their feeding appendages, and capture particles out of the water while swimming (*Pseudodiaptomus* spp., *Eurytemora affinis*) or slowly sinking through the water with intermittent upward hops (hop-and-sink, *Acartia* spp.). In this feeding mode the copepod "scans" the water for food particles (Kiørboe 2011) but does not filter the particles, since in the viscous fluid at that small scale the feeding appendages act as paddles rather than filters (Koehl and Strickler 1981). Particles may be captured by squeezing water out between the setae on the appendages, and particles may be actively grabbed or brushed away by a feeding appendage (Koehl and Strickler 1981, Kiørboe 2011).

Some copepods cruise through the water detecting prey organisms (*Acartiella*, *Tortanus*), while others hang motionless in the water and ambush swimming organisms that come near (*Oithona*

davisae, *Limnoithona*). The latter mode is effective at capturing motile organisms, most of which have some capability to detect and avoid a feeding current.

The food taxa consumed by copepods of a given species depends on food availability, size, swimming and escape behavior, chemical composition, and the availability of alternative food (Kiørboe 2011). The influence of chemical composition of the food consumed by copepods may be particularly important because the chemical composition of copepods is relatively consistent, particularly within a species, while that of their food can vary tremendously (Laspoumaderes et al. 2010). Constancy within a population implies strong homeostatic mechanisms for feeding and assimilation. This may involve active selection of food particles based on their chemical signals, or differential assimilation of compounds and nutrients depending on the requirements of the copepod for growth and maintenance. The complexity and, in most cases, invisibility of these selective processes makes predictions difficult about what a given species will eat in any situation, and helps to explain why the thousands of papers on feeding have not led to a general, predictive theory (Kiørboe 2011).

The suitability of diatoms as food for copepods is the subject of substantial work and considerable controversy. The world's most productive marine ecosystems are supported by intense diatom blooms, implying that these blooms are a valuable food source for copepods and other zooplankton. However, many studies have shown diatoms to be either nutritionally inadequate or even toxic to copepods (Ask et al. 2006, Ianora and Miralto 2010), although others have not (Irigoien et al. 2000, Sommer 2009). There is even considerable variability in suitability as food within a single diatom species, as shown in experiments with different clones of the diatom *Skeletonema costatum* being consumed by *Eurytemora affinis* (Ask et al. 2006), both important species in the SFE. This suggests that clonal differences or growth history of the diatoms may result in large differences in their suitability as food.

Vulnerability to predation Copepods are key organisms in pelagic foodwebs, which means many predators eat them. This has two important related consequences in estuaries. The first is that predation may exclude or limit penetration of coastal copepod species into estuaries (Kimmerer and McKinnon 1989, Kimmerer 1991, Ueda 1991), which are often regions of high biological activity and therefore high abundance of predatory organisms.

The second is that copepods that are successful in estuaries have evolved various strategies to avoid or minimize the effects of predation. A problem that small planktonic organisms face is that there are many modes of predation, each of which depends on different aspects of prey and can be avoided by different strategies (Brooks and Dodson 1965, Drenner et al. 1978, Viitasalo et al. 1998, Titelman and Kiørboe 2003). Of the strategies or mechanisms available to avoid or mitigate effects of predation, only rapid potential population growth would be effective against all predatory modes.

Generally, copepods are consumed by predators if their distributions (in salinity and vertically) and seasonal patterns overlap, the copepods are the right size to be consumed by the predator, and they can readily be detected (except for filter-feeders) and caught. Copepods vary greatly in

their detectability, which depends on size, pigmentation, and swimming behavior, and in the strength of their escape responses. There is little evidence that copepods vary in their palatability.

The most common mode of predation on larger stages of copepods in many estuaries is probably that by planktivorous fish, most of which detect prey visually and capture them one at a time, although some fish species can detect planktonic prey in the dark using the lateral line (Janssen et al. 1995). Either predatory mode is generally selective toward larger prey because of their higher detectability and possibly the greater net energy gain per individual consumed (Brooks and Dodson 1965), although active selection for one prey or another probably plays a minor role in planktivory (Luo et al. 1996). Mechanisms to avoid or reduce the impacts of visual planktivory include diel vertical migration (Bollens and Frost 1991) including migration to the bottom by day (Fancett and Kimmerer 1985), small size, translucence, and cryptic behavior resulting in poor detectability (Brooks and Dodson 1965, Gerritsen and Strickler 1977, Buskey 1984), delayed development to larger, more visible stages (Miller et al. 1977), and sensitivity to shear currents coupled with rapid escape responses (Buskey 1984, Fields and Yen 1997).

Examples of these mechanisms are common in the SFE. Mysids and amphipods underwent diel migration in the LSZ during 1994-1996 (Kimmerer et al. 1998, 2002). None of the copepods migrated dielily, perhaps because their other attributes eliminated the need for diel migration. Most of the copepods are small and the most abundant species (*Limnoithona tetraspina* and *Oithona davisae*) are the smallest (~0.5 μm total length), many are translucent in all life stages, and *L. tetraspina* is quiescent in the water and therefore difficult to detect hydromechanically. All of the calanoid copepods, notably *Eurytemora affinis*, *Pseudodiaptomus forbesi*, and *Acartiella sinensis*, have strong escape responses, and *Acartia* and probably *Acartiella* species have antennae that are well equipped to detect shear indicating an attack by a planktivore.

Filter feeders in the estuary include several fish that can switch between filtering and picking individual prey: northern anchovy *Engraulis mordax* and Pacific sardine *Sardinops sagax* in salty water, and threadfin shad *Dorosoma petenense* in freshwater. This predatory mode, generally used in areas of high abundance of food items too small to attack individually (< 1~mm, Leong and O'Connell 1969, Holanov and Tash 1978), is likely effective against smaller zooplankton with limited swimming capability such as *Limnoithona* spp. (Kimmerer 2006). In fact, the reduction in abundance of northern anchovy in the LSZ in 1987 probably opened the door to the establishment of *L. tetraspina* in an area of low predation risk by fish (Kimmerer 2006).

The other principal filter-feeding zooplanktivores in marine and estuarine systems are gelatinous predators, notably scypho- and hydromedusae and ctenophores. In the SFE all three are common in salty water and three species of hydromedusae are common in brackish water, but more abundant in sheltered sloughs and channels than the open waters (Mills and Rees 2000, Wintzer et al. 2011, L. Sullivan). Their filtration impact is unlikely to be large.

Clams also filter-feed and the introduced overbite clam *Corbula amurensis* is capable of consuming nauplii of several copepod species (Kimmerer et al. 1994, Kimmerer unpublished).

We have also observed adults of *Limnoithona tetraspina*, with their weak escape responses, being sucked into clam siphons in the laboratory. This consumption has a population-level effect for some species. *Eurytemora affinis*, largely confined to the Low-Salinity Zone, declined sharply in abundance during late spring-summer starting in 1987, which was attributed largely to consumption of nauplii by clams (Kimmerer et al. 1994). Ongoing analyses show high mortality of *Pseudodiaptomus forbesi* nauplii in the Low-Salinity Zone, which can be attributed to a combination of slow growth because of poor food conditions and consumption by clams.

Three predatory copepod species are sometimes abundant in the estuary (Table 5.3). The relatively low predation rate by fish and the lack of other planktivores may have provided the two introduced species an opportunity to thrive.

5.5. Current understanding of common species

This section presents additional information for some of the common species in and near the LSZ (see also Tables 5.2 and 5.3). Fig. 5.3 shows the abundance patterns in salinity and time of year for the common species for one or two ranges of years depending on when they were introduced or when abundance changed.

All of these species are consumed to some extent by delta smelt and other fishes that are most abundant in low-salinity waters. However, these species vary in their importance to diets of these fishes because of the degree of overlap between the copepods' salinity ranges and those of the fish. In addition, some of these copepods (*Oithona*, *Limnoithona* spp.) are small and difficult to see, and fish in general will attack larger prey when they are available (Table 5.3).

Acartia (Appendix 5.2) is a genus of marine to brackish species that are very abundant in most temperate estuaries and bays (e.g., Heinle 1966, Alcaraz 1983, Kimmerer and McKinnon 1985, Ueda 1991). *Acartia* species are not collected effectively by the IEP monitoring program because of the lack of sampling in Central and South Bay, but there is still clear evidence of a decrease in abundance of this genus in 1987, especially in summer (Kimmerer and Orsi 1996 and Figs. 5.1 and 5.3).

Eurytemora affinis is a member of a species complex, i.e., a group of closely related species that are very difficult to distinguish except by genetic analysis (Lee 2000). This group is numerically *Acartiella sinensis* preys on smaller copepods including all stages of *Limnoithona* and at least nauplii of *Pseudodiaptomus forbesi* (York et al. in revision, Slaughter and Kimmerer in prep.). In contrast to all of the other LSZ copepods, its reproductive rate appears to be high based on a handful of measurements, probably because its food is abundant. However, initial calculations show that its predatory impact on *L. tetraspina* is rather low. *A. sinensis* is often a common food for delta smelt in late summer (S. Slater, CDFG, pers. comm.).

Table 5.3. Key life history attributes for some common copepod species. Information not in references is unpublished by members of the Kimmerer laboratory at the Romberg Tiburon Center. "Food for fish" refers to the habitat where fish may consume these species, or their limited availability because of small size

Species	Reproduction	Feeding	Food for fish	Habitat/Notes	References
<i>Acartia</i> spp.	Broadcast	Omnivore; microzooplankton, phytoplankton	Marine	Three common species with similar life histories, IEP sampling does not distinguish. See text.	Carillo 1974, Trinast 1976, Landry 1978)
<i>Eurytemora affinis</i>	Sac	Filter-feeder; general omnivore	LSZ species (formerly abundant)	Species complex; formerly abundant in LSZ all year; now confined to winter-early spring	Lee 2000
<i>Pseudodiaptomus forbesi</i>	Sac	Filter-feeder; general omnivore	Freshwater and LSZ	Most abundant in freshwater during summer, transported to LSZ by mixing and advection	Orsi and Walter 1991
<i>P. marinus</i>	Sac	Filter-feeder; very general omnivore	Marine	Somewhat demersal (on bottom by day). Rapid growth rate but chronically food limited.	Orsi and Walter 1991, Liang and Uye 1997
<i>Sinocalanus doerrii</i>	Broadcast	Omnivore	Freshwater and LSZ	Genus reported as cannibalistic but in experiments it grew on a diet of algae	Orsi et al. 1983, Kimoto et al. 1986, Hada and Uye 1991,
<i>Tortanus dextrilobatus</i>	Broadcast	Ambush predator	Marine to brackish	Feeds on other copepods	Orsi and Ohtsuka 1999, Hooff and Bollens 2004
<i>Acartiella sinensis</i>	Broadcast	Cruising predator	LSZ	Feeds on other copepods, e.g., <i>L. tetraspina</i>	Orsi and Ohtsuka 1999, York et al. in revision
<i>Oithona davisae</i>	Sac	Ambush predator on microzooplankton, also consumes phytoplankton	Marine to LSZ; small size limits availability except for anchovies	Broader diet than previously believed. Introduced to several European estuaries.	Ferrari and Orsi 1984
<i>Limnoithona tetraspina</i>	Sac	Ambush predator on microzooplankton	LSZ; small size limits availability	Slow growth rate, chronically food-limited	Ferrari and Orsi 1984, Gould and Kimmerer 2010
<i>Limnoithona sinensis</i>	Sac	Infer from <i>L. tetraspina</i>	Freshwater; small size limits availability	No ecological studies	Ferrari and Orsi 1984
<i>Acanthocyclops vernalis</i>	Sac	Ambush predator	Freshwater and LSZ	Several other related cyclopids present, <i>A. vernalis</i> most abundant	Li and Li 1979
Harpacticoids	Sac		Various	Mostly juveniles of benthic species. Several pelagic species in more saline waters.	

Tortanus dextrilobatus adults feed on copepods up to nearly their own size (Hooff and Bollens 2004), and nauplii appear to feed on ciliates and small copepods such as *Limnoithona* (C. Craig). Experiments on *Acanthocyclops vernalis* have not been conducted here but in other places they too feed on copepods (Li and Li 1979). *Oithona davisae* adults and nauplii also have been reported not to feed on diatoms (non-motile; Uchima 1988, Henriksen et al. 2007), but we have found it to feed on a wide variety of prey including diatoms if they are available at high concentrations (R. Vogt).

Limnoithona comprises two species, both introduced to the SFE. *L. sinensis* was abundant in fresh to slightly brackish water following its introduction, but when *L. tetraspina* arrived it quickly became the numerical dominant in and near the LSZ, and displaced *L. sinensis*. Now, although both species co-occur in the estuary, the abundance of *L. sinensis* is much lower than that of *L. tetraspina*; the latter makes up 75% of the total *Limnoithona* at salinity < 0.5, where mean abundance is <1000 m⁻³, but 99% at salinity of 0.5-12 where this genus is more abundant. These small, cryptic copepods grow and develop slowly and have low reproductive rates (Gould and Kimmerer 2010). *L. tetraspina* feeds almost entirely on motile prey such as ciliate protozoans (Bouley and Kimmerer 2006, Gifford et al. 2007). They seem to be vulnerable to predation by clams at all life stages, based on laboratory observations. However, they also are not heavily consumed by fish owing to their small size and propensity to remain motionless in the water. Thus, reduced mortality of the later life stages may compensate for losses due to clam grazing.

Mysids are shrimp-like animals found throughout most temperate estuaries. The native mysid *Neomysis mercedis* was once so abundant and so important in the diet of young striped bass that it was actually the first planktonic organism sampled on a regular basis (Orsi and Mecum 1996). Its abundance crashed in 1987-1988, after which three new mysids were introduced, one of which (*Hyperacanthomysis longirostris*) is moderately abundant (Table 5.2). However, total mysid biomass declined by nearly 10-fold in summer of 1987, concurrent with the declines in chlorophyll and anchovy abundance.

Rotifers once numbered in the hundreds per liter in the LSZ and were even more abundant in the Delta. Their abundance in both regions has declined and they are now uncommon in the LSZ. This decline is likely a result of the overall decline in phytoplankton production partly due to clam grazing, as well as the direct grazing by clams on rotifers.

Microzooplankton Very little work has been done on microzooplankton, and no monitoring program collects them. Yet they are the next most important grazers on phytoplankton after clams (York et al. 2011, Kimmerer and Thompson in prep.), and at times the most important food of copepods (Rollwagen Bollens and Penry 2003, Bouley and Kimmerer 2006, Gifford et al. 2007). Consumption by clams can exceed their population growth rate, and as with *P. forbesi*, a subsidy through dispersion and advection from other parts of the estuary may be required to maintain the abundance of microzooplankton in the LSZ (Greene et al. 2011). Microzooplankton

are difficult to identify to species by microscopic examination, and most workers identify them to only very crude taxonomic levels.

5.6. Influences on abundance: reproduction, growth, and predation

As a group the zooplankton populations increase or decrease by the net of reproduction, growth/development rates, and mortality at all life stages including that due to transport losses of advection and dispersion. Progress has been made on measuring some of these population attributes in recent years.

Reproduction of copepods depends on the effect of temperature on biochemical processes, the ability to find mates, and on the availability of food of high enough quality for egg production. Egg development time, i.e., between egg laying and hatching, depends only on temperature (Corkett and McLaren 1970). This can be modeled as a negative exponential function of temperature, which matches quantitatively with predictions of the metabolic theory of ecology (Brown et al. 2004). Typical egg development times are 2-4 days at 15°C, and development time decreases about 2-fold for every ~6°C increase in temperature up to the thermal limit for the species.

Male copepods seek and follow females using either pheromones or hydromechanical signals. Pheromone tracking enhances the search capability of the male by over an order of magnitude (Choi and Kimmerer 2009), but some copepods (e.g., *Acartia* spp.) do not produce pheromones. The effective volume searched per unit of time by the males, together with ambient conditions of food supply, temperature, mortality, and dispersion determine the minimum population density from which a copepod population can recover (Choi and Kimmerer 2008, 2009). This minimum is generally below the annual population minima seen in the estuary but is probably an important factor in allowing new populations of introduced species to become established.

The biochemical food requirements for reproduction can be more exacting than those for growth of juveniles, as indicated by poor egg survival of copepods fed some diatoms and other nutritionally inadequate foods (Ianora and Poulet 1993). Because of these particular nutritional needs, reproductive rate often becomes food-limited before growth of juveniles. However, reproductive rate is usually higher and more sensitive to food in copepods that release their eggs than in those that carry egg sacs (Bunker and Hirst 2004).

Although contaminants may affect reproductive rate, such effects are likely to be sporadic rather than chronic because toxicity of water samples from the estuary is highly variable (Luoma et al. 1983, Werner et al. 2010). Thus, persistent depression of reproductive rates can usually be interpreted as evidence of food limitation, with some exceptions (see below).

Reproduction has been measured in a handful of studies which have shown evidence of food limitation in some species but not others. All three species of *Acartia* had widely varying egg production rates with peak rates during phytoplankton blooms (Kimmerer et al. 2005). Egg production of *Limnoithona tetraspina* in the LSZ during 2006-2007 was low (~2 eggs female⁻¹ d⁻¹) but this value was consistent with those for other oithonids and does not suggest food

limitation (Gould and Kimmerer 2010). By contrast, egg production rates of *Eurytemora affinis* in spring and *Pseudodiaptomus forbesi* in summer in the same study were consistently below estimated maxima for each species (Kimmerer et al. in prep.). A handful of measurements of egg production rate of *A. sinensis* during 2006-2010 showed rather high reproductive rate; although we do not know their maximum reproductive rate it does seem that they are less food-limited than the other species.

Development of copepods takes variable amounts of time for each life stage and has the same temperature dependence as eggs if food is plentiful. Growth and development are linked, in that molting from one stage to the next requires a certain amount of gain in weight, typically a factor of 1.2-1.5 from one stage to the next. Therefore temperature sets the lower limit of development time and food limitation can further extend it. Development time of *P. forbesi* in the laboratory from egg hatching to adult takes about 19 days at 15°C and 8 days at 22°C. Food-replete development times at 15°C are about 16d for *E. affinis* and 39d for *L. tetraspina* (Gould and Kimmerer 2010). After their terminal molt to adult, copepods begin using food energy for reproduction instead of growth.

Growth and development in the field have also been analyzed in a handful of studies. Growth of *L. tetraspina* in the estuary during 2006-2007 was consistently below the maximum determined in the laboratory (Gould and Kimmerer 2010). Growth rates of *E. affinis* and *P. forbesi* were also usually below their laboratory maxima during 2006-2007, particularly for *P. forbesi* in summer. None of the values of growth or reproductive rate of these three species were related to chlorophyll concentration, probably suggesting the importance of selective feeding on phytoplankton and on non-pigmented food organisms such as ciliates, and also the limited range of chlorophyll values resulting in poor statistical power to detect a response of growth to chlorophyll concentration.

Notwithstanding the apparent food-limitation of reproduction and growth discussed above, the copepod populations of the SFE are capable of very rapid net rates of increase. For example, the species that are common in summer and nearly absent in winter increase in abundance at rates of $\sim 10\% \text{ d}^{-1}$, by which their populations can double in only a week (Fig. 5.4). Note also that the abundance of *P. forbesi* in summer in freshwater is rather tightly constrained, considering its potential population growth rate. This probably reflects a strong negative feedback mechanism (i.e., density dependence) by which population growth rate is reduced when abundance is high. The cause underlying this mechanism is unknown but probably involves food limitation, and it apparently is not related to flow since abundance maxima are similar in wet and dry years. This population maximum occurs in freshwater, and abundance in the LSZ follows the same pattern but with greater variability.

Mortality is very difficult to estimate on field populations and the available methods are subject to considerable error (Aksnes and Ohman 1996). Generally mortality of populations that reproduce continuously can be estimated either through a vertical life table (Kimmerer and McKinnon 1987, Aksnes and Ohman 1996) or by fitting a population dynamics model to the

available data on life stage distributions (Bi et al. 2011). Mortality includes losses to predation, parasitism (Kimmerer and McKinnon 1990), disease (implied by results of Tang et al. 2006), and advection and dispersion away from the population center.

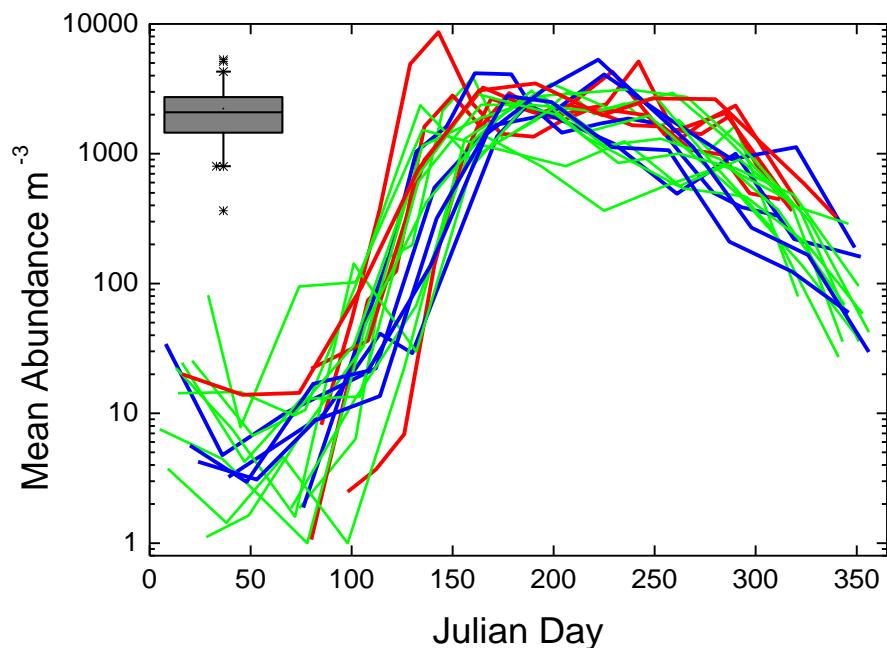


Figure 5.4. *Pseudodiaptomus forbesi* Mean abundance by day of the year for all samples in freshwater. Each line represents a single year from 1989 to 2008. Colors indicate wet (blue, 1993, 1995, 1998, 2005, 2006) and dry (red, 1990-1992, 1994, 2008) years and those between (green).

Grazing by clams can cause substantial mortality for microplankton including the nauplius stages of copepods; although nauplii of most copepod species have a strong escape response to clam siphons, they do not always escape. The initial decline in abundance of *E. affinis* was attributed mainly to grazing on the nauplii by *Corbula amurensis* (Kimmerer et al. 1994). The high proportion of young stages of *P. forbesi* in freshwater (Fig. 5.5) implies high mortality of adults resulting in a young population, while in the LSZ the high proportion of adults suggests high mortality of nauplii and low mortality of adults resulting in a senescing population (Slaughter and Kimmerer in prep.). These relationships are consistent with predation by planktivorous fish on the adults in the clearer waters of the eastern Delta, and losses of nauplii to clam grazing and advection/dispersion in the LSZ.

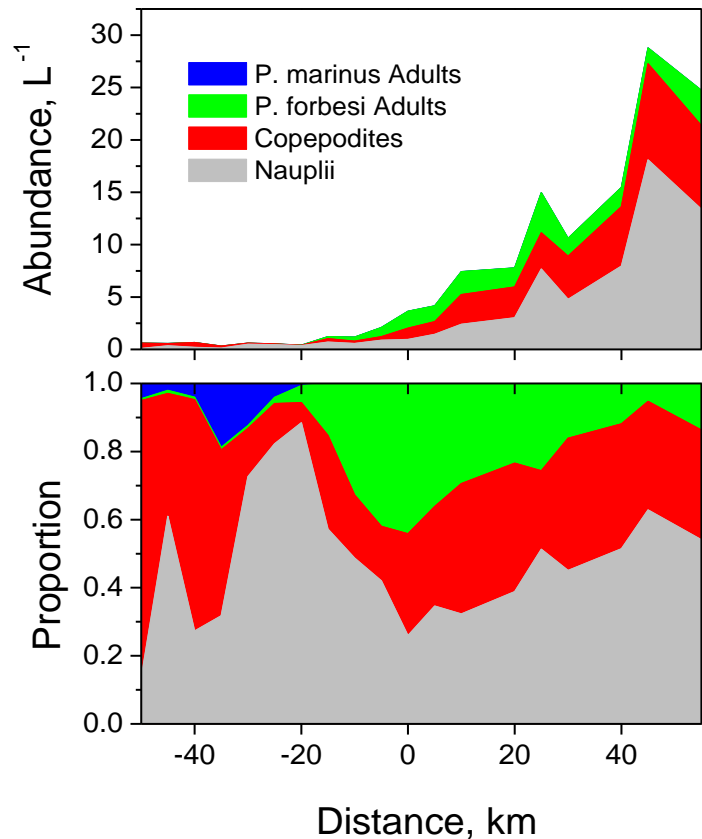


Figure 5.5. Distributions of gross life stages of *Pseudodiaptomus* species by distance from the 2 psu isohaline. A distance of 0 is essentially X2. Positive distances are based on station locations, and negative ones on salinity corrected to distance by the mean relationship of salinity to distance from 2 psu.

5.7. Influences on abundance: recent and future changes in the Delta

Several recent changes in the estuary may have affected population sizes of zooplankton.

Freshwater flow patterns Freshwater flow within the Delta and outflow from the Delta may be important for some zooplankton populations, although generally they do not respond strongly to flow (Kimmerer 2002). Residence time within the Delta is a key determinant of phytoplankton biomass (Jassby et al. 2002) and probably for zooplankton as well. Preliminary analyses do not show a relationship between abundance of common species (e.g., *P. forbesi*) and inflow, the principal determinant of residence time (see Fig. 5.4). However, the abundance of adult and juvenile *P. forbesi* in the LSZ is positively related to X2 (or outflow), presumably because advection increases with flow (Fig. 5.6).

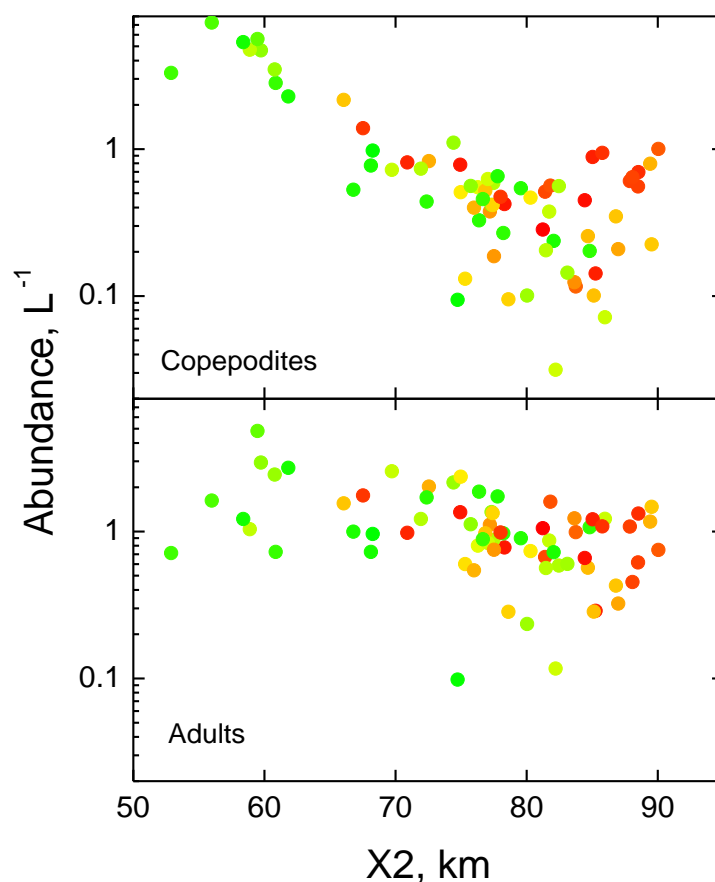


Figure 5.6. *Pseudodiaptomus forbesi*. Abundance in the LSZ for copepodites (including a small fraction of *P. marinus*) and adults as a function of X2.

Rates of export pumping from the Delta increased in the 1980s and have been high in most years and months, except during April-May of each year when export pumping is reduced to protect migrating salmon (Kimmerer 2004). Although export pumps in the south Delta remove over 50% of the incoming freshwater in dry periods, a more relevant measure for freshwater zooplankton is the fraction of the Delta's volume that is exported daily, which is up to about 3% (Kimmerer 2004). This is directly equivalent to a mortality rate, although only at a crude level and for the Delta as a whole. This rate is rather small compared to typical mortality rates we have estimated for copepods in the Delta, but could be important for slower-growing forms. Likewise a mass balance of phytoplankton in the Delta showed export losses to be considerable but a large unknown loss term, probably grazing, was much larger (Jassby et al. 2002). Thus for both phytoplankton and zooplankton export pumping appears to be a relatively small source of loss, and correlative analyses do not show an effect on copepods resident in the Delta (not shown). Export losses must be lower, and are probably negligible, for brackish-water copepods.

Predation rates on zooplankton can be inferred from the abundance of different kinds of predators. Abundance of visual planktivores (i.e., pelagic fishes) has declined, while that of other kinds of predators has increased (i.e., clams, jellyfish, predatory copepods, and centrarchid fishes in the Delta). The likely result of this change in dominant predatory modes is discussed above. Centrarchid fishes have increased in the Delta since about 1990 owing mainly to the increase in vegetated habitat with the spread of introduced waterweeds. Some species feed on zooplankton at least during early life stages, but nothing is known of their feeding rates or impact.

The turbidity of the water throughout the Delta and Suisun Bay has been decreasing over the last few decades (Kimmerer 2004, Schoellhamer 2011), allowing greater light penetration into the water. This has likely increased the ability of visually feeding planktivorous fish to find prey, but may also have reduced their ability to avoid predators (Feyrer et al. 2007). Rates of predation and their response to increasing water clarity have not been determined.

Toxic substances include natural toxins and contaminants. The principal natural toxins in the Delta come from summer-fall blooms of the toxic cyanobacterium *Microcystis aeruginosa* (Lehman et al. 2005, Ger et al. 2010). There has been no effective monitoring for *Microcystis*, mainly because the blooms take the form of large aggregates that are not well represented in phytoplankton samples taken by IEP and USGS sampling programs. However, anecdotally the frequency or intensity of blooms increased around 2000. In addition, microcystin, the toxin produced and released by some *Microcystis* strains, has been detected in the Delta during blooms (Lehman et al. 2005), and pilot monitoring measurements found microcystin throughout the LSZ, and in central and southern San Francisco Estuary (R. Kudela, unpublished data).

Microcystis has both toxic and non-toxic strains but both can impair survival of copepods that ingest them, apparently because of other metabolites besides microcystin (Ger et al. 2010). In laboratory experiments *P. forbesi* was better able than *E. affinis* to tolerate *Microcystis* in the diet. Studies are ongoing to examine the influence of *Microcystis* on *P. forbesi* in the Delta.

A contaminant of particular concern for copepods is ammonia released from wastewater treatment plants. Dissolved ammonia exists in two forms: the ammonium ion (NH_4^+) and un-ionized ammonia (NH_3). The proportions of each depend on pH: at a pH of 7.7, the median from IEP monitoring data from 1975-2012, about 3% of total ammonia is un-ionized and the rest is ammonium. Un-ionized ammonia is toxic to many marine organisms including fish, and its effects have been reported on crustaceans; bioassays with an amphipod showed frequent toxicity that may have been associated with ammonia in the lower Sacramento River (Werner et al. 2010).

There is little information in the literature about the effects of ammonia on zooplankton. *Acartia* spp. nauplii had a 48-hour LC50 value of 0.14-0.21 mg/L un-ionized ammonia (Sullivan and Ritacco 1985), which would correspond to ~4-6 mg/L (280-430 μM) total ammonia nitrogen at a pH of 7.8. Egg survival in *Acartia* was reduced after 9 days' exposure to 0.15 mg/L (11 μM) total

ammonia nitrogen (Buttino 1985), but there was no information in that paper on pH or the un-ionized fraction.

A recent report exploring toxicity of total ammonia to *P. forbesi* reported effects on survival through the life cycle at values as low as 0.36 mg/L ammonia-N (26 μ M, Teh et al. 2011). However, the numbers of copepods actually counted in that study were low (mean of 15 per replicate in controls) and replication error was high (e.g., the four replicates in the 0.36 mg/L treatment contained between 0 and 30 adults at 24 days). This report has also been criticized on several other grounds (Pacific EcoRisk, 2011) and its results should be treated cautiously until the work has been repeated with better replication.

Numerous samples from the estuary have exceeded the threshold for effects of 0.15 mg/L total ammonia N suggested by Buttino (1985) (Fig. 5.7). Roughly 12% of the samples taken in Suisun Bay since 1990 exceeded this limit. However, ammonia has a pronounced seasonal cycle by which it is highest in winter at stations far away from treatment plants. Presumably rates of nitrification and uptake by phytoplankton and macrophytes are reduced in winter, while close to the treatment plants the seasonal signal is weak presumably because the discharge from the treatment plants has little seasonal signal (see also Section W). Ammonia concentrations in only three samples out of 506 in Suisun Bay exceeded the above value during summers, when *P. forbesi* and delta smelt are present. Although this topic clearly needs more work, at the moment it would be difficult to either claim or rule out a population-level effect of ammonia toxicity on *P. forbesi* or other copepods.

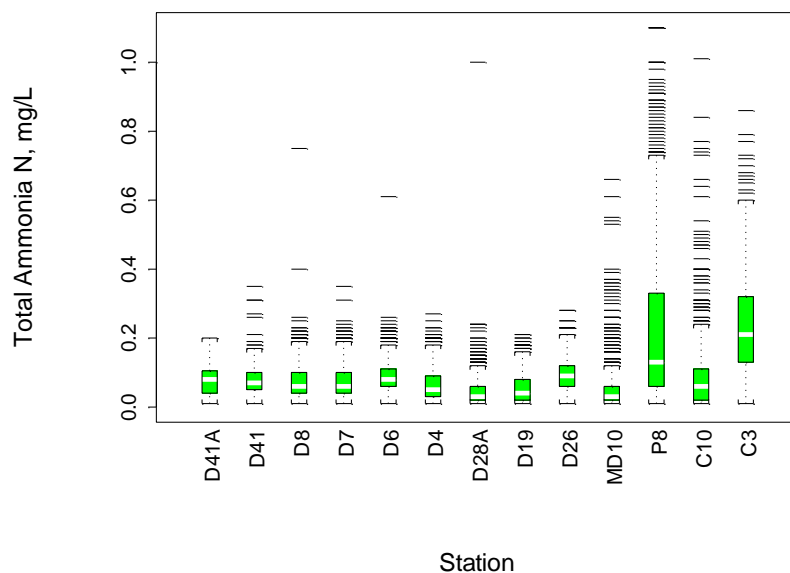


Figure 5.7. Total ammonia nitrogen in stations visited frequently in the IEP Environmental Monitoring Program. The stations have been ordered roughly from the San Pablo Bay to the eastern delta (http://www.water.ca.gov/bdma/images/Metadata-DiscreteWQ_stations.jpg). Station C3 is at Hood below the Sacramento Wastewater Treatment Plant, and P8 is in the Stockton Ship Channel. Note that the y axis has been cut off and 22 out of 553 values for P8 are above the upper limit (max 2.9).

The LSZ also receives numerous other contaminants from anthropogenic activities, including agro-chemicals such as pesticides and herbicides and compounds present in treated wastewater effluent (Hinton 1998, Werner et al. 2010). Data are limited on the potential effects of these compounds at ambient concentrations on copepods. Diazenon, an insecticide used extensively in the Central Valley, is found at concentrations below those that cause impairment to copepods, although cladocerans may be occasionally affected (Giddings et al. 2000).

Future changes The estuary of the future will almost certainly be different from what it is now. Great plans are afoot, as are climate change, human population growth, technological development, and quagga and zebra mussels. Although some changes can be forecasted (e.g., sea level rise, warming, change in runoff timing, Cloern et al. 2011; also restoration and modification to water diversion facilities in the Delta), others can only be anticipated without information about when they will occur (e.g., mussel invasions, massive levee failures in the Delta, Mount and Twiss 2005). Still others can be anticipated only in broad terms, including technological development (e.g., improvements in water use efficiency), economic shifts that change human activities around the estuary, and invasions by other high-impact species.

Given all of these potential changes, many of which are likely to affect zooplankton, it would be difficult to forecast their overall effect. It is easy to focus on changes with clear mechanisms for effects such as temperature, but the estuary is probably not close to thermal limits for any of the zooplankton species now resident here. The more substantial effects on zooplankton are likely to come from the arrival of mussels in the Delta (Caraco et al. 2006) and massive changes in the flow regime and physical configuration of the Delta, with corresponding changes in residence time and water clarity.

5.8. Pathways for effects of nutrients

Although nutrient concentrations are high enough not to limit phytoplankton growth except during strong blooms, they could affect phytoplankton and thereby zooplankton in several ways. Direct toxicity of ammonia is discussed above. High levels of nutrients or skewed nutrient ratios may stimulate harmful algal blooms, alter the chemical composition of food available to zooplankton, or affect the size distributions or suitability of phytoplankton as food for zooplankton.

High nutrient concentrations appear to be essential for the formation of *Microcystis* blooms, and ammonium appears to be somewhat better than nitrate at stimulating blooms (Moisander et al. 2009). Therefore the high nutrient concentrations in the estuary likely contribute to the blooms and to any resulting impairment of zooplankton (see above). In addition, ammonium has increased over the last 3 decades (Jassby 2008; see also Figure 6.4). However, it is unclear whether ammonium loading plays a particularly strong role in blooms. Ammonium levels have been high in Suisun Bay since as early as the 1970s (Figure 6.4), before the onset of *Microcystis* blooms in 1999 (Lehman et al. 2005). Low freshwater flow and high temperature may provide conditions favorable to blooms (Lehman et al. 2008), and in every year from 1999 to 2012 except 2006 and 2011 summer flows were very low. The extent of the bloom in 2006 has not

been reported but in 2011 a research group from SFSU and other universities found low abundance of *Microcystis*, yet ammonium levels were as high as in previous years.

Ammonium concentrations above a value of around 1-4 μM can inhibit nitrate uptake. Recent studies (Wilkerson et al. 2006, Dugdale et al. 2007, Parker et al. 2012a) argue that some phytoplankton, particularly diatoms, grow faster on nitrate than on ammonium in laboratory bioassays at high light levels, partly because concentrations in the estuary are higher and partly because diatoms can increase maximum uptake rate when ambient nitrate concentration is high (Parker et al. 2012a). It has thus been hypothesized that high concentrations of ammonium in the estuary, attributed primarily to discharge from wastewater treatment plants, can prevent phytoplankton from realizing their maximum growth rates. This could in turn limit phytoplankton biomass and copepod food supply. This effect has also been inferred from data collected in transects down the Sacramento River past the wastewater plant diffuser (Parker et al. 2012b). These studies and factors influencing N uptake and primary production are discussed further in Sections 2 and 3.

The principal unanswered question is the extent to which these nutrient effects have influenced the composition and productivity of phytoplankton, particularly in the LSZ and Suisun Bay. Glibert et al. (2011) claimed that nutrient composition and ratios have had a heavy influence on phytoplankton composition and productivity and thereby most of the long-term trends in the estuary. This claim ignores other obvious changes that have happened, most notably the introduction of *Potamocorbula*. At the time of that introduction, chlorophyll concentration, diatom production, mysid biomass, and the abundance of northern anchovy in the LSZ abruptly declined (Alpine and Cloern 1992, Orsi and Mecum 1996, Kimmerer 2005, 2006), and phytoplankton biomass and production have remained uniformly low since, except for occasional spring blooms (Kimmerer et al. 2012, Kimmerer and Thompson submitted). There is also evidence that phytoplankton community composition has indeed shifted considerably over the past 35 years in Suisun Bay (DWR-EMP data; Cloern and Dufford, 2005; Glibert et al., 2011 Senn et al., in preparation). However, the sharpest transition in species composition in Suisun occurred around 1987 (Senn et al., in preparation), coincident with the introduction of *Potamocorbula*, probably because of the strong, size-selective grazing pressure exerted by this clam.

Other long-term trends include increasing water clarity (Kimmerer 2004, Schoellhamer 2011), changes in circulation patterns in the Delta including an increase in export flows, an increasing extent of coverage by submerged macrophytes in the Delta (Brown and Michniuk 2007), and the introduction of numerous copepods into the estuary (Orsi and Ohtsuka 1999). These changes rule out any attempt to correlate long-term trends in pairs of variables without a good understanding of the underlying mechanisms.

Phytoplankton in the upper SFE are mainly subject to four key influences: nutrients as discussed above, water clarity, grazing by zooplankton and clams, and estuarine circulation. These factors operate in different ways, on different timescales in different locations, and with different

impacts on large and small phytoplankton. For example, light and nutrient availability operate on growth, and grazing operates on biomass. Studies of processes underlying individual factors and correlative evidence have been used to infer the importance of each factor, but no study has examined any of these factor in concert. Mixing and advection can cause plankton biomass to vary in ways that do not reflect local processes; for example, high biomass from a bloom in the Yolo Bypass can be advected into a turbid, deep, clam-rich area in Suisun Bay where a local bloom would be unlikely. Similarly, the effects of grazing by *C. amurensis* have been seen in monitoring data from stations far removed from the clams, presumably because of tidal dispersion (Kimmerer and Orsi 1996, Jassby et al. 2002). Thus, discriminating the actual effect of nutrients in the context of all the other factors will require a concerted effort including experimental work and modeling.

Apart from potential influences of nutrients on productivity (and therefore availability) and size composition of phytoplankton, it is plausible that the chemical composition of phytoplankton has changed with the changes in nutrient ratios and species composition, as argued by Glibert et al. (2011). Most zooplankton have strong homeostatic mechanisms for maintaining their biochemical composition even as that of their diet varies widely. However, faced with a diet of very different composition than itself, an animal must either reject food that is low in the required chemical components, or consume large quantities of the food and eliminate the excess of the less-needed components. Both mechanisms result in inefficiency compared to consuming a more balanced diet, consistent with what Glibert et al (2011) propose as a cascading effect due to altered nutrient concentrations or N:P ratios.

The actual magnitude of this putative effect of stoichiometry has not been determined. These effects are likely to be small compared to the obvious and documented effects of the introductions of clams and copepods, which cannot reasonably be linked to nutrient conditions in the estuary.

5.9. Needs for research and monitoring

It is always easy, but rarely helpful, for researchers to list research topics that they consider important. A more useful approach is to consider what information is needed to resolve key issues that have implications for management or planning. Here are a few that are not yet being pursued effectively:

Effects of nutrients The wastewater treatment plants may be forced to upgrade treatment, and part of the reason is the potential for negative influences of ammonium on phytoplankton discussed above. The ambiguity in the magnitude of this effect in relation to other influences on phytoplankton, and therefore in its effect on zooplankton, suggest the need for a coordinated program of laboratory research and modeling.

Effects of freshwater clams The clam *Corbicula fluminea* has a major impact on phytoplankton in some parts of the Delta (Lopez et al. 2006), but its impact on zooplankton has not been examined. Ongoing modeling efforts will be able to assess the likely effects of changes in

physical configuration and residence time on zooplankton, but clam grazing is potentially large missing piece of the population dynamics picture.

Importance of peripheral habitats to the foodweb All of the zooplankton monitoring has occurred in channels or far from shores. Yet, many of the fishes of concern can feed in nearshore habitats. These habitats should be sampled for a better understanding of the food environment for delta smelt and other fishes.

Effects of restored marsh The Bay-Delta Conservation Plan calls for extensive restoration of tidal marsh throughout the upper estuary. Part of the justification is that marshes may serve as sources of food organisms for fishes of the open water, but this assumption has not been tested. In fact, shallow, nearshore areas can be sinks for phytoplankton and zooplankton because of consumption by clams and small resident planktivores. Some research on extant tidal marshes, both natural and restored, would help to resolve this issue.

Monitoring needs Although the IEP monitoring program has proved to be very valuable, the missing pieces discussed above should be addressed for completeness. Microzooplankton are not difficult to monitor and some monitoring of their abundance should be added to the program because of their importance as food for copepods. For the same reason monitoring of chlorophyll should include size fractionation at 5 μm , the approximate lower limit for efficient grazing by both zooplankton and clams. Similarly, given the potential importance of phytoplankton community composition, and the fact that composition is analyzed monthly at many DWR-EMP stations, consistent and sufficient size data should also be acquired to allow for conversion to biomass (or biovolume) estimates.

Depending on the results of sampling in peripheral habitats (above), some monitoring of these habitats may be warranted. Finally, extending the seaward limit of the monitoring program at least to Central Bay would be a valuable addition to the program, and could be done in conjunction with the ongoing San Francisco Bay Study.

5.10. References

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6. Synthesis of ambient water quality and loading data in Suisun Bay

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6.1. Introduction

The IEP's conceptual model for the Pelagic Organism Decline (POD) recognizes that multiple factors may be acting in concert to degrade habitat and contribute to the declining abundances of pelagic fish species in Suisun Bay and the Delta (Baxter et al., 2010, NRC 2012). Recent studies in Suisun Bay and the Delta have hypothesized that increases in anthropogenic nutrient loads over the past few decades, in particular ammonium (NH_4^+), are exerting adverse pressures on the system's phytoplankton and copepod communities (Section 3, Section 4, Section 5) that in turn affect pelagic fish via bottom-up pressure through the food web.

Understanding the underlying causes of habitat degradation and the POD in Suisun Bay and the Delta requires a broad and integrated analysis of all potential drivers, and an assessment of their relative importance. In addition to the need to better understand the hypothesized NH_4^+ -related mechanisms through which adverse pressures are exerted (Sections 3, 4 and 5), a better understanding of NH_4^+ concentrations, sources, and fate in Suisun Bay is necessary in order to inform near-term decisions related to managing nutrient loads. The goals of Section 6 are to

- Synthesize existing data on ambient NH_4^+ levels in Suisun Bay from long-term monitoring programs and special studies, including characterizing seasonal, temporal and spatial variations in observed concentrations
- Develop estimates of major nutrient loads to Suisun Bay, including loads from the Delta, treated wastewater effluent, and stormwater runoff based on currently available data;
- Characterize the seasonal and long-term variability of major NH_4^+ sources, and assess their relative importance
- Explore the underlying causes of spatial, seasonal, or temporal variations in NH_4^+ concentrations
- Explore how ambient NH_4^+ concentrations compare with various thresholds or guidance levels that studies have suggested may impair beneficial uses.

To address these goals, we compiled and analyzed data from USGS and DWR/IEP long-term monthly monitoring programs over the period 1975-2011, and recent studies that collected samples at higher spatial and temporal resolution. NH_4^+ data was compared with thresholds associated with hypothesized NH_4^+ -inhibition of primary production and toxicity to copepods. We also estimated loads from the Delta, publicly owned wastewater treatment works (POTWs), and stormwater into Suisun Bay during this period. Finally, we used a basic 1-box mass balance model to explore the potential underlying causes of seasonal and temporal trends in NH_4^+ . While other aspects of nutrient cycling in Suisun Bay – e.g., changes in loads, concentrations and forms of nutrients (NH_4^+ : NO_3^- , N:P) – also ultimately need detailed analysis, this section focuses primarily on NH_4^+ .

6.2. Approach:

6.2.1. Ambient water quality data

Nutrient concentration data were obtained from multiple sources (Table 6.1; Figure 6.1). Long-term monthly water quality monitoring data in Suisun Bay were obtained from both California's Department of Water Resources/Interagency Ecological Program Environmental Monitoring Program (DWR/IEP)³ and U.S. Geological Survey (USGS)⁴. Monthly concentration data for nutrients and related parameters (i.e. temperature, turbidity, salinity) were available from DWR/IEP stations throughout Suisun Bay over the period 1975-2011. While monitoring at some stations ceased in 1995, stations D6, D7 and D8 have continuous records from 1975-2011. The USGS San Francisco Bay Water Quality research program has also been carrying out a monthly sampling campaign along a transect through Suisun Bay to Rio Vista during over this time period. The USGS collects discrete water samples for nutrients at only 3 stations in this region, and sampling for nutrients was sporadic prior to 2006. Both DWR/IEP and USGS almost exclusively report combined $\text{NO}_3^- + \text{NO}_2^-$ rather than individual concentrations. During a brief period when DWR/IEP reported NO_3^- and NO_2^- separately, NO_3^- comprised >95% of $\text{NO}_3^- + \text{NO}_2^-$, and will hereafter be referred to as NO_3^- . Stations D6, D7 and D8 had the most complete long-term records available data record (Figs. 6.2).

Additional data from recent studies in Suisun Bay, carried out by San Francisco State University's Romberg Tiburon Center (RTC), were also considered. RTC collected nutrient and chlorophyll data at 7-9 locations within Suisun Bay on a near-weekly basis for April-September 2010 and April-July 2011.

While total ammonia is actually present as both NH_4^+ and NH_3 in natural waters, at the pH typically observed in Suisun Bay (~7.7), the vast majority (>95%) of total ammonia is present as NH_4^+ . We therefore refer to it throughout this section as NH_4^+ .

³ <http://www.water.ca.gov/bdma/meta/Discrete/data.cfm>

⁴ <http://sfbay.wr.usgs.gov/access/wqdata/>

Table 6.1. Available water quality data from DWR/IEP and USGS stations in Suisun Bay. The number of available data points is indicated by n.

Station Name	Source	NH ₄ ⁺	NO ₃ ⁻ ⁽³⁾	TKN	Organic N	DIP	TP	Chl-a
DWR/IEP Stations¹								
D6	DWR/IEP	1975-2011 n=426	1975-2011 n=438	1975-2011 n=438	1975-2011 n=424	1975-2011 n=437	1975-2011 n=437	1975-2011 n=431
D7	DWR/IEP	1975-2011 n=416	1975-2011 n=434	1975-2011 n=435	1975-2011 n=422	1975-2011 n=434	1975-2011 n=432	1975-2011 n=424
D8	DWR/IEP	1975-2011 n=420	1975-2011 n=436	1975-2011 n=436	1975-2011 n=416	1975-2011 n=434	1975-2011 n=435	1975-2011 n=427
D9	DWR/IEP	1975-1995 n=240	1975-1995 n=248	1975-1995 n=249	1975-1995 n=239	1975-1995 n=248	1975-1995 n=248	1975-1995 n=242
D2	DWR/IEP	1975 n=11	1975 n=10	1975 n=11	1975 n=11	1975 n=12	1975 n=12	1975 n=10
D10	DWR/IEP	1975-1995 n=233	1975-1995 n=249	1975-1995 n=249	1975-1995 n=235	1975-1995 n=248	1975-1995 n=249	1975-2011 n=431
S42	DWR/IEP	1975-1984 n=69	1975-1984 n=71	1975-1984 n=71	1975-1984 n=71	1975-1984 n=71	1975-1984 n=71	1975-1984 n=69
USGS Stations²								
3	USGS	1975-2005 n=129 2006-2011 n=62	1975-2005 n=133 2006-2011 n=62	—	—	1975-2005 n=136 2006-2011 n=62	—	1977-1980 n=41 1988-2011 n=244
6	USGS	1975-2005 n=123 2006-2011 n=64	1975-2005 n=130 2006-2011 n=60	—	—	1975-2005 n=136 2006-2011 n=60	—	1977-1980 n=43 1988-2011 n=224
9	USGS	1975-2005 n=131 2006-2011 n=63	1975-2005 n=137 2006-2011 n=62	—	—	1975-2005 n=143 2006-2011 n=62	—	1977-1980 n=43 1988-2011 n=246

¹ <http://www.water.ca.gov/bdms/meta/Discrete/data.cfm>

² <http://sfbay.wr.usgs.gov/access/wqdata/>

³ Both DWR/IEP and USGS report NO₃⁻ + NO₂⁻ combined concentrations. During a brief period when DWR/IEP reported each separately, NO₃⁻ + NO₂⁻ was more than 95% NO₃⁻ and therefore is abbreviated to NO₃⁻ throughout this document.

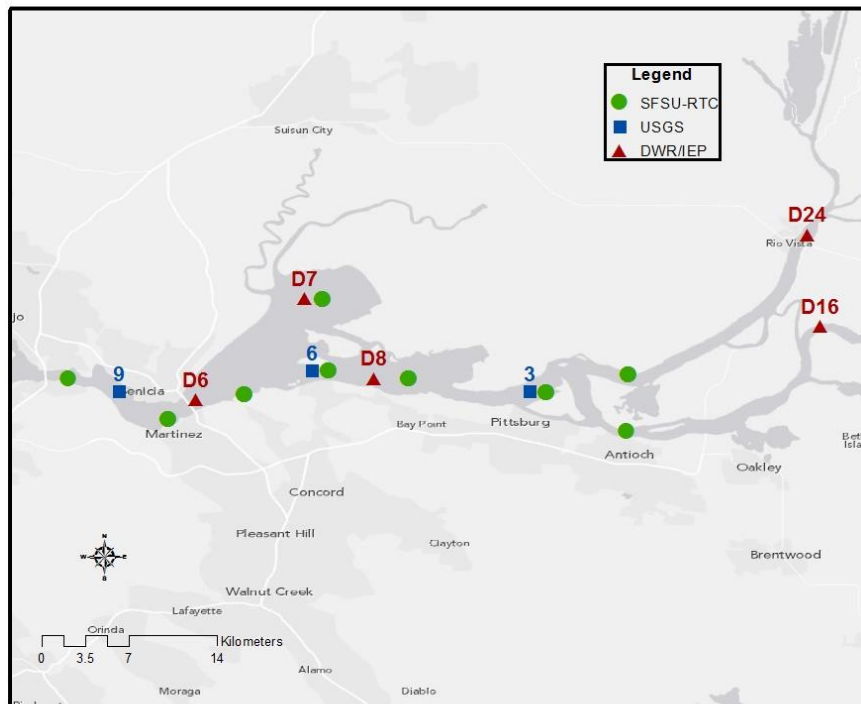


Figure 6.1. Location of DWR/IEP (red triangles), USGS (blue square) and SFSU-RTC (green circle) monitoring stations with nutrient data available.

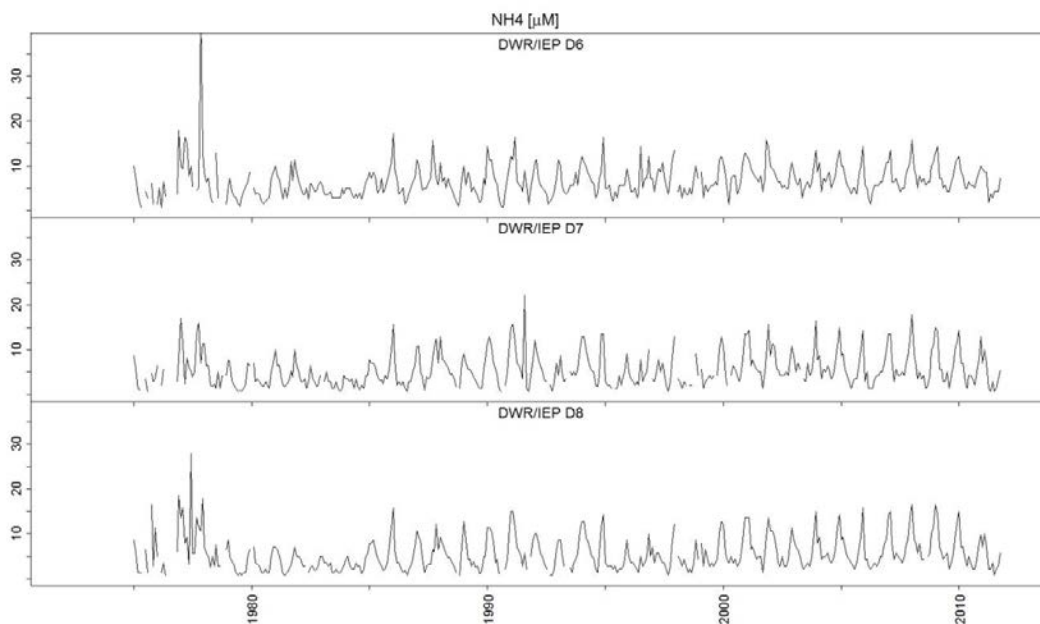


Figure 6.2. Time-series of available NH_4^+ data in μM at key Suisun Bay DWR/IEP stations. Stations D6, D7 and D8 had the most continuous record of data of all DWR/IEP or USGS stations in Suisun Bay (see Fig. A.6.1.1)

6.2.2. Nutrient Loads

Monthly-average nutrient loads to Suisun Bay from Delta influx were calculated for the period of 1975-2011 by adapting the approach used by Jassby and Cloern (2000) to quantify organic matter loads to Suisun Bay. The approach combines monthly concentration data from two DWR/IEP stations in the western Delta (D16 and D24) with monthly flow estimates at Rio Vista (DAYFLOW value Q_{rio}) and Twitchell Island (DAYFLOW value Q_{west}) to estimate Delta efflux into Suisun Bay. These load estimates account for nutrient loads originating from Sacramento Regional Wastewater Treatment Plant (SRWTP) since the stations used for the flow and concentration data are located ~100 km downstream of SRWTP. Monthly water quality data was available on a continuous basis from 1975-1995 at stations D24 and D16, the same stations used by Jassby and Cloern (2000); thus, continuous time series for NH_4^+ and NO_3^- loads could be calculated for this time period. However, nutrient measurements were dropped at both of these stations in 1995. Regular sampling at a USGS station (USGS657) that is co-located with D24 began in 2006; however D16 was not replaced. We addressed the data gaps (1995-2006 for D24/USGS657; 1995-2011 for D16) by performing a multivariate linear regression of D24 and D16 concentration data with data from nearby stations for the period 1975-1995, and used the best combination of stations to estimate concentrations for the missing time periods. Loads from station D24/USGS657, located on the main stem of the Sacramento River, typically accounted for >95% of loads; thus the loss of station D16 introduced only minor uncertainty to the overall load magnitude, and estimates are reasonably well constrained for 1975-1995 and for 2006-2011.

All of the stations used to estimate loads (both by Jassby and Cloern (2000) and in this report) are between 10 and 30km upstream of the mouth of Suisun Bay. To explore the sensitivity of load estimates to station location, we calculated loads using both these upstream stations (D24/D16) and one closer to the mouth of Suisun Bay (D4) for a period when data were available at both sites (1975-1995). DIN load estimates do not differ substantially when different stations are used. NH_4^+ load estimates do, however, appear to be sensitive to choice of station, suggesting that transformations do occur along this stretch (Figure A.6.2.3). The relative difference between estimates is largest during warmer, lower flow months (April-October), and negligible during other months. Since loads from direct POTW inputs to Suisun Bay are comparable to or substantially greater than loads from the Delta during those months (discussed in Section 6.4.2), the influence of any bias from station choice on total estimated NH_4^+ loads (i.e., from combined sources) is least pronounced during this time. For consistency with the approach developed by Jassby and Cloern (2002), the estimates of NH_4^+ loads leaving the Delta and entering Suisun are based on D24/D16, and the implications of that station choice are discussed below where relevant. For DIN, the choice of stations makes no apparent difference in calculated loads.

Nutrient loads from POTW's were estimated using existing self-reported effluent concentration and flow data from the following agencies that discharge directly into Suisun Bay: Central Contra Costa County Sanitation District (CCCSD), Delta Diablo Sanitation District (DDSD) and Fairfield-Suisun Sewer District (FSSD). Both the period of record and the measurement frequency varied by discharger. In general, flow data was ample, while nutrient data was available on a less frequent basis. Most dischargers measured NH_4^+ concentration in effluent on a monthly basis; CCCSD was the exception in that they measured NH_4^+ on a daily basis for the past 20 years. When flow data was available, but nutrient concentration data was not, we used best estimates for NH_4^+ and NO_3^- concentration from the literature, specific to treatment type, in order to estimate nutrient load. For plants that do not nitrify, NH_4^+ was assumed to be 25 mg L^{-1} ; for plants that do nitrify, NH_4^+ was assumed $<1 \text{ mg L}^{-1}$. Using a combination of actual and estimated data, CCCSD loads were estimated from 1975-present, DDSD loads from 1991-present and FSSD loads from 2004-present. Details on available data can be found in Table 6.2. CCCSD nitrified effluent during several brief trial periods (1977-1982, 1987-1988), during which time NH_4^+ loads differed from the majority of loads over the period of record. Time series of NH_4^+ loads including these periods are presented in Figs. A.6.2.1.5 and A.6.2.1.6, but data included below focus on 1990-2011.

Table 6.2 Available effluent water quality data from major wastewater dischargers into Suisun Bay: Central Contra Costa Sanitary District (CCCSD), Delta Diablo Sanitary District (DDSD) and Fairfield Suisun Sanitary District (FSSD)

	Flow	NH_4^+	NO_3^-	Total P
CCCSD	<u>1975-1978</u> 3-4x/month	<u>1975-1978</u> 3-4x/month	<u>1993-2011</u> 3-4x/month	<u>1975-2011</u> 3-4x/month
	<u>1979-2011</u> Daily	<u>1979-2011</u> Daily		
DDSD	<u>1991-2011</u> Daily	<u>1992-1993</u> Monthly	<u>1992-1993</u> Monthly	<u>1992-1993</u> Monthly
		<u>2007-2011</u> Monthly	<u>5/2007-8/2007</u> Monthly	
FSSD	<u>2004-2012</u> Daily	<u>2004-2012</u> 3-4x/month	<u>2004-2012</u> 3-4x/month	<u>2004-2012</u> 3-4x/month

Currently there is limited data, and no calibrated models, for estimating stormwater nutrient loads in the Bay Area. In the absence of existing estimates for stormwater loads to Suisun Bay, we generated order of magnitude monthly stormwater load estimates for comparison with loads from other sources. Stormwater loads were calculated for the period 1975-2011 using monthly average rainfall data, a weighted-average runoff

coefficient (based on land-use) and representative stormwater nutrient concentrations from the literature. The approach is described in more detail in Appendix 6.3. Watersheds that drain into Suisun Bay were first identified, and their land area and the percent land use compositions were computed using ArcGIS (Fig. A.6.3.1). Using this information along with land-use specific runoff coefficients from literature (Lent and McKee, 2011), we calculated lower-bound and upper-bound weighted-average runoff coefficients. Monthly-average stormwater loads were computed using monthly average rainfall data from National Weather Service, watershed area, weighted-average upper- and lower-bound runoff coefficients, and stormwater nutrient concentrations based on both field measurements from the Bay Area and estimates from literature (e.g., McKee and Gluchowski, 2011).

Some nutrient sources were not estimated because of limited current availability of data. There are additional anthropogenic discharges to Suisun Bay, including small POTW's (Mountain View Sanitary District), refineries (Martinez Refining Company, Valero Refining Company and Valero Beneficia Refinery) and industrial dischargers (Dow Chemical Company, Rhodia Basic Chemicals). Based on the size and average flow from these discharges, their loads are expected to be negligible compared to the Delta loads and POTW loads, and they were therefore not included in load estimates. Internal nutrient loads from sediment flux were also not included because their magnitude is poorly known, but are discussed in Section 6.4.2.

6.2.3. Data Analysis

We evaluated seasonal, temporal and spatial variations of ambient NH_4^+ concentrations in Suisun Bay, focusing on DWR/IEP stations D6, D7 and D8 because of both the completeness of their data record and because they allowed for some spatial comparison among stations in different regions of Suisun Bay.. To visualize long-term and seasonal variations in 30+ year time series, data was aggregated into three eras (1975-1986, 1987-1997 and 1998-2011). Within each era, nutrient concentration data was averaged by month. These specific eras were selected to i) account for any effects of the *Corbula amurensis* clam invasion in 1986 on nutrient and chlorophyll levels; and ii) divide the human high-population-growth period of 1987-2011 into two eras. To quantify long-term changes in concentrations, we compared monthly values over time and calculated the Theil slope. In this method, the slope is calculated between each possible combination of points (in this case, individual monthly values), and the median slope determined (Jassby 2002). Statistical significance of these trends was evaluated based on the Kendall tau test ($p < 0.05$ was considered significant). Lastly, we compared observed NH_4^+ concentrations over this period of record to threshold concentrations hypothesized to inhibit phytoplankton production (Dugdale et al, 2007) and be toxic to copepods (Teh et al, 2011).

Seasonal and temporal variations in nutrient loads into Suisun Bay were also evaluated. Loads were analyzed in a similar manner as nutrient concentration data, utilizing time series plots and also changes in monthly-average concentrations over time. The eras used for presenting load data were 1975-1986, 1987-1995, 1996-2005, 2006-2011. These eras differ somewhat from those used for ambient nutrient concentration analysis because of the changes in data availability in 1995 and 2006. When possible, though, we maintained the latter eras for the analysis of loads from wastewater dischargers.

To characterize the fate of NH_4^+ within Suisun Bay and factors influencing seasonal variations in NH_4^+ concentration, a 1-box mass balance model was developed that treated Suisun Bay as a well-mixed control volume. Data analysis for the box model focused on the period 2006-2011 because loads from important sources were best characterized during this time. Loads into the system included advective Delta efflux, wastewater discharge and tidal exchange. Loads out included tidal exchange and advective efflux out of Suisun. The monthly well-mixed concentrations within Suisun Bay were calculated as the average of D6, D7 and D8. A first-order source or sink term was also included. Additional details on the structure of the mass balance are given in Appendix 6.4.

6.3. Results

6.3.1. Long-term monitoring of nutrient concentrations 1975-2011

Analysis of long-term trends in NH_4^+ concentrations focused primarily on DWR/IEP stations D6, D7 and D8 because of the continuous record of data from 1975-2011 (Figure 6.2, Table 6.2).

As evident in time series plots at D6, D7, and D8 (Fig. 6.2), NH_4^+ concentrations exhibited pronounced seasonality and a gradual increase in baseline levels between 1975-2011. The seasonality and the long-term increases are more evident in Fig. 6.3, where monthly-average NH_4^+ concentrations at each station are presented for three eras. Over the period of 1975-2011, NH_4^+ concentrations increased at D6, D7, and D8 in nearly all months, with statistically significant increases observed during Oct-Dec at all sites and during May-June at D6 and D7. Under current conditions (i.e., 1998-2011), a 2-4 fold increase in NH_4^+ between low-flow (May-October) and high-flow months (November - April) was consistently observed at D6, D7, and D8. NH_4^+ concentrations tended to be 25-75% higher at D6 than at both D7 and D8 during multiple months (Fig. A.6.1.4).

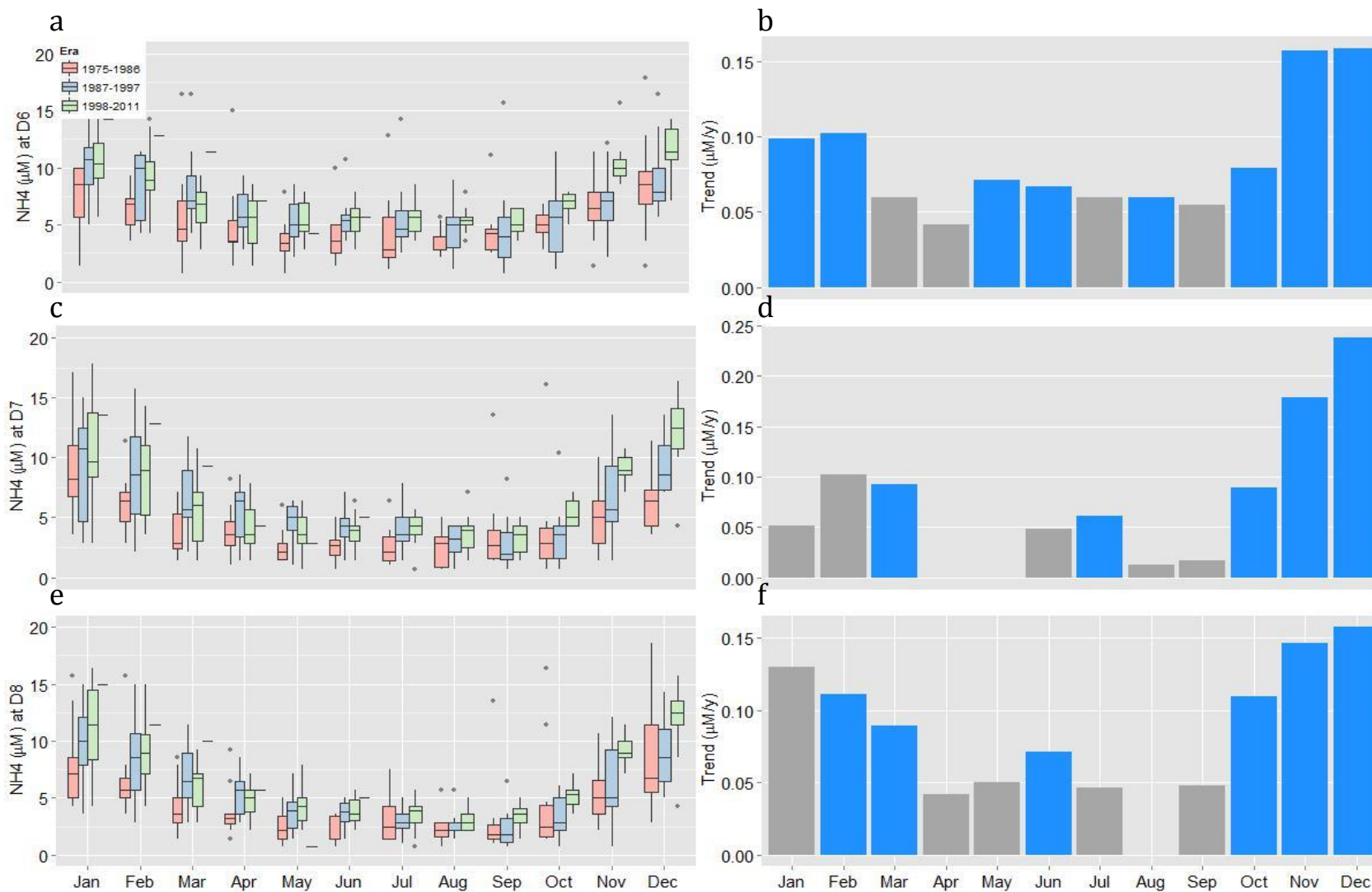


Figure 6.3. Seasonal and temporal variations in NH_4^+ at DWR/IEP stations D6, D7 and D8. Data were first aggregated into three eras (1975-1986, 1987-1997 and 1998-2011), and then averaged by month within each era (panels a, c and e). The boxes extend to the 25th and 75th percentile, the whiskers extend to the highest (or lowest) value within additional 1.5x the interquartile range from the 75th (or 25th) percentiles, and any outliers are shown with dots. Long-term trends were characterized by the Theil slope (see description in Section 6.2.3) (panels b, d and f). Blue bars indicate statistically significant trends with $p < 0.05$ as determined by the Kendall Tau test.

6.3.2. RTC Field Studies: Spring 2010 and 2011

Researchers from RTC performed field studies in Suisun Bay in Spring 2010 (Dugdale et al. 2012) and 2011 (unpublished) to investigate nutrient uptake rates and phytoplankton growth rates in Suisun Bay. The NH_4^+ data were collected at higher spatial and temporal frequency than DWR/IEP data and provide complementary information to the information presented above. An overview of the interpretation of 2010 results with respect to NH_4^+ inhibition of primary production is reported in Dugdale et al. (2012) and discussed briefly in Section 3.

Contour plots of NH_4^+ concentrations during weekly sampling along an east-west transect (in the channel) of Suisun Bay during Spring 2010 illustrate two 3-4 week periods of low NH_4^+ concentrations ($< 4 \mu\text{mol L}^{-1}$) along stretches of 20-30 km (Figure 6.8). Low NH_4^+ concentrations ($< 2 \mu\text{mol L}^{-1}$) were also observed at D7, and persisted at $\leq 2 \mu\text{mol L}^{-1}$ during most of April and May 2010 (Fig. A.3.1). While depressed NH_4^+ levels ($3\text{--}4 \mu\text{mol L}^{-1}$) were generally evident in the monthly DWR time-series data at D7 and D8 and at USGS stations during this time period (Fig. 6.2), the nearly-depleted NH_4^+ levels ($1\text{--}2 \mu\text{mol L}^{-1}$) were not necessarily evident, nor do they give a clear impression of the size and duration of this zone.

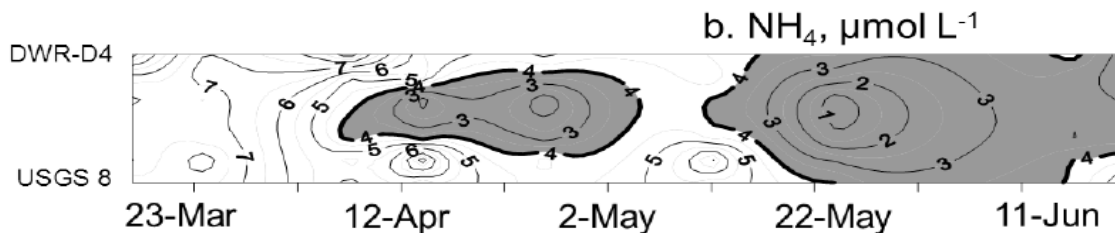


Figure 6.4 NH_4^+ contour plots of data collected by SFSU-RTC during Spring 2010 in Suisun Bay. Data were collected on 9 days at 7 stations along a roughly linear transect through Suisun Bay between DWR/IEP-D4 and USGS-8. (DWR/IEP D7 not included here – see Fig. A.3.1) Figure from Dugdale et al (2012).

The RTC data for 2011 have not yet been published in a peer reviewed paper, but were provided by RTC for this report. NH_4^+ concentrations were less than $4 \mu\text{mol L}^{-1}$ for most of April and May 2011 along the main east-west channel (Fig. 6.5). At D7, NH_4^+ concentrations were $\leq 2 \mu\text{mol L}^{-1}$ throughout April and May 2011 (Fig A.3.2). High flows occurred in Spring 2011, which resulted in short residence times of 6 days or less..

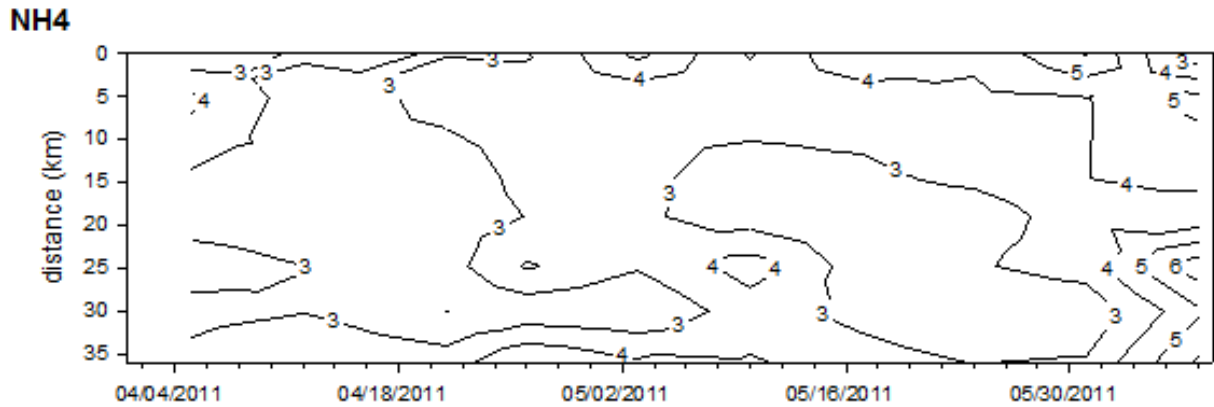


Figure 6.5. NH_4^+ , contour plots of data collected by SFSU-RTC on 9 dates during Spring 2011 in Suisun Bay. Data were collected at 7 stations along a roughly linear transect through Suisun Bay between DWR/IEP-D4 and USGS-8. (Samples were also collected at DWR/IEP D7, but are not included in this plot. See Fig A.3.2 for D7 data)

6.3.3. Load estimates

Delta efflux NH_4^+ loads to Suisun Bay exhibited strong seasonal and interannual variability (Fig. 6.6). Delta NH_4^+ loads to Suisun Bay were highest during high flow months (Dec-Mar; Fig. 6.6, Fig. 6.7). The vast majority of the estimated Delta load came from Sacramento River inputs flowing past Rio Vista, as opposed to flows coming from the central or southern Delta (Fig. A.6.1.6). Visual inspection suggests, not surprisingly, that interannual variability in river flow exerted a strong influence on NH_4^+ loads (Fig. 6.6)

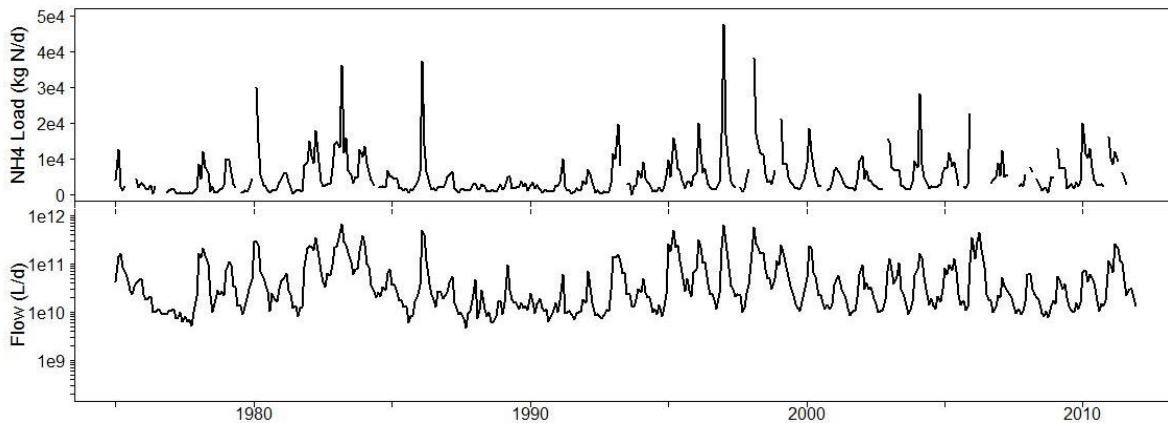


Figure 6.6 Time series of estimated (a) NH_4^+ loads and (b) flows into Suisun Bay from the Delta. Loads were estimated using flow data from DWR DAYFLOW and concentration data from DWR/IEP stations

Between 1975 and 2011, statistically significant increases in NH_4^+ loads were observed during 7 months (Fig. 6.7b), although this increase was not uniform over the entire period (Fig. 6.7a). Between 1975-1995, there was little evidence of increased NH_4^+ loads; in fact, loads decreased in several months over this period (Fig. 6.7a). However, daily NH_4^+ loads increased in all months from 1987-1995 to 1996-2005, with the largest increases occurring during high flow-months. Increases in NH_4^+ loads were also evident during some months between 1996-2005 to 2006-2011. There was a 3-4x difference in NH_4^+ loads between summer and winter, and this seasonality was observed over all eras from 1975-2011.

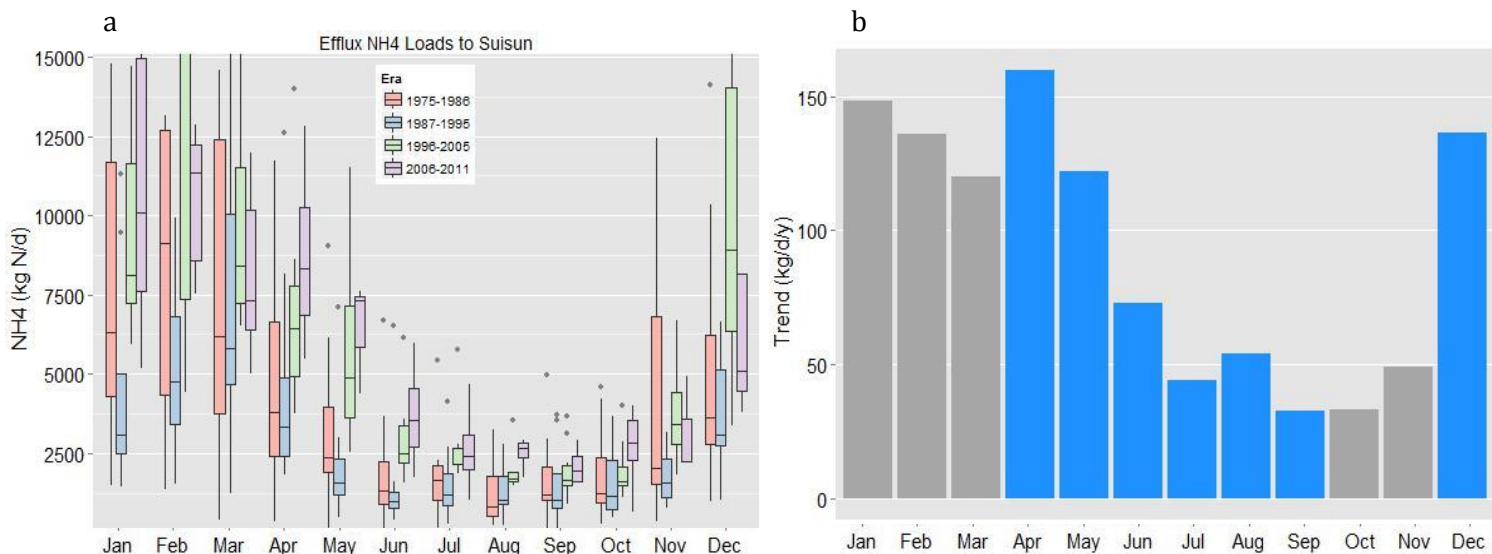


Figure 6.7 Seasonal and temporal variations in Delta efflux NH_4^+ loads to Suisun Bay. Data were first aggregated into three eras (1975-1986, 1987-1997 and 1998-2011), and then averaged by month within each era (panel a). Long-term trends were characterized by the Theil slope (see description in Section 6.2.3) (panel b). Blue bars indicate statistically significant trends with $p < 0.05$ as determined by the Kendall Tau test.

Direct discharge loads to Suisun Bay from 3 POTWs were also estimated. Two POTWs, CCCSD and DDSO, contributed most of the wastewater NH_4^+ loads to Suisun Bay, with loads from CCCSD being ~3 times those from DDSO. The third, FSSD, performs nitrification and their NH_4^+ loads were on average less than 1% of the other two combined and are therefore not included here. During the period of 1990-2011, CCCSD's daily loads varied over a large range, from 20-7350 kg N d⁻¹, with a mean of 2970 kg N d⁻¹ (Fig. 6.8). Loads tended to be highest in January-April, although mean values varied by only 20% between the highest and lowest months. CCCSD's annual-average loads increased by ~20% between 1989 and 2011, with statistically significant increases in monthly-averaged loads observed in all months (Fig. A.6.1.7). Given the proximity of CCCSD's discharge to Carquinez Straits, there is uncertainty about the proportion of

CCCSD's load that is mixed into Suisun Bay and how much is advected downstream before mixing. This point is discussed further in Section 6.4.2.

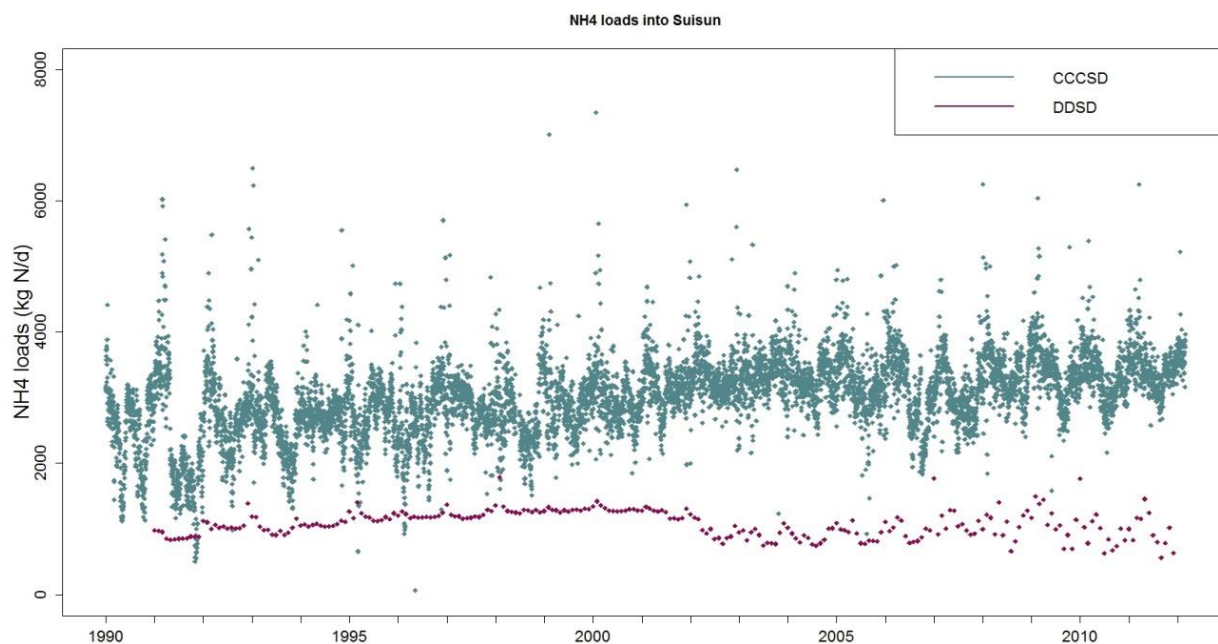


Figure 6.8. Time series of NH_4^+ effluent loads from the two major NH_4^+ dischargers to Suisun Bay: CCCSD and DDS. Data for trial periods of nitrification at CCCSD (1977-1982, 1987-1988) are presented in Figure A.6.1.3. Nitrification processes at FSSD reduce NH_4 loads to approximately 1% of the other two dischargers and are therefore not included here.

DDS's average NH_4^+ load was 1080 kg N d^{-1} , for 1991-2011, with loads ranging from 560 to 1790 kg N d^{-1} (Fig. 6.12). Monthly-averaged NH_4^+ loads from DDS exhibited no consistent change from 1991-2011 (Fig. A.6.1.7d), and loads tended to be higher in Dec-Jun than other months (Fig. A.6.1.7c).

The total watershed area that drains directly to Suisun Bay is 1500 km^2 (Fig. A.6.3.1). The northern and southern combined watersheds had similar upper- and lower-bound weighted average runoff coefficients of 0.40 and 0.15 (Fairfield watershed) and 0.42 and 0.22 (Concord watershed). Calculated NH_4^+ loads from the watersheds were essentially zero during dry periods (Fig. 6.10). During high flow periods, maximum NH_4^+ loads were $200\text{-}600 \text{ kg N d}^{-1}$, which is 80-90% lower than POTW loads and more than an order of magnitude lower than Delta loads during the same months.

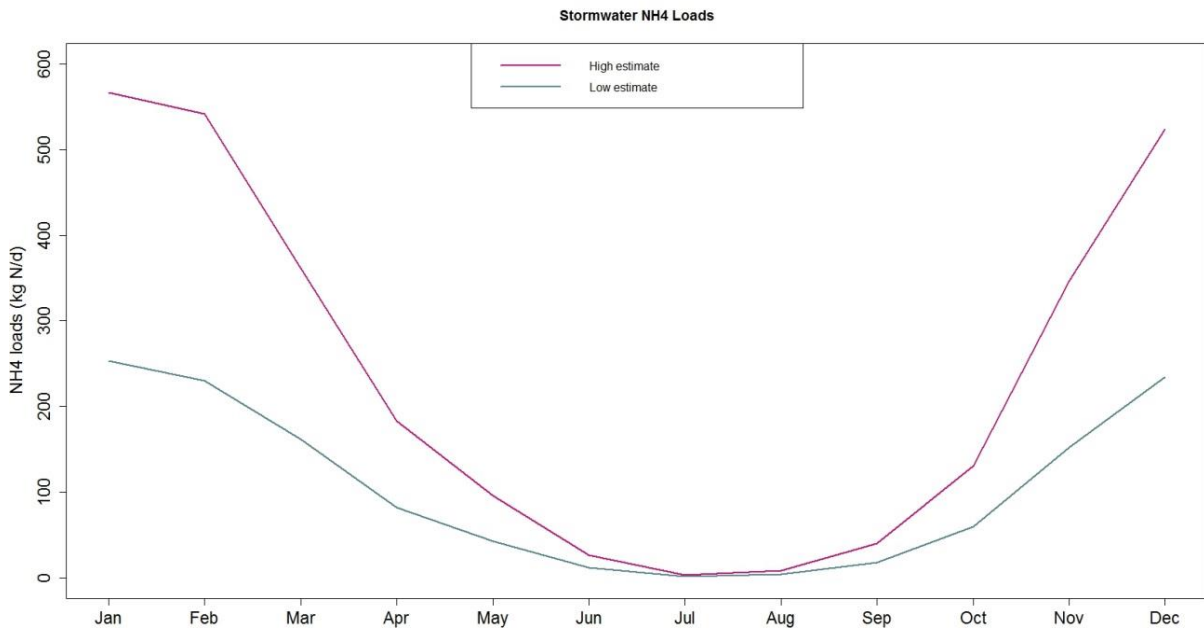


Figure 6.9. Estimated stormwater NH₄⁺ loads from two watersheds that drain directly into Suisun Bay. Loads were estimated using monthly average precipitation values, average runoff coefficient for each watershed (weighted by land-use), watershed area and stormwater NH₄⁺ concentrations from the literature. See Appendix 6.3 for further information.

6.4. Discussion

6.4.1. Comparison of NH₄⁺ concentrations with hypothesized thresholds

The IEP's conceptual model for the POD identifies multiple hypothesized factors that could contribute to ecosystem decline. (Baxter et al. 2010). Elevated NH₄⁺ concentration in Suisun Bay is among those hypothesized factors, with the hypothesized impact resulting from a) inhibition of primary production and thereby lower food supply (e.g., Dugdale et al., 2007; Section 3); b) selection of phytoplankton assemblages that are poorer food quality (Glibert et al., 2011); or c) direct chronic toxicity to copepods that decreases their reproductive success or the survival of offspring to adult forms. NH₄⁺ loads to Suisun Bay have increased significantly over the past few decades (Fig. 6.6 and 6.8). The increased loads have been accompanied by significantly increased NH₄⁺ concentrations within Suisun Bay during certain times of the year (Fig. 6.3). In this section observed NH₄⁺ concentrations in Suisun Bay are compared with NH₄⁺ thresholds that recent studies have identified as having adverse impacting primary production or copepod survival. As noted above, this overall report, and in particular this sub-section, will address the potential roles that NH₄⁺ plays in inhibiting primary production and having toxic effects on copepods. Other nutrient related effects (including shifts in phytoplankton community composition) will be explored in a subsequent report.

According to the conceptual proposed by RTC researchers (Section 3), at NH_4^+ concentrations above $4 \mu\text{mol L}^{-1}$ the uptake of NO_3^- by phytoplankton is substantially inhibited, resulting in lower primary production rates (Dugdale et al., 2007). Although this conceptual model also indicates that $4 \mu\text{mol L}^{-1}$ is not a ‘bright-line’ threshold, and that NO_3^- uptake and phytoplankton productivity are also inhibited at lower levels of NH_4^+ (down to $\sim 1 \mu\text{mol L}^{-1}$; Parker et al., 2012), we will use the $4 \mu\text{mol L}^{-1}$ value here because it is the most frequently discussed value. Similar conclusions are reached when ambient concentrations are compared to either 1 or $4 \mu\text{mol L}^{-1}$. Here we compare ambient concentrations in April-October, when higher chl-a levels were most typically observed prior to 1987, to this $4 \mu\text{mol L}^{-1}$ value. Between 1975-1986, NH_4^+ levels exceeded $4 \mu\text{mol L}^{-1}$ in 44% of the monthly observations at D6, D7, and D8 (Table 6.3). Between 1987-1997, the $4 \mu\text{mol L}^{-1}$ threshold was exceeded in 70% of monthly observations. More recently, from 1998-2011, ambient NH_4^+ concentrations exceeded $4 \mu\text{mol L}^{-1}$ the vast majority of the time (87%). Thus, the frequency with which a $4 \mu\text{mol L}^{-1}$ threshold has been exceeded between April-October has approximately doubled over the past 35 years (Table 6.3).

Table 6.3. Comparison of ambient NH_4^+ concentrations in Suisun Bay in three eras (1975-1986, 1987-1997 and 1998-2011) to relevant environmental thresholds.

		D6				D7				D8			
		#	Tot	%	Mean	#	Tot	%	Mean	#	Tot	%	Mean
> 4 μM (Apr-Oct)	1975-1986	32	73	44%	4.3	15	75	20%	3.2	13	75	17%	3.4
	1987-1997	54	77	70%	5.4	36	72	50%	4.3	25	70	36%	3.5
	1998-2011	85	98	87%	5.6	54	93	58%	4.0	50	97	52%	4.0
> 11 μM (all months)	1975-1986	9	130	6.9%	5.8	7	131	5.3%	4.4	11	131	8.3%	4.8
	1987-1997	17	132	13%	6.7	15	126	12%	5.9	13	125	10%	5.5
	1998-2011	17	164	10%	7.2	18	159	11%	6.1	20	163	12%	6.2
> 26 μM (all months)	1975-1986	1	130	0.8%	5.8	0	131	0%	4.4	1	131	0.7%	4.8
	1987-1997	0	132	0%	6.7	0	126	0%	5.9	0	125	0%	5.5
	1998-2011	0	164	0%	7.2	0	159	0%	6.1	0	163	0%	6.2

Ambient NH_4^+ concentrations at D6, D7, and D8 were also compared to the $26 \mu\text{mol L}^{-1}$ value identified by Teh et al (2011) as the lowest observed effect level (LOEL) for chronic NH_4^+ toxicity to copepods. Copepods have complex life-cycles and are present year round, albeit in varying abundance. Therefore, the $26 \mu\text{mol L}^{-1}$ value was compared with concentrations over the entire year from 1975-2011. Ambient concentrations at stations D6 and D8 only exceed $26 \mu\text{mol L}^{-1}$ once at each of these stations during the

period 1975-2011 (both occurred in 1977) (Table 6.3). There have been limited studies of NH_4^+ toxicity to copepods (Section 4 and 5). One other study of ammonia toxicity to copepods was found, and that study observed chronic toxicity at levels as low as $11 \mu\text{mol L}^{-1}$ (Buttino 1994)., although no pH was specified with this threshold so the partitioning between NH_4^+ and NH_3 is unknown. This value is based on only a single study, and its relevance for Suisun Bay is unknown. NH_4^+ concentrations at D6, D7, and D8 exceeded $11 \mu\text{mol L}^{-1}$ 11% of time between 1998-2011, which was approximately twice as frequent as between 1975-1986.

The above comparisons should be viewed with caution for two main reasons. First, none of these thresholds has been rigorously established. The NH_4^+ -inhibition hypothesis requires further testing (Section 2 and 3); in addition, if it is found to be an important mechanism that limits primary production rates, a protective NH_4^+ value needs to be established through more investigation and could conceivably be lower than $4 \mu\text{mol L}^{-1}$. The copepod toxicity study by Teh et al. (2011) has not yet been replicated, and repeating the experiment may be warranted, both to confirm the original findings and to establish a NOEL. It would also be important for some experiments to be carried out at salinity ranges relevant to Suisun Bay. Copepod sensitivity to NH_4^+ may be salinity-dependent, since toxicity is hypothesized to be exerted through the Na/K transporter, and Na^+ and K^+ levels vary with salinity (ref; S. Teh, pers. comm.). In addition, although Teh et al. (2011) has been evaluated and critiqued as part of discharge permits' public process, it has not to our knowledge undergone peer review.

Second, while NH_4^+ levels at the stations discussed in this report may represent average conditions observed in Suisun Bay, they likely do not represent the highest concentrations in the system, since no data from near-field sampling around POTW outfalls has been included. Undiluted treated wastewater effluent that did not undergo nitrification contains NH_4^+ at concentrations of $\sim 1500 \mu\text{mol L}^{-1}$. Dilutions of ~ 60 -fold are needed to reduce effluent to $< 26 \mu\text{mol L}^{-1}$. This is not an especially large dilution, and likely happens over small distances from outfalls because of strong mixing in Suisun Bay. Nonetheless, if such data exist, it may be worthwhile to compare near-field observations with thresholds, while considering the sensitivity of the habitat within the areas of lower dilution (e.g., its size or location).

6.4.2. Seasonal and temporal trends in NH_4^+ concentrations and loads

NH_4^+ concentrations in Suisun Bay have increased by approximately 50% in several months between 1975-2011 (Fig. 6.3). Statistically significant increases were observed during October-December at D6, D7, and D8, with statistically significant increases also detected in May-June at D6 and D8. NH_4^+ concentrations exhibited strong seasonality over the entire period of record, with ~ 2 -fold higher concentrations observed in January and December than in June-September. This section examines the long-term record of

estimated loads from the Delta to Suisun and loads from POTWs to identify potential causes of both the temporal increase in and pronounced seasonality of NH_4^+ concentrations. .

Since 1975, NH_4^+ loads from the Delta to Suisun Bay have increased substantially (Fig. 6.7), with most of the increase occurring after 1995. On an annual basis, the mean (± 1 s.d.) loads entering Suisun Bay from the Delta were $5790 \pm 1840 \text{ kg N d}^{-1}$ from 2006-2011, and $4060 \pm 2660 \text{ kg N d}^{-1}$ from 1975-1995. NH_4^+ loads exhibited strong seasonality (Fig 6.6, Fig 6.7), as did the magnitude of the increase between pre-1995 and post-1995. Since most of the Delta-derived NH_4^+ load entering Suisun came from the Sacramento River (Fig. A.6.1.6), and most of the NH_4^+ transported along the lower Sacramento River originated at SRWTP (Parker et al. 2012; Foe 2010), increased loads from SRWTP were presumably responsible for most of the observed load increase to Suisun Bay. SRWTP's NH_4^+ loads increased by more than a factor of 2 between 1986 and 2005, with most of that increase occurring after 1995 (Jassby 2008). During the months most relevant for spring phytoplankton blooms (i.e., April and May), mean NH_4^+ loads increased by $5000\text{-}6000 \text{ kg d}^{-1}$ over the period 1975-2011, with most of this increase coming after 1995. This is comparable to the NH_4^+ load increase at SRWTP during this time (Jassby 2008). Present day loads from SRWTP (annual average = $13200 \text{ kg N d}^{-1}$ for 2006-2011) are much larger than the loads entering Suisun from the Delta (Fig. 6.6). As has been demonstrated in other studies (Foe 2010; Parker et al., 2012), much of SRWTP's NH_4^+ load undergoes nitrification en route to Suisun Bay, and a substantial loss of NH_4^+ is consistent with our estimated loads entering Suisun Bay (Fig. 6.6). To preliminarily assess the Delta's role in modulating nutrient loads to Suisun Bay, we also calculated the total monthly NH_4^+ loads that enter the Delta from all major tributaries (Sacramento, San Joaquin, and smaller eastern tributaries), and the amounts that leave the Delta (both to Suisun and via water exports) for the period of 1975-2011, again following the approach described in Jassby and Cloern (2000). On an annual basis, 65% of NH_4^+ was removed within the Delta either by nitrification or uptake by phytoplankton. This value increased up to 90% during some low flow periods. Thus, the Delta acts as a substantial biogeochemical reactor, and its efficiency at transforming NH_4^+ appears to vary seasonally, likely due to factors such as residence time and temperature.

Loads from CCCSD also increased by $\sim 800 \text{ kg N d}^{-1}$ between the early 1990s and 2011 (mean 1990-1995 = 2620 kg N d^{-1} ; mean 2008-2011 = 3380 kg N d^{-1}). CCCSD's loads exhibited strong seasonality; however monthly-average deviations from the annual average were typically less than 20%. DDSD was the third largest NH_4^+ source to Suisun Bay, but its NH_4^+ loads have remained relatively constant since 1990, followed by

stormwater loads, which initial estimates suggest contribute less than 5% of NH_4^+ loads during wet periods.

Identifying which sources have contributed most to the observed increases in NH_4^+ concentrations in Suisun Bay (Fig. 6.3) is not straightforward, because of the large seasonal variation in loads from the Delta. Fig. 6.10 illustrates the magnitudes of NH_4^+ loads from the Delta and from direct POTW discharges to Suisun Bay from 2006-2011. During wet months in most years, Delta loads substantially exceeded direct POTW loads. However, POTW loads were comparable to or exceeded those from the Delta during most spring, summer, and fall months (assuming 100% of CCCSD discharge mixed into Suisun Bay). The increase in Delta loads from pre-1995 to those observed 2005-2011 was large (several thousand kg N d^{-1}) in January-June (Fig 6.7) relative to the increase from CCCSD (several hundred kg N d^{-1}) over the same time period (Fig. A.6.1.7). However, in the remaining months the increases from the two sources were more comparable in magnitude. In addition, the extent to which CCCSD's plume mixes into Suisun Bay needs to be considered. Since CCCSD discharges close to Carquinez Strait, an unknown portion of its effluent plume may be advected downstream before mixing into Suisun Bay, thereby potentially decreasing CCCSD's actual contribution to ambient concentrations in Suisun Bay. The higher NH_4^+ concentrations observed at D6 compared to both D7 and D8, and at USGS7 and USGS 8 relative to other stations further east during the RTC spring sampling campaigns, are consistent with incomplete mixing; however the spatial difference in concentration (a few micromolar) is a fairly modest local increase, considering that the NH_4^+ concentration in CCCSD's effluent was approximately $1500 \mu\text{mol L}^{-1}$. Finally, "internal" sources of NH_4^+ , namely NH_4^+ flux from the sediments, need to be taken into consideration. While this source is not necessarily expected to have changed substantially over time, its magnitude is currently poorly constrained, and it would likely exhibit strong seasonal variations (e.g., due to changes in temperature and organic matter respiration rates, and changes in delivery of fresh organic matter to sediments). A recent study of sediment nutrient fluxes in Suisun Bay and the Delta found that NH_4^+ fluxes varied substantially in space and season, and in light vs. dark conditions (Cornwell et al., submitted). Based on the limited data specific to Suisun in this study, the NH_4^+ fluxes from the sediments to the water column could be on the order of thousands of kg N d^{-1} , and thus potentially comparable in magnitude to POTW loads discharging directly to Suisun Bay. It therefore seems that better constrained estimates of this load, and improved mechanistic understanding of the factors that control its spatial and seasonal variability, are needed. Sediments can also be a substantial NH_4^+ sink (nitrification at the sediment:water interface) in some systems, and also needs to be considered.

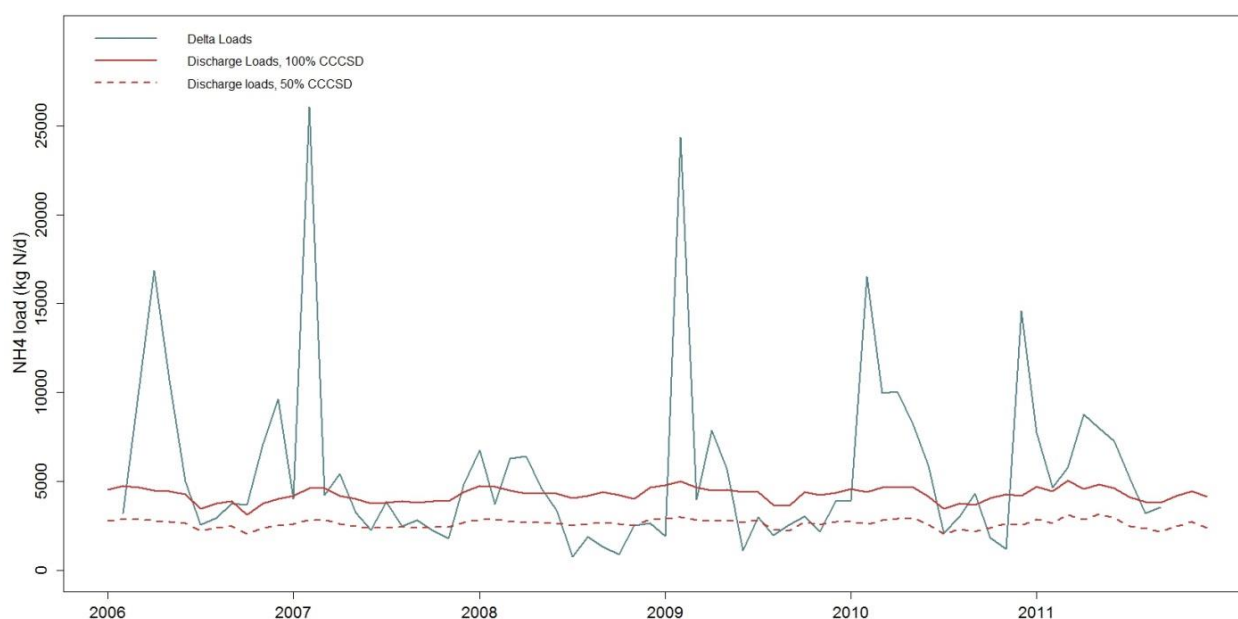


Figure 6.10. Comparison of Delta efflux NH_4^+ loads to Suisun Bay (blue line) and direct POTW discharge NH_4^+ loads assuming 100% mixing of CCCSD effluent (red solid line) and 50% mixing of CCCSD effluent (red dashed line) for the period 2006-2011. During high-flow periods, loads are dominated by the Delta, however during low flow periods discharge loads are comparable to or exceed Delta efflux loads. For a more detailed description of how Delta efflux loads were calculated, see Appendix 6.2

6.4.3. Examining NH_4^+ fate in Suisun Bay

NH_4^+ concentrations in Suisun Bay varied seasonally by as much as a factor of 2-3 between low-flow and high-flow months, a general pattern that has remained similar over the past 35 years (Fig. 6.2). This seasonal variation cannot be explained by seasonal variations in NH_4^+ loads alone: when current NH_4^+ loads from the Delta and Suisun Bay POTWs were considered along with typical flushing rates during spring, summer, and fall, the predicted NH_4^+ concentration was on the order of $20 \mu\text{mol L}^{-1}$ (assuming conservative behavior), as compared to the observed levels $3\text{--}6 \mu\text{mol L}^{-1}$ from May-September (Fig. 6.3). This large difference between predicted and measured concentration is especially relevant within this concentration range of $3\text{--}20 \mu\text{mol L}^{-1}$, considering the levels at which NH_4^+ is hypothesized to inhibit primary production ($>2\text{--}4 \mu\text{mol L}^{-1}$) and have toxic effects on copepods ($\text{LOEL} = 26 \mu\text{mol L}^{-1}$). To further explore the seasonal variations in NH_4^+ concentrations and NH_4^+ fate, we developed a basic 1-box model for Suisun Bay. Data analysis with the box model focused on 2006-2011, when data from all load sources was most certain, and also on the months April-October, when residence time in Suisun Bay tends to be longest and when phytoplankton blooms have been historically observed. The analysis considered several load terms, including:

loads from the Delta, POTW loads, advective loads out of Suisun Bay through the Carquinez Straits, and tidal exchange (See Appendix 6.4 for details). A first order term (source or sink) was also included.

During April-October of 2006-2011, the box model results suggest that on average only 25% of the NH_4^+ that entered Suisun Bay actually exited as NH_4^+ through the Carquinez Straits (outgoing loads were based on estimated advective transport and tidal exchange combined; Fig 6.11). By difference, ~75% of NH_4^+ that entered Suisun Bay was either transformed (e.g., nitrification) or taken up by phytoplankton. We tested the sensitivity of the model to the proportion of CCCSD's load that is assumed to mix completely into Suisun Bay: even when 50% of CCCSD discharge is assumed to be directly transported downstream, and not mix into Suisun, approximately 70% of the NH_4^+ still needs to undergo transformation or loss within Suisun Bay in order to explain the observed concentrations. The first order rate constant required to explain the transformation/loss of NH_4^+ during low-flow periods was in the range of 0.1-0.3 d^{-1} (Fig. A.6.4.7), which is comparable in magnitude to nitrification rates typically used in water quality models (e.g., ~0.1 d^{-1} ; J Fitzpatrick, HDR, pers. comm.). The mass balance did not include NH_4^+ loads due to flux from the sediments; therefore, the calculated NH_4^+ loss rate constants may in fact be lower-bound values. The magnitudes of the transformation/loss term and downstream transport term varied within a given year (Fig. 6.11). As expected, as flow decreased from April-October, and residence time increased, the magnitude of downstream transport decreased. It was initially somewhat surprising to see that the magnitude of transformation/loss was actually larger in April and May than in later months when residence times were longer and temperatures warmer, which would tend to favor higher rates of nitrification. One plausible hypothesis is that the higher transformations/loss rate in April and May were due in part to phytoplankton uptake. April and May are the months during which phytoplankton growth rates have typically been greatest in Suisun Bay (Kimmerer and Thompson, in press), and when blooms were generally observed prior to 1987 and now occur occasionally (e.g., blooms in 2001 and 2010 documented by Dugdale et al, 2007 and Dugdale et al. 2012). Although chl-a levels tended to remain low in April and May over 2006-2011 (except the 2010 bloom; Dugdale et al., 2012), the low chl-a levels could be readily explained by estimated clam grazing and microzooplankton grazing, which typically exceeded or matched gross primary production rates (Kimmerer and Thompson, in press).

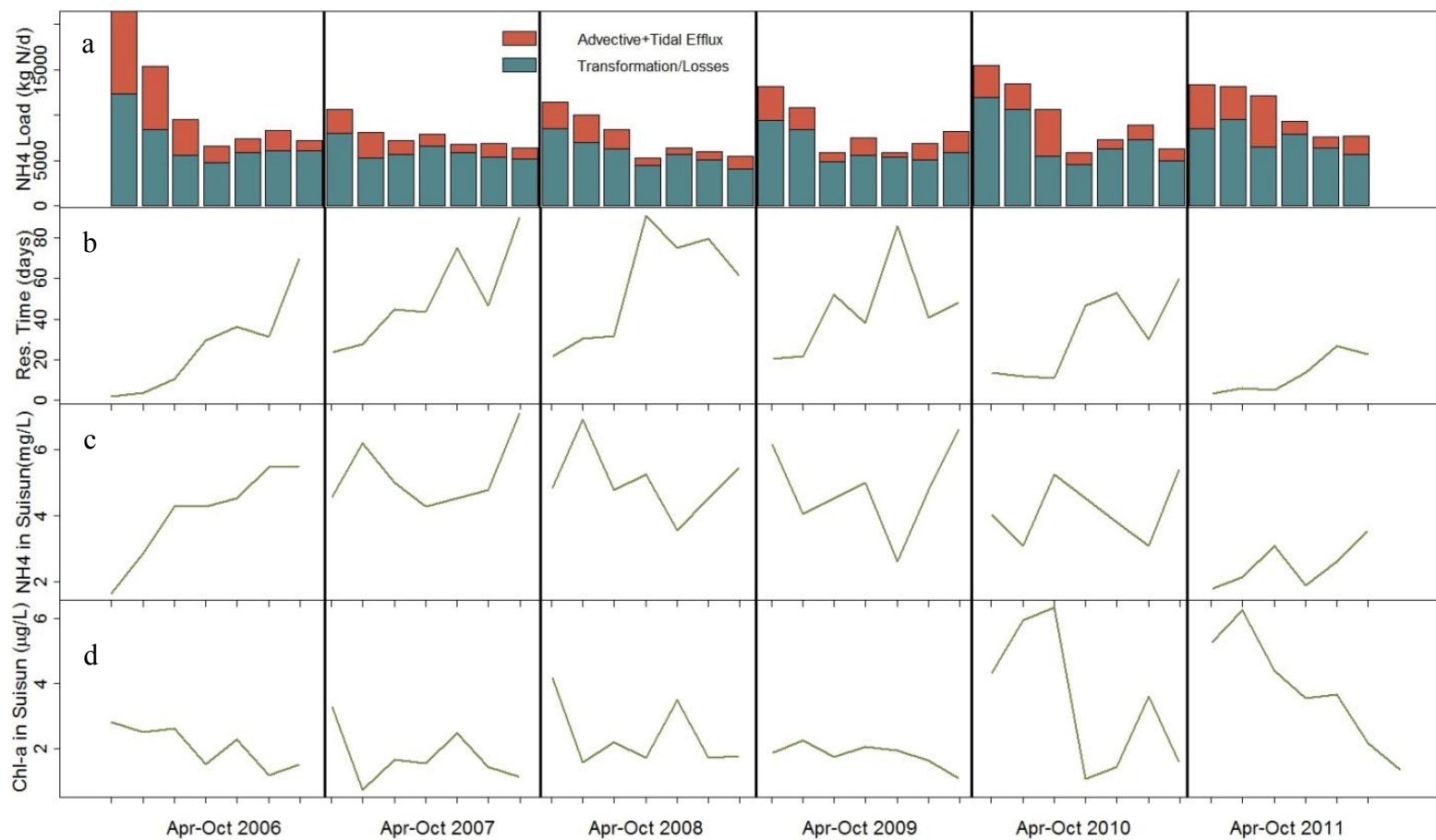


Figure 6.11. a) Comparison of the fate of NH₄⁺ entering Suisun Bay during April-October 2006-2011. Transformations/losses are always greater than advective/tidal efflux, particularly in the late summer months. b) Residence time in Suisun Bay. Residence time was calculated by dividing the volume of Suisun Bay (6.5×10^{11} L) by daily advective flows. c) NH₄⁺ concentrations in Suisun Bay (average of DWR/IEP stations D6, D7 and D8). d) Chl-a concentrations in Suisun Bay (average of DWR/IEP stations D6, D7 and D8).

We evaluated the uncertainties introduced by the simplifying assumptions of the 1-box model, and discussion of those points is in Appendix 6.4. Overall, though, despite the inherent limitations of a 1-box model, the mass balance results suggest that transformations/losses within Suisun Bay play an important role in determining ambient NH_4^+ concentrations during low-flow months. Better characterizing these processes, including seasonal and temporal variability, will require modeling Suisun Bay on finer spatial and temporal scales, and likely some field investigations. The comparison of Delta efflux NH_4^+ loads estimated using different stations suggested that total NH_4^+ loads were actually somewhat lower than those used in the box model. This in turn means the calculated transformation/loss rates during low-flow periods are overestimates. However, even if actual Delta efflux loads were considerably lower than those used in the box model, the overall conclusion that substantial transformation/loss occurs within Suisun Bay does not change, since Delta efflux loads represented ~30% of total loads during the months considered. Nonetheless, this uncertainty underscores the point that improved load estimates may be needed.

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7. Recommended next steps

The recommendations identified here are not intended to be comprehensive, but rather communicate some broad suggestions that became clear during the writing of this report.

1. General: A coordinated nutrient science plan should be established for Suisun Bay and the Delta, with clearly articulated scientific questions, recommended experiments or monitoring, and a prioritization of work. There are currently numerous nutrient-related studies being conducted in Suisun and the Delta. However, the work is being carried out in more of a patchwork fashion, funded or directed by different organizations, and with limited overarching prioritization and coordination. This does not necessarily require a new entity. Instead, the development of a Delta-Suisun nutrient science plan could be coordinated among the Bay-wide nutrient strategy participants, the IEP, and other entities. Developing such a coordinated nutrient science program is consistent with recent recommendations in the Delta Plan V6.0.

2. NH_4^+ inhibition hypothesis:

2.a To identify the specific science questions and the types of studies needed to better understand the hypothesized mechanisms of the NH_4^+ -inhibition conceptual model, it would be both helpful and efficient to convene a science panel. This panel should consist of regional scientists working on phytoplankton ecology and nutrient issues in the Bay, as well as outside experts. The panel would explore the detailed evidence from studies in San Francisco Bay and literature from other systems and identify: science issues on which there is consensus among the panelists; outstanding scientific questions; and studies that need to be carried out to address the outstanding questions. It is recommended that the panel develop a brief consensus document summarizing their observations and recommendations. Such a document could be a key component of the Suisun nutrient science plan mentioned above.

2.b. In parallel with any field or experimental studies, modeling work should be carried out to evaluate the potential quantitative importance of NH_4^+ -inhibition at the ecosystem scale, relative to other factors known to play important roles in limiting primary production rates (e.g., light limitation) or biomass accumulation (clam grazing, residence time) in Suisun Bay. Thus far, this issue has not been adequately investigated. Such an analysis could be carried with relatively basic biogeochemical models and existing data, and using current parameterizations of the proposed mechanisms. These modeling efforts have benefits well beyond testing the NH_4^+ hypothesis, in that they will simultaneously yield tools for quantitatively synthesizing existing nutrient and phytoplankton data in Suisun Bay and other embayments, identifying data and monitoring needs, and informing the broader modeling strategy for the Bay.

3. NH_4^+ toxicity to copepods:

If toxicity to copepods from NH_4^+ will be among the issues that will inform nutrient management decisions in Suisun Bay, it would worthwhile to conduct further investigations. While the copepod toxicity study by Teh et al. (2011) was carefully executed, it has not yet been replicated. Furthermore, although there is some support for the proposed toxicity mechanism in the literature, only a handful of studies have been published on NH_4^+ toxicity to aquatic invertebrates, and none of those studies used copepods as the test organism. Prior to beginning work it would be valuable to have the study design peer reviewed, and to have broad buy-in among regulators and stakeholders (see recommendation #1). Teh et al. (2011) observed an effect in the lowest dosed samples, and treatments at lower NH_4^+ concentrations would be needed to establish a no observed effect level (NOEL). In addition, treatments using salinity and pH ranges relevant to Suisun Bay would be needed, since copepod sensitivity to NH_4^+ could vary with salinity. While other more nuanced questions and complex study designs may eventually be warranted (e.g., effect of food limitation plus NH_4^+), replicating the chronic toxicity experiment first, and determining if similar or different thresholds are observed, is a logical next step.

Appendices

Appendix 2

A.2.1 Photosynthesis

A.2.1.1 Light Harvesting and Photosystem II (PSII)

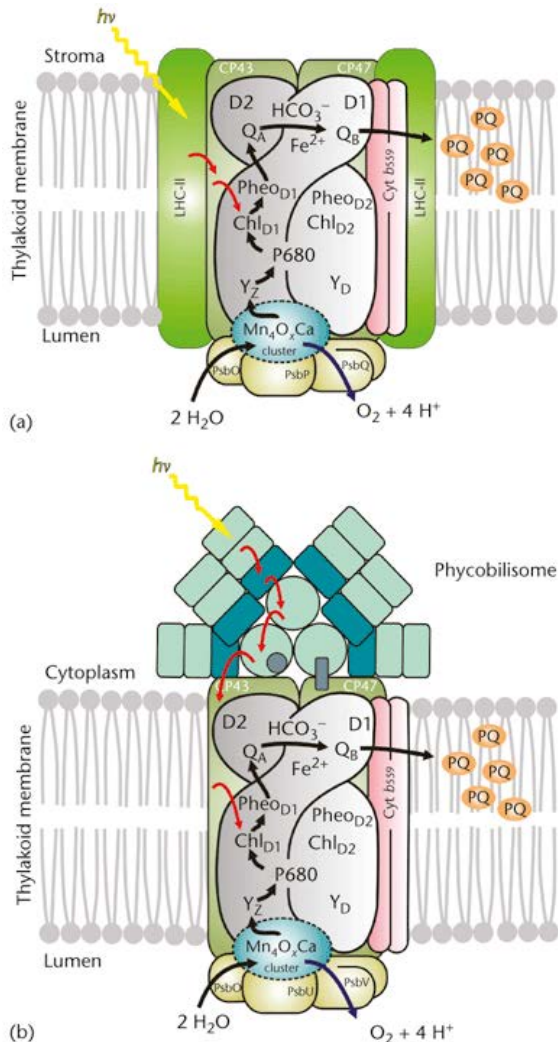


Figure A.2.1 Reproduced from Govindjee et al. 2010; PSII in a) eukaryotes and b) prokaryotes. The core of PSII is composed of the proteins D1 and D2 that bind P680, a pair of chlorophylls, and Chl_{D1}, the primary electron donor. Together, these chlorophylls make up the reaction center. CP43 and CP47 constitute the interior PSII antenna system (not to be confused with the peripheral LHCII and Phycobilisome antennae) and Mn₄O₂Ca is the manganese cluster involved in splitting and removing electrons from water.

The central structure for light energy conversion is the photosystem, a macromolecular pigment-protein complex integral to the thylakoid membrane. Photosystem II (PS II) consists of two key components, a peripheral antenna containing chlorophyll *a* (Chl *a*) and other pigment molecules that absorb light, and a reaction center at the core of the complex (Govindjee et al. 2012). The light absorbed in the antenna is transferred to the reaction center (Fig. 1, red arrows) where an electron in the Chl *a* molecule becomes excited and the first steps of electron transport occur (Fig. 1, black arrows). In eukaryotic phytoplankton, the light harvesting complex II antenna (labeled LHC-II in Fig. 1a) is integral to the thylakoid, just as the core is, but in most prokaryotes, the phycobilisome antenna rests on top of the thylakoid membrane (Fig. 1b). While the core complex is highly conserved among all classes of photosynthetic organisms, the antenna are diversified in terms of their structure and the types of pigments they bind (Ballottari et al. 2012).

After the electron in the reaction center becomes excited it's transferred to the plastoquinone pool (PQ) and on down the electron chain to the final electron acceptor NADP⁺. Along the way, energy from the electrons is used to push protons to the lumen side of the thylakoid membrane. With continued electron transfer down the chain, protons accumulating in the lumen build up a cross-membrane potential that is subsequently used to power ATP synthesis. Meanwhile, the electron originally lost from Chl *a* in the reaction center is replaced by an electron extracted from water; four photons are required to remove four electrons from two water molecules to produce one molecule of oxygen (Govindjee et al. 2010).

A.2.1.2 Photoprotection and stress

PSII is the only photosystem that catalyzes the splitting of water and the production of oxygen in photosynthetic organisms. With this distinction also comes great danger when the cell absorbs more energy than it can use to power C- fixation. In that case, the entire electron chain may back-up resulting in the release of free electrons that combine with oxygen to produce oxygen radicals that damage the PSII proteins (Vass et al. 1992, Clarke et al. 1993). To avoid this situation, photosynthetic cells have several built-in “electron valves” (e.g. Bailey et al. 2008, Zehr and Kudela 2009). One of the most effective occurs at the point where light energy is harvested; instead of being funneled to the reaction center, it can be dissipated as heat by the accessory pigments in the antenna (Ballottari et al. 2012). In diatoms, this involves the action of carotenoid xanthophyll cycle pigments (Nymark et al. 2009). This radically slows the flow of electrons through the electron transport chain and decreases the efficiency of the conversion of light energy to ATP, also known as the quantum yield of PSII (or yield). The decrease in yield occurs in all photosynthetic cells in response to any stressor that affects C-fixation and can be measured reliably with an instrument that measures variable fluorescence.

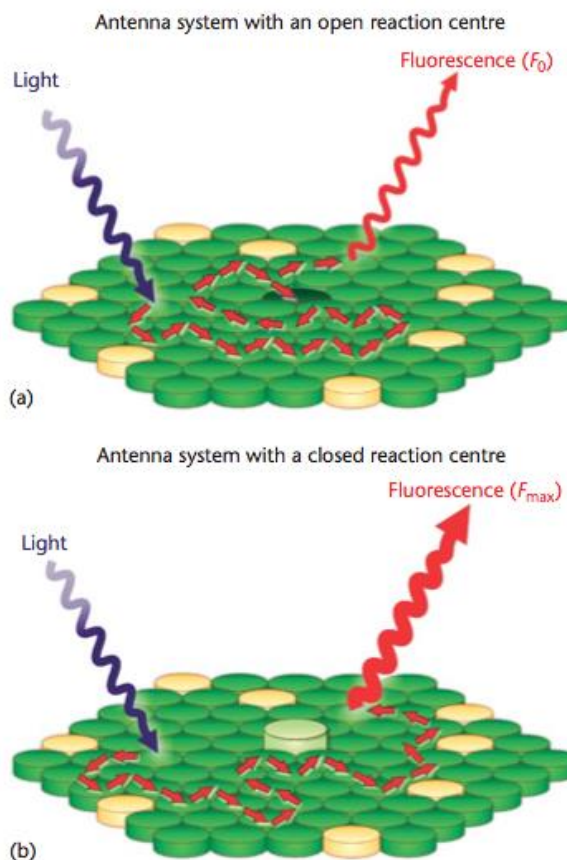


Figure A.2.2 Reproduced from Govindjee et al. 2010; Excitation energy transfer among Chl *a* and carotenoid molecules in a “generic” LHC II antenna (small red arrows). Green disks represent Chl *a* molecules and orange disks represent accessory pigment molecules. a) Energy transfer to an open reaction center with minimal fluorescence emission and b) energy transfer to a closed reaction

A.2.1.3 PSII efficiency and fluorescence

In addition to generating ATP and heat, light energy can be dissipated as fluorescence. In an optimally functioning photosynthetic cell, up to 90% of the harvested light energy is used to excite electrons in the PSII reaction centers and very little is dissipated as heat or fluorescence. As the cell becomes more stressed, less of the energy is used for photochemistry and more is dissipated as heat. The

amount of energy that goes into photochemistry can be measured by channeling it into fluorescence. This is accomplished by closing all reaction centers in the cell at once; the resulting increase in fluorescence corresponds to the amount of light energy that would otherwise be used to excite electrons (Fig. 2). By subtracting the baseline fluorescence (F_0) from this maximal fluorescence (F_m), one arrives at variable fluorescence ($F_m - F_0 = F_v$) which provides a direct estimate of the quantum yield of PSII. Because the architecture of the PSII core, and therefore fluorescence emission, are highly conserved, the range in variable fluorescence is similar across all photosynthetic taxa. When cells are stressed, yield and variable fluorescence rapidly decline. Typically, F_v is expressed as a fraction of F_m . This quotient, F_v/F_m , varies from 0 to about 0.65 in marine phytoplankton depending on the baseline fluorescence reading (Kromkamp and Forster 2003). Phycobilin-containing cyanobacteria tend to have a slightly greater baseline reading (F_0), therefore F_v/F_m typically ranges between 0-0.5 (Cambell et al. 1998).

Variable fluorescence is widely used in phytoplankton ecology to document environmental stress such as toxicity from herbicides (Fai et al. 2007, Choi et al. 2012), nutrient limitation (Geider et al. 1993, Young and Beardall 2003, Berg et al. 2008, Kudela 2008, Parkhill et al. 2012), high light or UV exposure (Six et al. 2004, 2007, Berg et al. 2011) and oxidative stress (Drabkova et al. 2007), to mention a few.

A.2.1.4 Photoacclimation

Dissipation of excess light energy as heat (or fluorescence) are short-term responses. A photosynthetic cell can also acclimate to changes in irradiance over the longer term by adjusting the size of its peripheral antenna to capture more or less energy. Under persistent high light, the cell will acclimate by shedding Chl *a* in order to decrease its antenna size. Photoacclimation occurs on the order of hours and is not only affected by changes in irradiance, but also by changes in nutrients and temperature. With constant irradiance, a decrease in nutrient concentration will have the same physiological effect as an increase in light intensity because the energy capture will be in excess of C fixation as the cell slows its growth. To avoid photoinhibition (loss of photosynthetic function due to PSII damage in excess of cell's capacity of repair) under persistent nutrient limitation, cells will decrease their Chl *a* cell⁻¹ (LaRoche et al. 1993, Graziano et al. 1996). With constant irradiance and nutrient concentration, an increase in temperature will increase the growth rate of phytoplankton and therefore their energy needs. To compensate, the cell will increase its antenna size and Chl *a* cell⁻¹ (Geider 1987). Changes in Chl *a* cell⁻¹ as a function of irradiance, nutrient concentration and temperature substantially influences the C:chl *a* ratio (C:Chl) of the cell. and decreases exponentially with increased temperature (and growth rate) at constant light level (Fig. 3).

A.2.2 Carbon Fixation

A.2.2.1 The Calvin Cycle

As mentioned above, photochemistry leads to a) the build-up of a proton-motive force and subsequent production of ATP via photophosphorylation and b) reducing power in the form of NADPH. The link between C fixation (the process of attaching gaseous CO₂ to a C skeleton) and photochemistry becomes

evident when we examine the energetics of C fixation. It takes electrons from two NADPH carriers and 3 ATP molecules to fix a single CO_2 molecule in the Calvin cycle (Fig. 3). Therefore, C fixation would grind to a halt without a continuous supply of reducing power and ATP from the light reactions (Fig. 3).

The rate-limiting step in C fixation is the Ribulose-1,5- Bisphosphate Carboxylase/Oxygenase (Rubisco) enzyme, which catalyzes the first step in the Calvin cycle. This is because Rubisco is notoriously slow and catalyzes 3 molecules per second compared with 1000 molecules per second for a typical reaction. To make up for this the cell has to produce large quantities of the enzyme. The cell also has to make sure that all the Rubisco enzymes are saturated with CO_2 to prevent oxygen from binding to its active site. This may be a problem for marine phytoplankton that live in habitats where the concentration of CO_2 found in seawater can limit phytoplankton C fixation (Riebesell et al. 1993). To deal with this issue, most marine phytoplankton evolved what is collectively known as C concentrating mechanisms (CCM). Rather than depend on the diffusion of CO_2 across the plasma membrane, phytoplankton actively take up bicarbonate (HCO_3^-) occurring at a concentration of 2 mmol/L in seawater. Inside the cell, HCO_3^- is converted to CO_2 in close proximity to Rubisco in order to saturate the enzyme reaction (Krooth et al. 2008).

A.2.2.2 Beta carboxylation

Phytoplankton can also directly fix HCO_3^- to organic C. This pathway, mediated by the enzymes phosphoenolpyruvate carboxylase (PEPC) and pyruvate carboxylase (PYC) catalyze the reaction of HCO_3^- to either phosphoenolpyruvate or pyruvate, respectively, to form oxaloacetate (OAA). These enzymes are primarily targeted to the mitochondria, ubiquitous in marine phytoplankton, and may be involved in supplying OAA to the Tricarboxylic Acid (TCA) cycle to counter the drain of 2-oxoglutarate (also called α -ketoglutarate) C skeletons to the NH_4^+ assimilation/amino acid biosynthesis pathway (Fig. 4, Guy et al. 1989, Vanlerberghe et al. 1990, Kroth et al. 2008). Because the TCA cycle generates electrons for the respiratory/mitochondrial electron transport chain, NH_4^+ assimilation has the potential of reducing this electron flow, and therefore ATP production, unless there is a source of OAA to the TCA cycle (Guy et al. 1989).

A.2.3 Nitrogen assimilation

C and N assimilation are tightly linked because they share the flow of energy from light, and because fixed C provides skeletons for N assimilation. Additional energy for N reduction is supplied from respiration of fixed C (Fig. 3)

NH_4^+ is key to N assimilation and the first steps of amino acid biosynthesis because this is the only form of N that can be attached to oxoglutarate (also known as α -ketoglutarate), supplied by the TCA cycle, to produce the amino acid glutamate via the action of the enzymes glutamine synthetase (GS) and glutamate synthase (GOGAT). These reactions require input of reductant and ATP (Fig. 3). Multiple forms of the GS/GOGAT enzymes, localized both to the cytosol and to the chloroplast, exist in phytoplankton (i.e. Huppe and Turpin 1994). Recent genome sequencing efforts have demonstrated that

in diatoms, the plastid-localized set is comprised of GSII and a ferredoxin-dependent form of GOGAT (Fd-GOGAT), thought to be responsible for the assimilation of NH_4^+ produced by NO_3^- reduction (Hockin et al. 2012). A second, GSIII and NADPH-dependent GOGAT set is localized outside the chloroplast and might assimilate NH_4^+ produced by cellular processes (Fig. 3), uptake and deamination of organic N sources (amino acids, purines etc) and direct NH_4^+ uptake (Mock et al. 2008, Brown et al. 2009, Hockin et al. 2012).

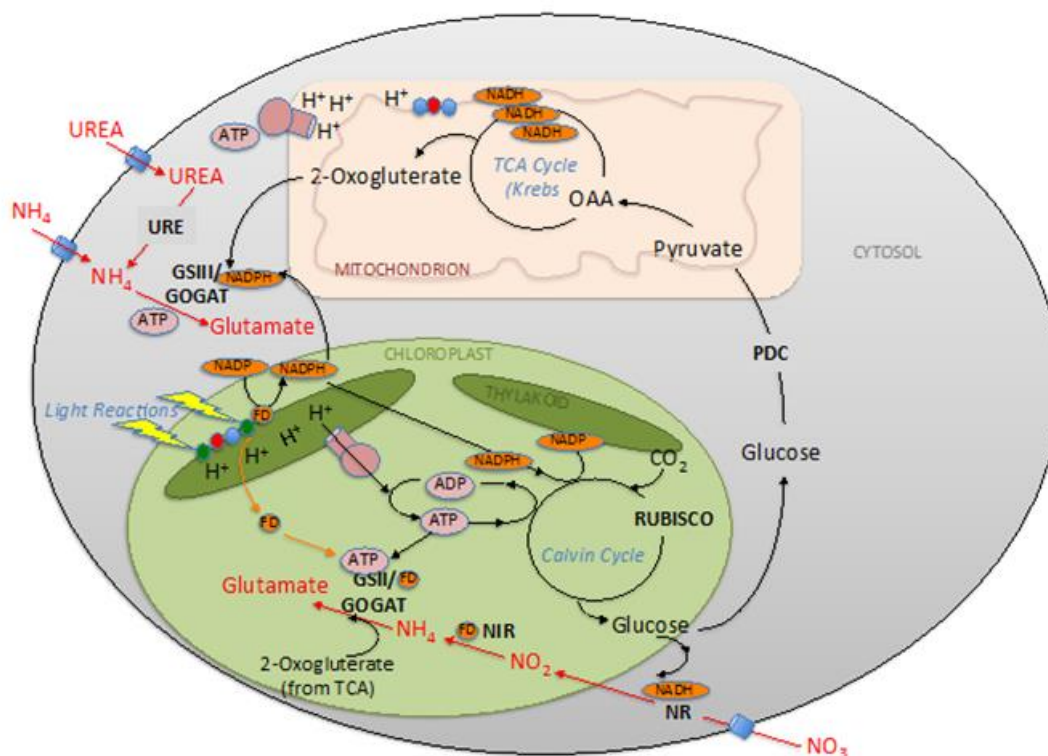


Figure A.2.3. Interactions between carbon metabolism (black lines) and nitrogen metabolism (red lines) in a photosynthetic cell. Electron carriers in orange, ATP in pink, ATP synthase enzyme in pink, FD=ferredoxin, NADPH=nicotinamide adenine dinucleotide phosphate, ATP=adenosine triphosphate, NR=nitrate reductase, NiR=nitrite reductase, GS/GOGAT=glutamine synthetase and glutamate synthase, URE=urease, PDC=pyruvate dehydrogenase complex, OAA=oxaloacetate, TCA=Tricarboxylic Acid Cycle, also known as the Krebs cycle. Green oval is chloroplast with thylakoid membranes, peach rectangle is mitochondrion and grey is cytosol of the cell. **Nitrate Reduction:** The first step, catalyzed by NR, reduces NO_3^- to nitrite (NO_2^-) by the addition of two electrons from NADH (most marine phytoplankton) or NADPH (green algae and terrestrial plants), and takes place in the cytosol. Subsequently, NO_2^- is transported into the chloroplast where it's reduced by NiR to NH_4^+ with six electrons donated from FD. **Ammonium assimilation/chloroplast:** NH_4^+ reduced from NO_3^- is subsequently combined with 2-oxoglutarate via GSII/Fd-GOGAT requiring reductant from FD and ATP to produce glutamate. **Cytosol:** Glutamate is also synthesized from NH_4^+ in the cytosol via GSIII/NADPH-GOGAT.

A.2.3.1 Kinetics of nutrient uptake and the role of transporters

Recent advances in the molecular biology of transport proteins in phytoplankton give insights into the factors that drive variation in K_s and in the shape of uptake hyperbolas. For example, it has been established that nitrate (NO_3^-) uptake in phytoplankton reflect what is known for vascular plants and is mediated by high affinity transporters (HATS) and low affinity transporters (LATS) of the *Nrt2* and *Nrt1* gene families, respectively (Galvan and Fernandez 2001). The high affinity transporter protein NRT2 operates at NO_3^- concentrations upto 60-100 $\mu\text{moles L}^{-1}$ but will typically saturate around 40 $\mu\text{moles L}^{-1}$ in marine phytoplankton (Lomas and Glibert 2000, Collos et al. 2005). The 40-100 $\mu\text{mole L}^{-1}$ range of NO_3^- represents the upper limit of what phytoplankton species are exposed to in marine and coastal environments, therefore regulation of the NRT2 HATS transporter proteins are of primary interest for growth of marine phytoplankton. The number of *Nrt2* genes, which encode the NRT2 transporter proteins, vary in marine and estuarine phytoplankton genomes. Some, like the diatom *Thalassiosira weissflogii* contain six nearly identical *Nrt2* gene copies while others like the harmful alga *Aureococcus anophagefferens* contain only one copy (Song and Ward 2007, Berg et al. 2008). The *Nrt2* genes are inducible and are transcribed in response to NO_3^- concentrations, N starvation, and are inhibited in response to NH_4^+ (Navarro et al. 1996, Hildebrand and Dahlin 2000, Galvan and Fernandez 2001, He et al. 2004).

Above external NO_3^- concentrations of 100 $\mu\text{moles L}^{-1}$, LATS may be induced in marine and estuarine phytoplankton resulting in biphasic kinetics (Dortch et al. 1991, Collos et al. 1992). LATS typically display linear kinetics and remain unsaturated upto several hundred $\mu\text{moles L}^{-1}$ (Dortch et al. 1991, Collos et al. 1992, Collos et al. 2005). Induction of LATS in chlorophytes and vascular plants occur at much greater NO_3^- concentrations than in marine phytoplankton, typically above 1 mmoles L^{-1} (Galvan and Fernandez 2001). To our knowledge, only a couple of estuaries in the world have concentrations of NO_3^- high enough to induce LATS for NO_3^- in marine phytoplankton (Frankignoulle et al. 1996, Collos et al. 1997).

Uptake of NH_4^+ is regulated by the high affinity ammonium transporter AMT1 which mediates active transport by coupling NH_3 influx to a H^+ gradient, or transports charged NH_4^+ without co-transport with H^+ (von Wiren et al. 2000, Loque et al. 2007, 2009). An additional high affinity transporter family, AMT2, with sequence identity close to that in yeast and bacteria, has also been identified in vascular plants (Sohlenkamp et al. 2002) but so far not in phytoplankton. The AMT1 family include upto eight *AMT1* genes (*AMT1;1-8*) in the chlorophyte *Chlamydomonas reinhardtii* that are expressed differentially in response to NH_4^+ (Gonzalez Ballester et al. 2004). Some are expressed in response to N starvation and to the presence of NO_3^- , whereas others are expressed constitutively regardless of N substrate or concentration (Gonzalez Ballester et al. 2004). In the estuarine diatom *Cylindrotheca fusiformis*, Hildebrand (2005) observed the *AMT1* transporter gene to be expressed constitutively explaining why maximal rates of NH_4^+ uptake are commonly observed regardless of how low external NH_4^+ reach, resulting in uptake hyperbolas that differ markedly from that of NO_3^- (McCarthy et al. 1975, Kudela and Cochlan 2000).

Biphasic kinetics of NH_4^+ uptake has also been observed in vascular plants across root hairs (Ullrich et al. 1984, Wang et al. 1993). The LATS that mediate NH_4^+ transport are hypothesized to be passive potassium (K^+) channels and aquaporins (Ullrich et al. 1984). In contrast with AMTs, which are very

selective for NH_4^+ , these channels do not differentiate between K^+ and NH_4^+ . In marine and freshwater phytoplankton, LATS for NH_4^+ uptake have not been characterized but are assumed to be related to passive K^+ channels as in vascular plants (Gonzalez Ballester et al. 2004). However, investigations with marine phytoplankton cultures have not uncovered biphasic uptake kinetics for NH_4^+ in a similar manner to that for NO_3^- . That may be because NH_4^+ concentrations above the saturation point for the AMT1 transporters can be toxic to phytoplankton cells.

Uptake of inorganic phosphate (PO_4^{3-}) as a function of PO_4^{3-} concentration looks different still compared with NO_3^- and NH_4^+ . Short term PO_4^{3-} uptake kinetics displays two inflection points at low PO_4^{3-} concentrations, in a concentration range that is much lower than that predicted to involve a low affinity transporter (Persson et al. 1999). Depending on growth rate, these inflection points occur at 1 and 4 $\mu\text{moles PO}_4^{3-}$ at high growth rates and 3 and 6 $\mu\text{moles PO}_4^{3-}$ at low growth rates (Laws et al. 2011). In yeast, high affinity PO_4^{3-} transport is controlled by two transporters, PHO5/PHO89 and PHO4/PHO89, which are H^+ - and Na^+ -coupled transporters, respectively. Both are controlled by external PO_4^{3-} concentration (Versaw and Metzenberg 1995, Bun-ya et al. 1997, Martinez et al. 1998,). Only the Na^+ -coupled PHO4 transporter type has been characterized in eukaryotic marine phytoplankton (Chung et al. 2003, Wurch et al. 2011). For example, the pelagophyte *Aureococcus anophagefferens* has two distinct PHO4-like transporters whose transcripts and protein expression profiles are regulated differentially by PO_4^{3-} . Both are expressed increasingly, at both the mRNA and protein levels, as P becomes limiting but one transporter protein is expressed at a higher level under acute P limitation than the other (Wurch et al. 2011).

Uptake of silicate (Si) by phytoplankton as a function of its concentration typically follows Michaelis-Menten type kinetics with half saturation constants varying from 0.2-7.7 $\mu\text{g at Si L}^{-1}$ at maximum Si concentrations varying from 30-160 $\mu\text{moles L}^{-1}$ in a range of diatom species (Paasche 1973, Azam et al. 1974, Sullivan 1976, Leynaert et al. 2004, Milligan et al. 2004). A few studies have demonstrated biphasic or sigmoidal kinetics at greater concentrations of Si (Azam and Volcani 1974, Nelson et al. 1976, Brzezinski 1992, Thamtracoln and Hildebrand 2008). Diatoms possess at least three silicon transport genes in their genomes that encode three distinct transport proteins (SITs) responsible for the range of kinetics observed for Si (Hildebrand et al. 1997, Thamtracoln and Hildebrand 2007). The kinetics vary depending on whether the cells have been starved for Si or not, on the time frame of measurement, and on the cell's growth phase (Thamtracoln and Hildebrand 2007, 2008). For example, measurements of uptake kinetics after 2 minutes in Si-sufficient cells produce non-saturable kinetics, but measurements in the same cells after 30 minutes or longer exhibit Michaelis-Menten type saturable kinetics. The transition between non-saturable and saturable Si kinetics is through to be mediated by intracellular Si and cell wall Si incorporation (Thamtracoln and Hildebrand 2008). Moreover, the 2-minute uptake rate varies depending on the growth stage with the highest rates observed during valve formation (G2/M phase) and lowest rates prior to the S phase (Thamtracoln and Hildebrand 2007).

In summary, over a narrow concentration range transport and uptake of a nutrient can follow Michaelis-Menten, sigmoidal or other kinetics, whereas over broader concentration ranges transport cannot be fit to a single kinetic model due to induction of different transporters with increasing concentration. As a result, biphasic kinetics result over larger concentration ranges. Moreover, constitutive expression of a transporter, as in the case of one or more of the AMT1 transporters, precludes uptake of NH_4^+ from following a kinetic model. Accordingly, "uptake kinetics" which are controlled at

the level of transport cannot be used interchangeably with “growth kinetics” which are controlled by environmental variables such as irradiance, temperature and nutrients, in addition to species-specific differences in growth rates (Eppley et al. 1969). Moreover, the kinetics of uptake and growth as a function of nutrient concentration are measured over different time scales, minutes versus days, respectively. Important to note is that measurements of uptake kinetics after minutes that produce non-saturable kinetics will produce saturable kinetics in the same cells after a longer incubation period. The transition between non-saturable (or any other type of uptake kinetic) and saturable kinetics with time is mediated by feedback between intracellular processes and transport into the cell (Thamatrakoln and Hildebrand 2007).

Appendix 3

Appendix 3.1 Additional Figures

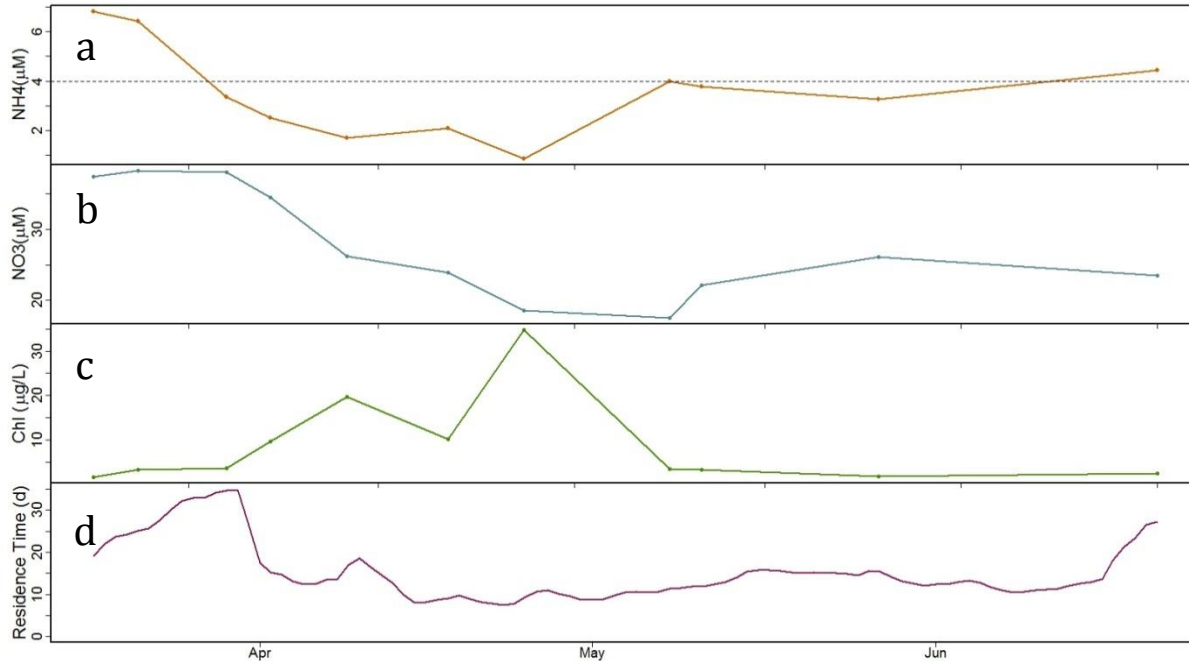


Fig A.3.1 Time series of NH_4^+ , NO_3^- and chlorophyll-a data collected by SFSU-RTC near DWR/IEP station D7 on 11 dates during Spring and Summer 2010 in Suisun Bay. The dashed line in panel a is at 4 μM , the concentration believed to inhibit NO_3^- uptake and limit primary production (Dugdale et. al, 2007). Residence time was calculated by dividing the volume of Suisun Bay (6.54×10^{11} L) by daily advective flows

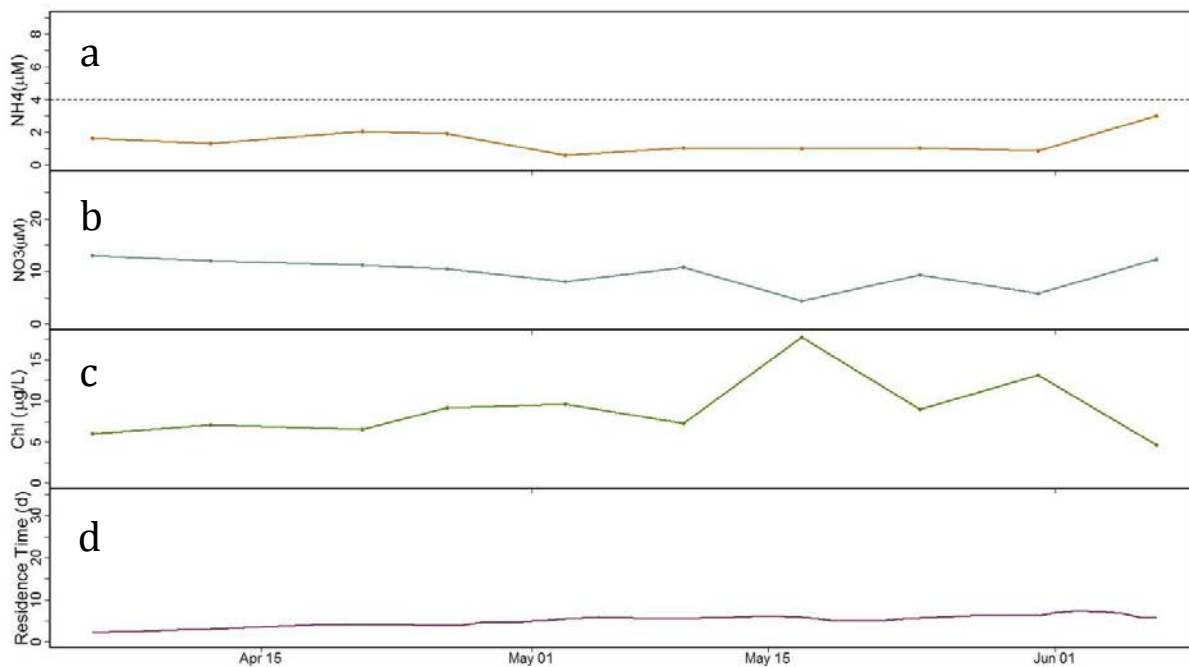


Figure A.3.2 Time series of NH_4^+ , NO_3^- and chlorophyll-a data collected by SFSU-RTC near DWR/IEP Station D7 on 10 dates during Spring 2011 in Suisun Bay. Data presented here were not included in Figure 6.10. The dashed line in panel a is at 4 μM , the concentration believed to inhibit NO_3^- uptake and limit primary production (Dugdale et. al, 2007). Residence time was calculated by dividing the volume of Suisun Bay (6.54×10^{11} L) by daily advective flows

Appendix 5

Appendix 5.1 Sources of Knowledge

We would know nothing about the ecology of copepods in the San Francisco Estuary were it not for the foresight that led to the long-term IEP monitoring program, the high quality of the work done by that program, and the persistent support in the agencies for maintaining it. Nevertheless, most of our knowledge of copepod ecology more generally comes from laboratory experiments. These generally have one of two objectives: to determine what the organism can do, or to determine what it is actually doing in the estuary.

For the first objective, investigators may examine the sensory capability of the organism, its swimming, feeding, and mating behavior, its maximum growth or development rate, its metabolic and nutritional requirements, or its sensitivity to water quality. These sorts of experiments often use copepods obtained from cultures to remove the signal of past environmental variability and allow a focus on the animal's capabilities (e.g., Ger et al. 2010). Many species of copepod have been cultured in many different laboratories worldwide, and some of these cultures have been maintained for years. Typically cultures are fed a mixture of phytoplankton although, in some cases, single phytoplankton or microzooplankton species have proved to be adequate food for the entire life cycle of some species (Stoettrup et al. 1986).

For the second class of experiments, copepods are collected in the estuary and transported to the laboratory for setting up experiments. This is the approach used in most studies to determine feeding, growth, and development rates under environmentally realistic conditions (e.g., Kimmerer and McKinnon 1987, Bouley and Kimmerer 2006, Gifford et al. 2007).

Information from laboratory studies is often extended to field conditions using models of various processes or of population dynamics. For example, models can be used to examine development (Gentleman et al. 2008) or to estimate mortality rates from the distributions of life stages in the field, if their development times are known (Kimmerer and McKinnon 1987, Aksnes and Ohman 1996). Models have also been used to estimate how mechanisms for mate-searching affect the minimum population density from which a population of copepods can recover (Kiørboe 2007, Choi and Kimmerer 2008), and to examine the flow or chemical field around a swimming copepod (Bearon and Magar 2010, Jiang and Kiørboe 2011). Life-cycle models are rarer than models of individual processes, but recently individual-based models are coming into use for copepods (e.g., Dur et al. 2009), and we are preparing an IBM of *Pseudodiaptomus forbesi*.

Appendix 5.2. Notes on *Acartia* species and their relatives

The family Acartiidae includes two genera present in the SFE, *Acartia* and *Acartiella*. Species within subgenera of *Acartia* can be difficult to tell apart morphologically, and molecular evidence is mounting that cryptic speciation is ubiquitous within the subgenera *Acanthacartia* and *Acartiura* (McKinnon et al. 1992, Caudill and Bucklin 2004, Chen and Hare 2008). These subgenera are represented in the San Francisco Estuary by two and one species respectively. *A. (Acanthacartia) californiensis* is the only one whose name is correct. The species identified as *A. (Acanthacartia) tonsa* based on morphology is almost certainly a different species from the one so named from the east and Gulf coasts of North America and Europe (Caudill and Bucklin 2004). Copepods from the east and west coasts identified as *A. (Acartiura) clausi* were unable to interbreed (Carrillo et al. 1974). Painter (1966), Caskey (1976), and Ambler et al. (1985) referred to *A. clausi*, but the species in the SFE more closely resembles *A. hudsonica* (Bradford 1976). We have examined several specimens from the 1978-1981 samples, all of which match the description of *A. hudsonica* but not that of *A. clausi*. Collections from nearby Tomales Bay contained these species as well as *A. (Acartiura) omorii* (Kimmerer 1993), and it is possible that this species has occurred in SFE as well.

Ambler et al. (1985) reported a seasonal cycle in which *A. "clausi"* was very abundant in winter, and *A. californiensis* in summer, with total abundance of *Acartia* spp. rather constant throughout the year, while *A. tonsa* was uncommon. In contrast, samples taken in 1999-2002 showed *A. hudsonica* to be abundant throughout the year, with *A. californiensis* and *A. tonsa* also present all year and abundant at times (Kimmerer et al. 2005). Unfortunately the IEP monitoring program does not distinguish among the species present or sample their entire habitat, so there is no way now to determine when this change happened or why.

Acartiella superficially resembles *Acartia*, but is actually a very different organism. It is most abundant in the LSZ in late summer to fall and rare at other times and places (Fig. 3). Morphologically it is obviously a predator based on the shape of its mouthparts (Tranter and Abraham 1971), and its long antennae suggest a capability to detect rather large organisms from a distance. Nothing is published on its ecology, but experiments show that it consumes other copepods (York et al. in revision).

Appendix 6

Appendix 6.1: Additional Figures

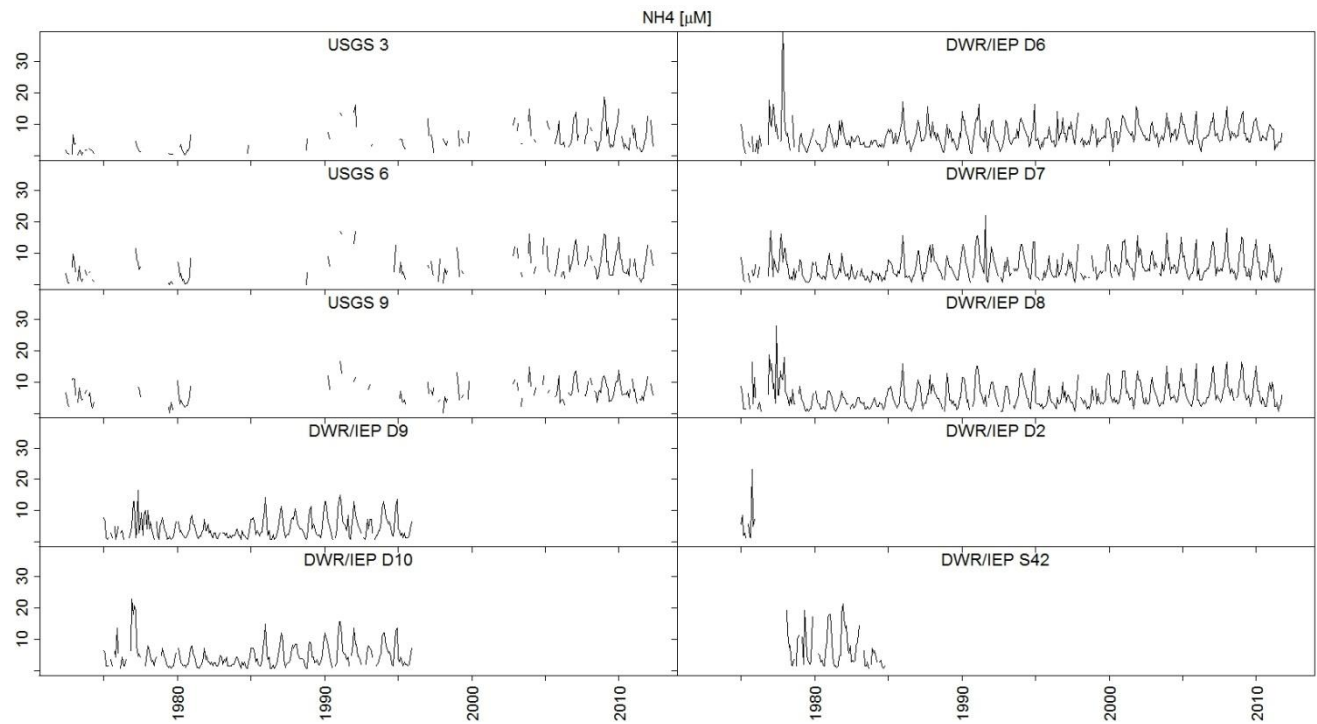


Fig A.6.1.1 Available NH_4^+ data for all DWR/IEP¹ and USGS² stations in Suisun Bay. DWR/IEP stations D6, D7 and D8 have the most complete record and are presented in greater detail in Figure 6.1.

¹<http://www.water.ca.gov/bdma/meta/Discrete/data.cfm>

²<http://sfbay.wr.usgs.gov/access/wqdata/>

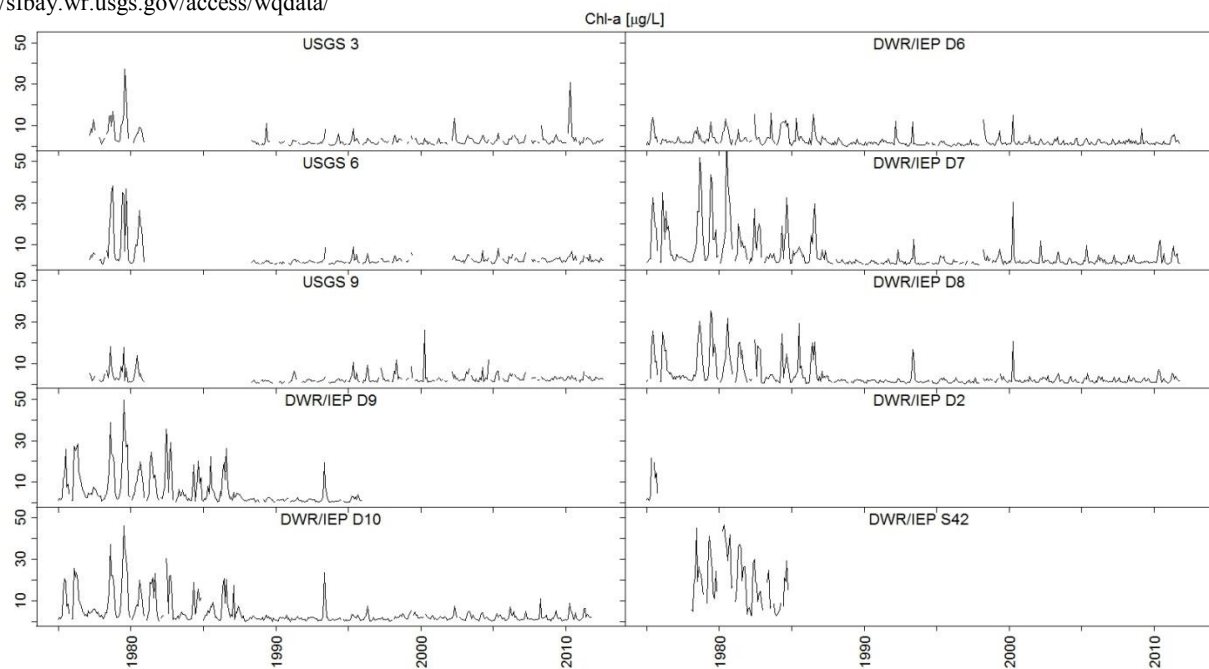


Fig A.6.1.2 Available chlorophyll-*a* data for all DWR/IEP and USGS stations in Suisun Bay

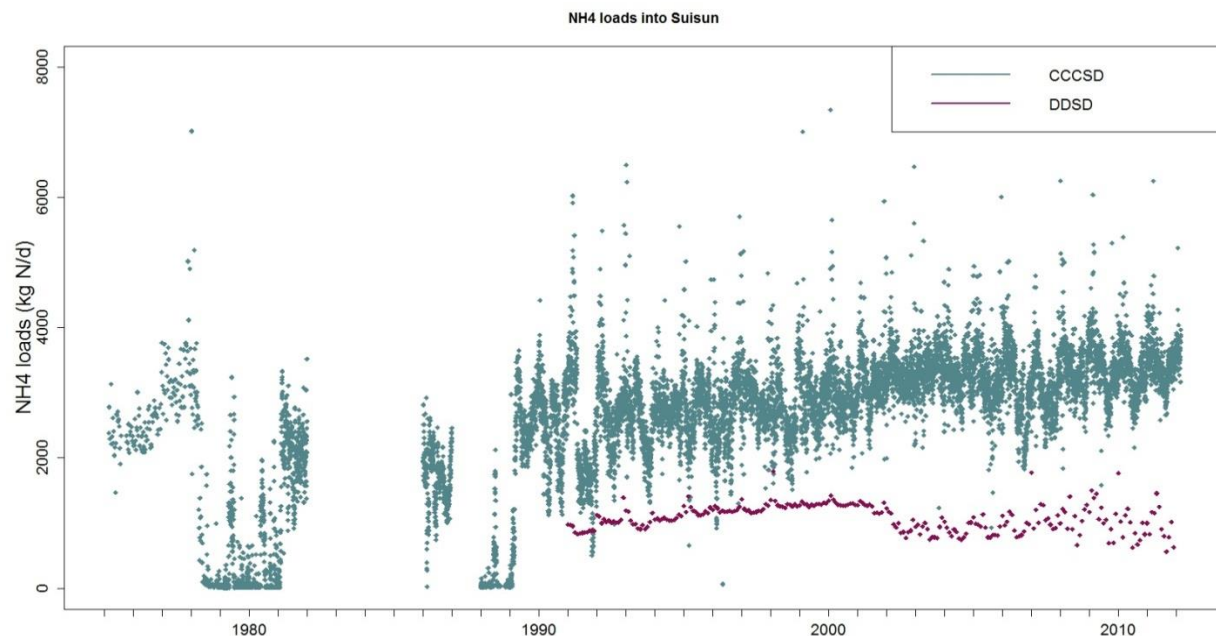


Fig A.6.1.3 Effluent NH_4^+ loads from the two major NH_4^+ dischargers to Suisun Bay, CCCSD and DDSD, including trial periods of nitrification at CCCSD (1977-1982, 1987-1988). Nitrification processes at FSSD reduce NH_4^+ loads to approximately 1% of the other two dischargers and are therefore not included here.

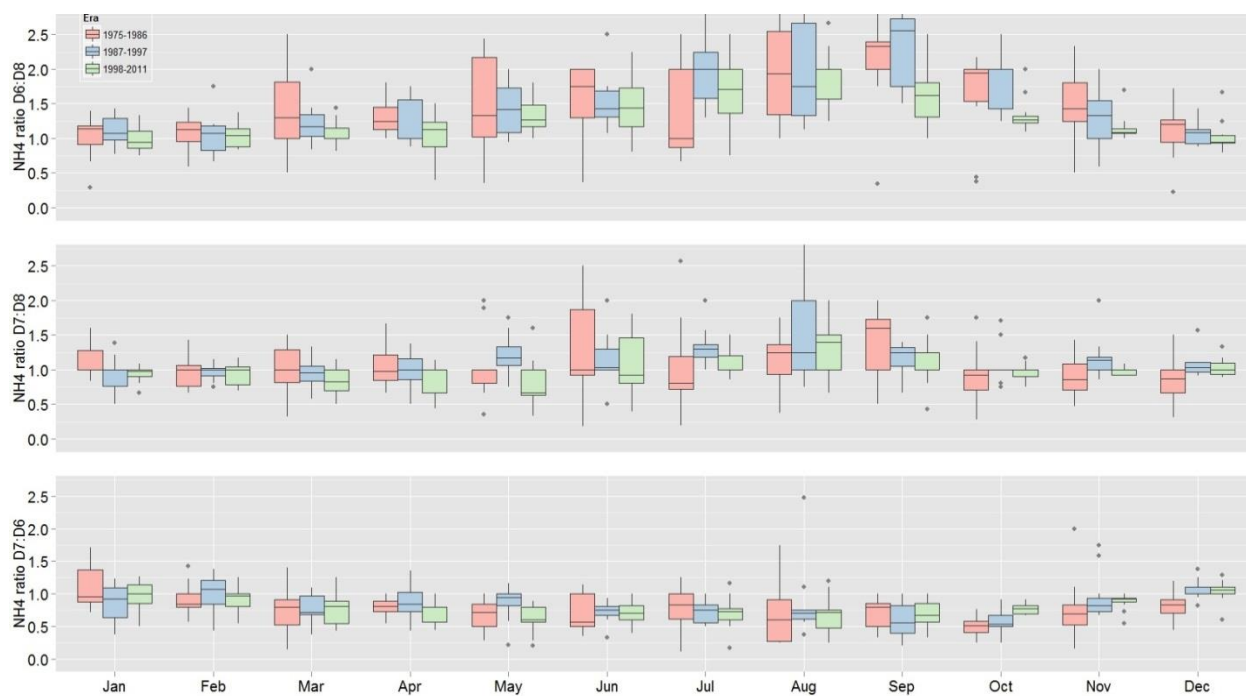


Fig A.6.1.4 Ratio of NH_4^+ between each of the three key stations in Suisun Bay. This analysis will indicate spatial variability of NH_4^+ concentrations in Suisun Bay. Data were first aggregated into three eras (1975-1986, 1987-1997 and 1998-2011), and then averaged by month within each era

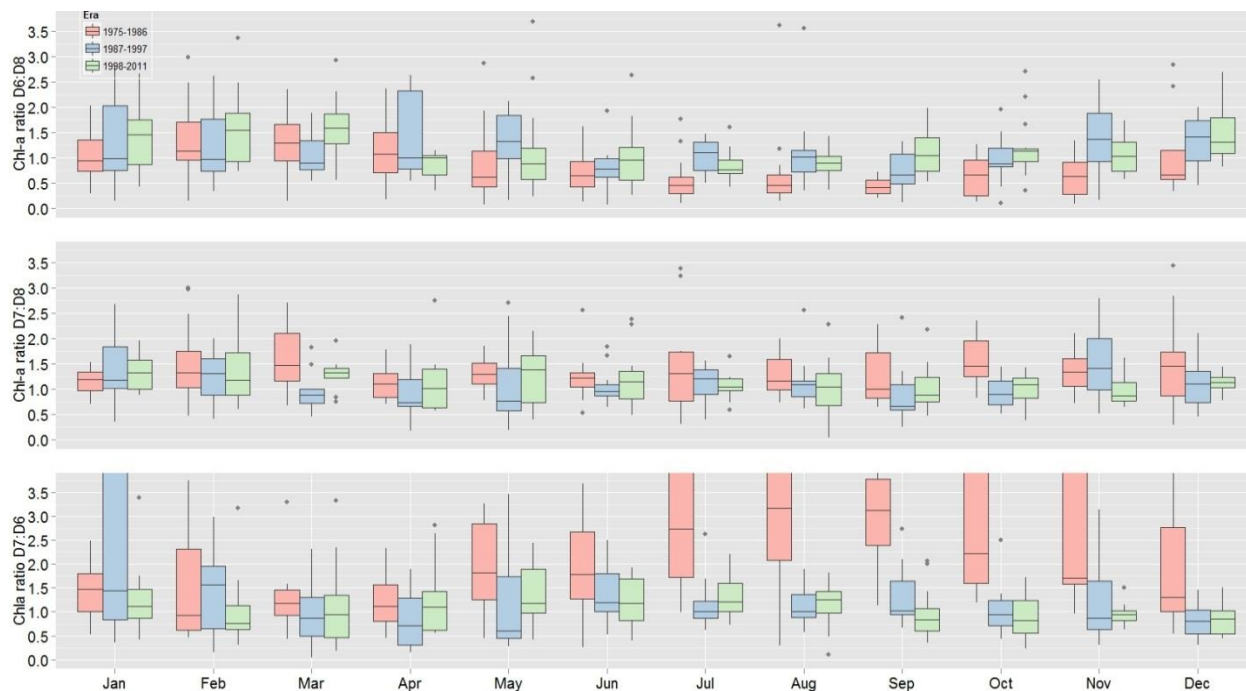


Fig A.6.1.5 Ratio of Chl-a between each of the three key stations in Suisun Bay. This analysis will indicate spatial variability of chl-a concentrations in Suisun Bay. Abrupt changes in chlorophyll-a concentrations brought about by the invasion of the *Corbula amurensis* clam is reflected in this figure. Data were first aggregated into three eras (1975-1986, 1987-1997 and 1998-2011), and then averaged by month within each era

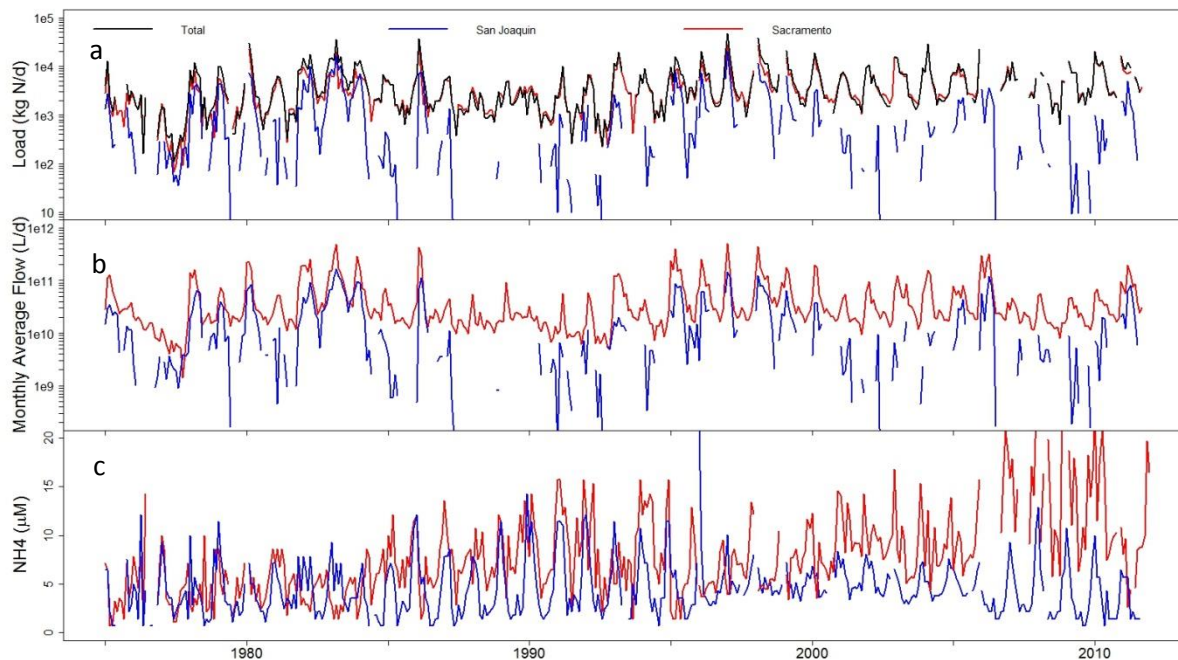


Fig A.6.1.6 Estimates of Delta efflux NH_4^+ loads into Suisun Bay (panel a), Delta flow through Suisun Bay (panel b) and concentrations measured in the Sacramento and San Joaquin Rivers just prior to entering the Delta. Contributions from the Sacramento River are shown in red, and those from the San Joaquin River are shown in blue. Loads are dominated by the Sacramento River (panel a), which includes effluent from Sacramento Regional Water Treatment Plant. Calculations were performed in a similar manner to those used by Jassby and Cloern (2000) to estimate organic matter loads to Suisun Bay. Details of calculations of these loads can be found in Appendix 6.2.

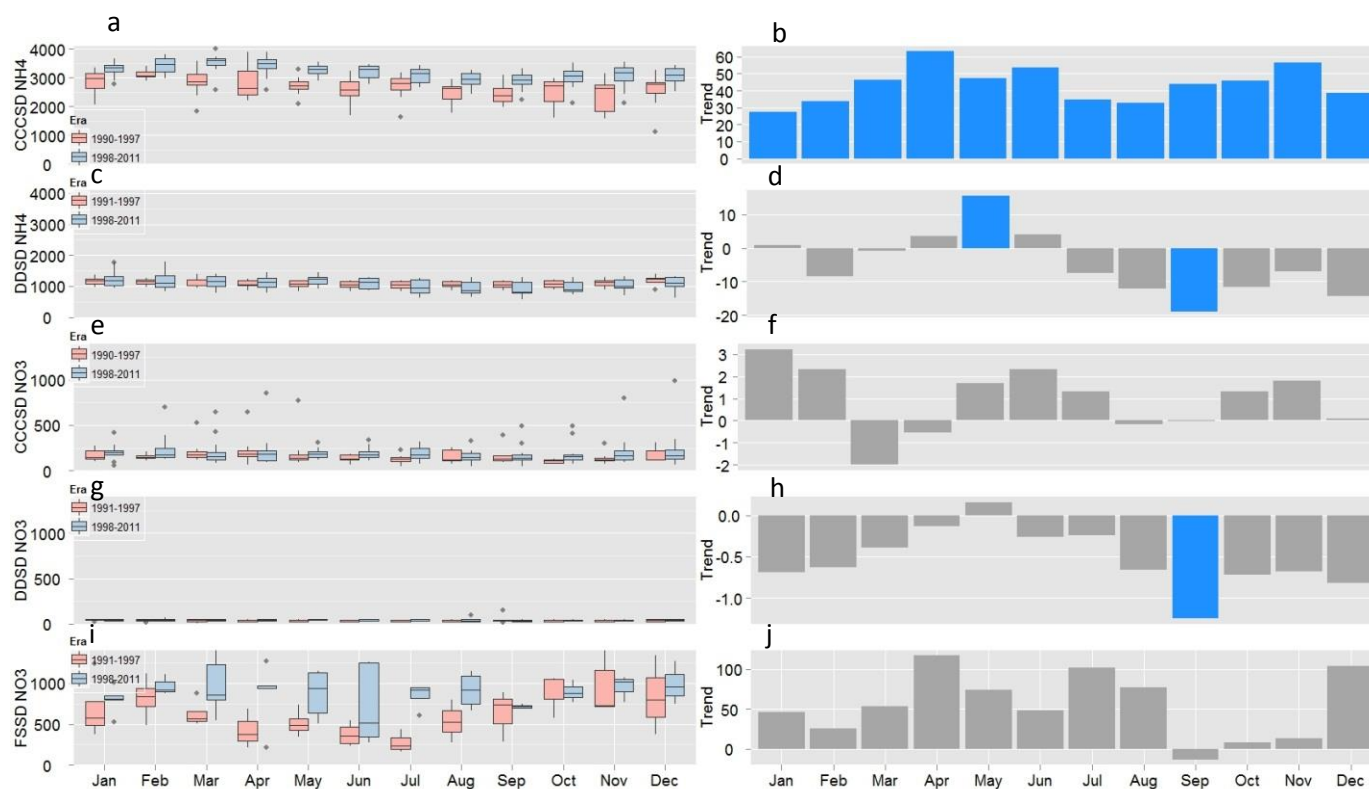


Fig A.6.1.7 Seasonal and long-term variability in effluent NH_4^+ and NO_3^- loads from the three major dischargers to Suisun Bay, CCCSD, DDSD and FSSD, after trial periods of nitrification at CCCSD (1977-1982, 1987-1988). Data was a combination of self-reported effluent flow and either actual measured concentrations, or approximate effluent concentrations from the literature (see section 6.2.2 for further details). Nitrification processes at FSSD reduce NH_4^+ loads to approximately 1% of the other two dischargers and are therefore not included here. Loads (kg N d^{-1}) were first aggregated into two eras (which varied based on data availability of the individual discharger), and then averaged by month within each era (panels a,c,e,g, and i). Long-term trends were characterized by the Theil slope ($\text{kg d}^{-1} \text{y}^{-1}$) (see description in Section 6.2.3) (panels b,d,f,h and j). Blue bars indicate statistically significant trends with $p < 0.05$ as determined by the Kendall Tau test.

Appendix 6.2: Estimating Delta Efflux Loads

The approach for calculating nutrient loads from the Delta into Suisun Bay was adapted from an approach used by Jassby and Cloern (2000). We quantified loads past Rio Vista (representing flow originating in the Sacramento River, Q_{rio}) and loads past Twitchell Island (representing flow originating in the San Joaquin River, Q_{west}), and combined these to estimate total load on a monthly average basis

$$Load = Q_{west}C_{west} + Q_{rio}C_{rio}$$

Flow:

Flow values were taken from DWR DAYFLOW records. Both Q_{west} and Q_{rio} are calculated values, using actual measured flows at gages throughout the Delta. Flow values were available daily, and we took a monthly average to calculate monthly average loads.

Q_{west} :

$$Q_{WEST} = Q_{SJR} + C_{SMR} + Q_{MOKE} + Q_{MISC} + Q_{XGEO} - Q_{EXPORTS} - Q_{MISDV} - 0.65 (Q_{GCD} - Q_{PREC})$$

Q_{rio}

$$Q_{RIO} = Q_{SAC} + Q_{YOLO} - Q_{XGEO} - 0.28 (Q_{GCD} - Q_{PREC})$$

Concentration:

DWR/IEP and USGS conduct monthly water quality monitoring in the Delta, and we combined these concentrations with monthly-averaged flow to produce monthly-averaged estimates of load. Stations used for C_{west} and C_{rio} varied throughout the period of 1975-2011 because of changes in station operation (Table A.2.1). Between 1975 and 1975, DWR/IEP station D24 was used for C_{rio} and DWR/IEP station D16 was used to represent for C_{west} . Unfortunately, monitoring at both of these stations ceased in 1995, and we were forced to substitute using stations whose monitoring continued past 1995. We performed multivariate linear regressions of D24 and D16 data from 1975-1995 against data from nearby stations from the same period in order to develop the substitutions that would be used post-1995. Starting in 2006, we made single-station substitutions for both C_{west} and C_{rio} . At this time, nutrient monitoring intensified at DWR/IEP station D19 and began at USGS station 657, which is nearly collocated with DWR/IEP D24. Details on stations substitutions can be found in the table below. Locations of stations relative to Q_{west} and Q_{rio} , as well as relative to each other, can be found in Figure A.2.2

Uncertainty:

Although the method used to estimate Delta efflux loads was the same as was previous used by Jassby and Cloern (2000) to estimate organic matter loads to Suisun Bay, there is some uncertainty associated with the constituent data sets used in this calculation. Q_{west} and Q_{rio} are both calculated values, not directly measured by flow gages. Although the formula used to calculate these terms is frequently reviewed and

revised by DWR (as recently as 2012), a calculated value will never be as accurate as one that is measured. The DWR/IEP and USGS stations used are not continuous over the entire period 1975-2011. There are stations with continuous data from 1975-1995 (D16 and D24), which are also nearly collocated with DAYFLOW locations of Q_{west} and Q_{rio} , however both of these stations were dropped in 1995. A USGS station (657) that is nearly identical to the location of station D24 began monitoring for nutrients in 2006, but there were gaps in the record from 1995-2006 (at the former station D24) and from 1995-2011 (at the former station D19). Multivariate linear regressions from nearby stations filled these gaps with varying levels of accuracy (see r^2 values in Table A.6.2.1), but this station substitution introduces additional uncertainty into these estimates. Additionally, all of these stations are located 10km to 30km upstream from the mouth of Suisun Bay, and it is possible for nutrient loads to change along this distance due to transformation or loss. To explore the sensitivity of load estimates to station location, we calculated loads using both these upstream stations (D24/D16) and one closer to the mouth of Suisun Bay (D4) for a period when data were available at both sites (1975-1995). On average, NH_4^+ load estimates decrease by ~30% (approximately 860 kg/d N) and NO_3^- load estimates increase by ~10% (approximately 850 kg/d N) between these two locations, but DIN and DIP loads are virtually unchanged. While NH_4^+ transformations along this distance are more significant during warmer summer months, direct POTW NH_4^+ loads to Suisun Bay already dominated over Delta efflux loads during these times. Therefore, while our exact estimates forms of N exported from the Delta to Suisun Bay are somewhat sensitive to station locations used in calculations, our overall conclusions, as well as overall DIN and DIP estimates, are reasonable. In spite of data gaps, the estimates made here are believed to be reliable as order of magnitude approximations and further modeling efforts in the Delta could help refine these estimates further.

References:

- Jassby, A.D., and Cloern, J.E. (2000) Organic matter sources and rehabilitation of the Sacramento-San Joaquin Delta (California, USA). *Aquatic Conservation: Marine and Freshwater Ecosystems* 10: 323–352.

Tables and Figures:

		C_{west}	C_{rio}
1975-1995	NH4	D16 ¹	D24 ¹
	NO3+NO2	D16 ¹	D24 ¹
1996-2005	NH4	$0.311 * D26 + 0.235 * D28A + 0.320 * D4 - 0.001$ $r^2 = 0.77$	$0.165 * C3 + 0.551 * D4 + 0.022$ $r^2 = 0.52$
	NO3+NO2	$0.5305 * D26 + 0.1613 * D28A + 0.3812 * D4 - 0.020$ $r^2 = 0.93$	$0.200 * C3 + 0.809 * D4 - 0.023$ $r^2 = 0.85$
2006-2011	NH4	D19 $r^2 = 0.81$	USGS 657 ²
	NO3+NO2	D19 $r^2 = 0.84$	USGS 657 ²

Table A.6.2.1 DWR/IEP and USGS water quality monitoring stations used in combination with DWR DAYFLOW values Q_{west} and Q_{rio} to approximate Delta loads. After 1995, when both station D24 and D16 were dropped, there were gaps in the record that were filled by multivariate linear regression from nearby stations whose monitoring continued past 1995 (the resulting linear equation and r^2 values are shown here).

¹Stations used by Jassby and Cloern (2000)

²Regression against D24 not possible because data from these two stations never coexisted

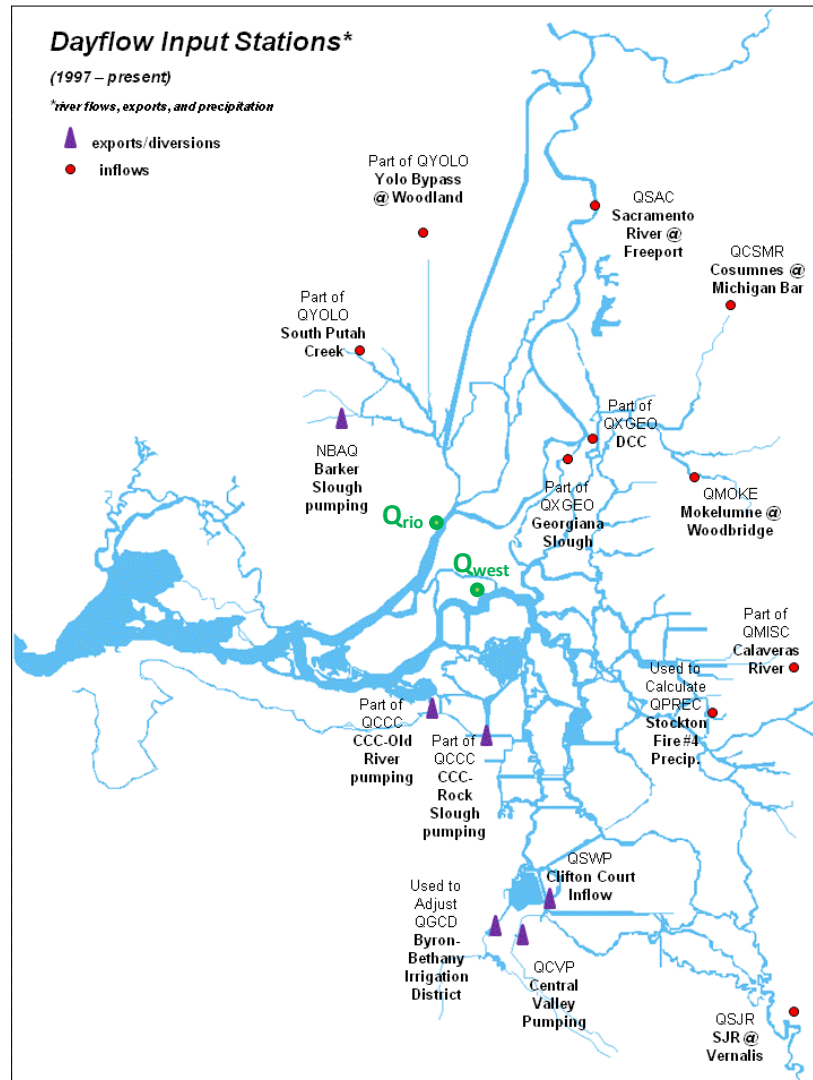


Figure A.6.2.1 Location DWR DAYFLOW gages (indicated by purple triangles). The values used in our estimation, Q_{west} and Q_{rio} , are calculated according to the following formulas and give approximation of flow past the points indicated above.

$$Q_{WEST} = Q_{SJR} + C_{SMR} + Q_{MOKE} + Q_{MISC} + Q_{XGEO} - Q_{EXPORTS} - Q_{MISDV} - 0.65 (Q_{GCD} - Q_{PREC})$$

$$Q_{RIO} = Q_{SAC} + Q_{YOLO} - Q_{XGEO} - 0.28 (Q_{GCD} - Q_{PREC})$$

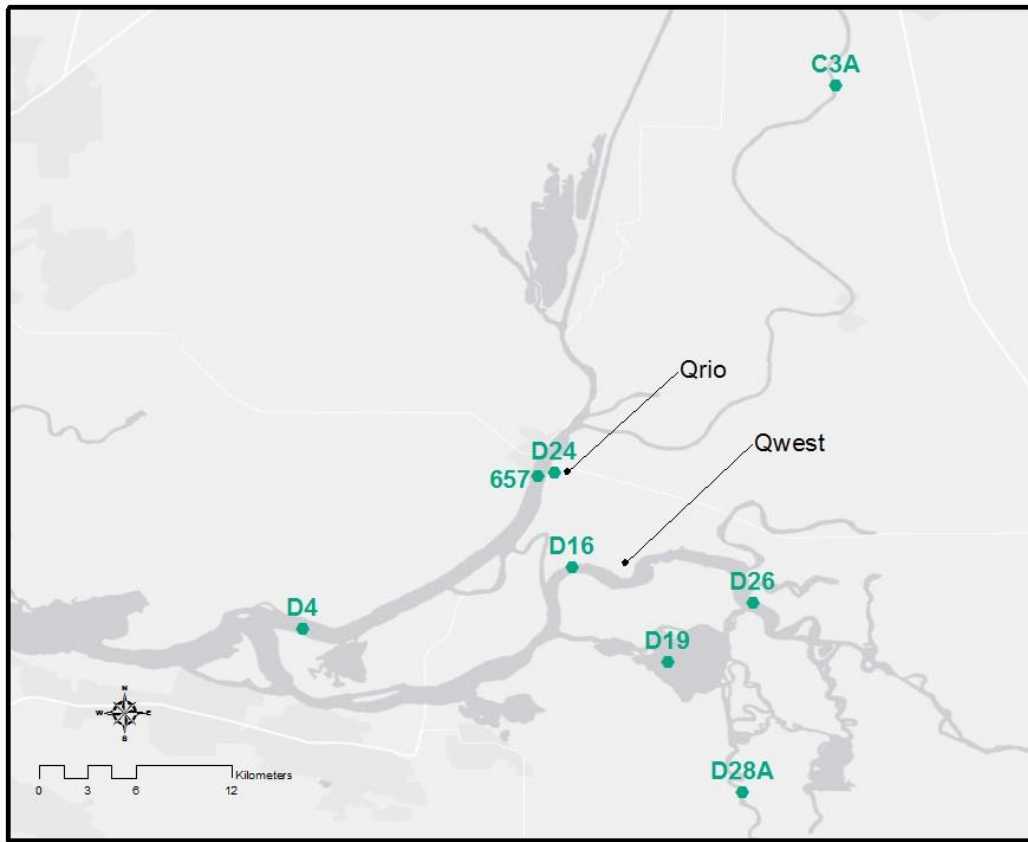


Figure A.6.2.2 Location of DWR/IEP and USGS water quality stations used in Delta loads estimate, as well as location of flow estimates.

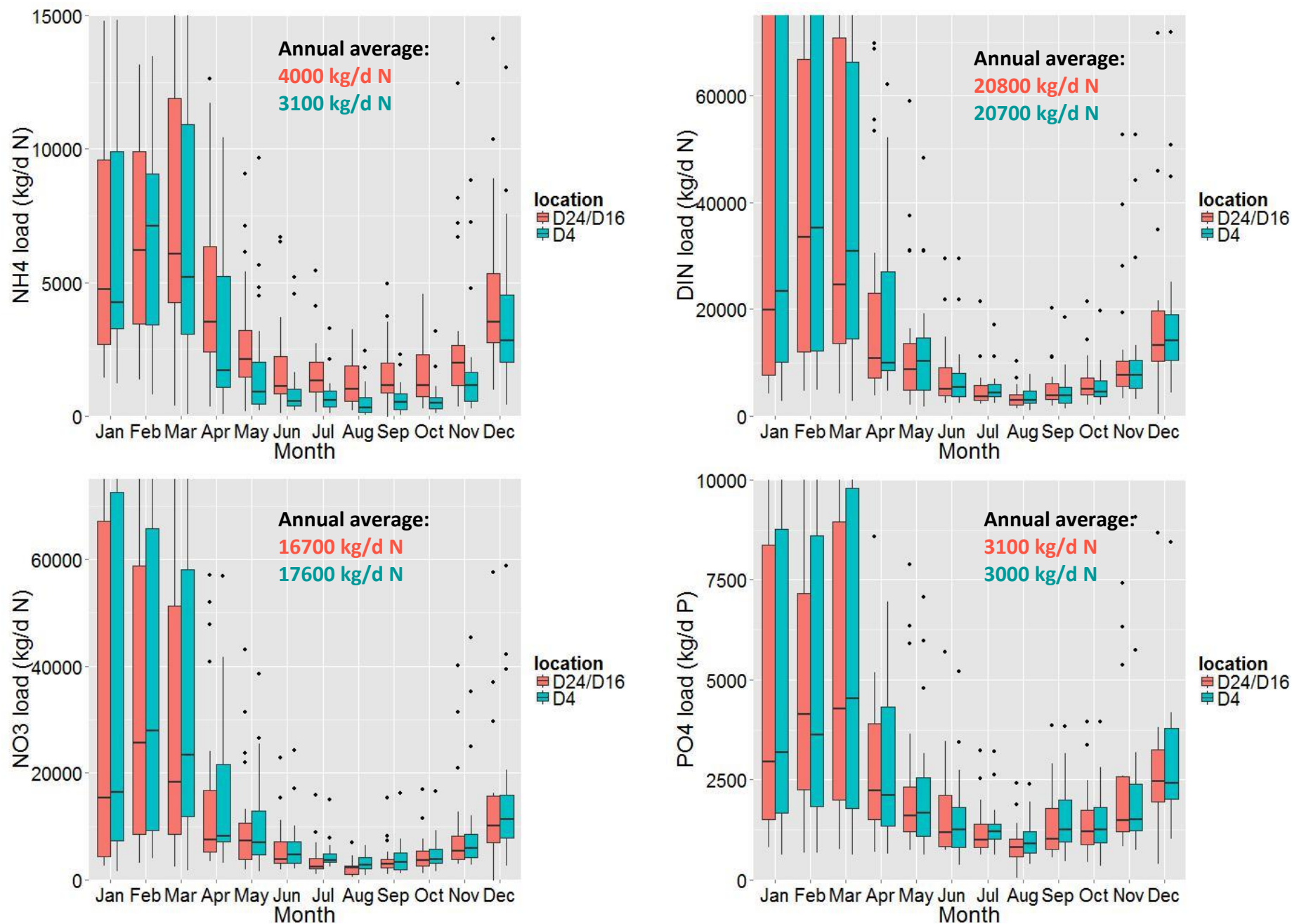


Fig A.6.2.3 Comparisons of Delta effluent load estimates when stations D24/D16 are used in calculations (those used in Jassby and Cloern, 2000 and in this report) vs. when station D4 is used (closer to the mouth of Suisun Bay). While on average, the NH_4^+ load decreases by 30% (approximately 860 kg/d N) and the NO_3^- load increases by 10% (approximately 850 kg/d N) between these two locations, DIN loads were virtually unchanged. Note the different scales on the y-axis.

Appendix 6.3: Estimating Stormwater Loads

Our approach to calculating stormwater loads is the following:

$$Load = P * RC * A * C$$

where

P = precipitation

RC = runoff coefficient

A = area of watershed

C = concentration of NH₄ or NO₃

Precipitation:

We used monthly values of precipitation for the entire Bay Area averaged over the period 1914-2005 (available from the Western Regional Climate Center) (Table 6.3.1).

Runoff Coefficients:

We calculated a single weighted-average runoff coefficient for each of the two watersheds that drain into Suisun Bay (Figure A.6.3.1). We calculated the % of each watershed in each land-use bin (agriculture, commercial, industrial, open, residential, transportation and water; Figure A.3.1) and then multiplied by a land-use specific runoff coefficient (Lent and McKee, 2011; Table A.6.3.2) in the following way:

$$RC = \%_{agr}RC_{agr} + \%_{comm}RC_{comm} + \%_{ind}RC_{ind} + \dots$$

We used a low and high estimate of runoff coefficients bound the uncertainty of our estimates.

Area:

Concord watershed had a total area of 654 km², and Fairfield watershed had a total area of 867 km².

Concentration:

In 2010, 8 Bay Area watersheds were monitored for nutrients in both the wet season and the dry season (McKee and Gluchowski, 2011). We used the average of these different sites as our representative concentrations in our calculations (Table A.6.3.3)

References:

Lent, M.A. and McKee, L.J., 2011. Development of regional contaminant load estimates for San Francisco Bay Area tributaries based on annual scale Rainfall-Runoff and Volume-

McKee, L.J., and Gluchowski, D.C, 2011. Improved nutrient load estimates for wastewater, stormwater and atmospheric deposition to South San Francisco Bay (South of the Bay Bridge). A Watershed Program report prepared for the Bay Area Clean Water Agencies (BACWA). San Francisco Estuary Institute, Oakland CA.

Tables and Figures:

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
0.112	0.096	0.071	0.035	0.001	0.003	0.001	0.001	0.006	0.026	0.066	0.104

Table A.6.3.1 Average monthly precipitation values used to estimate stormwater runoff loads, in meters (available from the Western Regional Climate Center)

Land Use Type	Lower-Bound Runoff Coefficient	Upper-Bound Runoff Coefficient	Concord % Land Use	Fairfield % Land Use
Water	0	0	1.2%	4.7%
Open	0.09	0.34	53%	51%
Residential	0.2	0.39	26%	6.6%
Industrial	0.5	0.6	4.5%	1.6%
Commercial	0.5	0.6	6.3%	1.6%
Transportation	0.78	0.83	8.7%	5.5%
Agriculture	0.12	0.46	0.3%	29%
Weighted-average Runoff Coefficient:			Concord low: 0.22 Concord high: 0.42	Fairfield low: 0.15 Fairfield high: 0.40

Table A.6.3.2 Land-use specific run-off coefficients (Lent and McKee, 2011) were used in combination with % landuse in each Suisun Bay watershed to estimate an upper- and lower-bound runoff coefficient for the entire watershed

	Average wet season (Oct-Apr)	Average dry season (May-Sep)
NH4	0.332 mg/L	0.254 mg/L
NO3	1.01 mg/L	0.95 mg/L

Table A.6.3.3 Wet and dry season concentrations of NH4 and NO3 in stormwater (measured in 8 Bay Area watersheds in 2011, McKee and Gluchowski 2011).

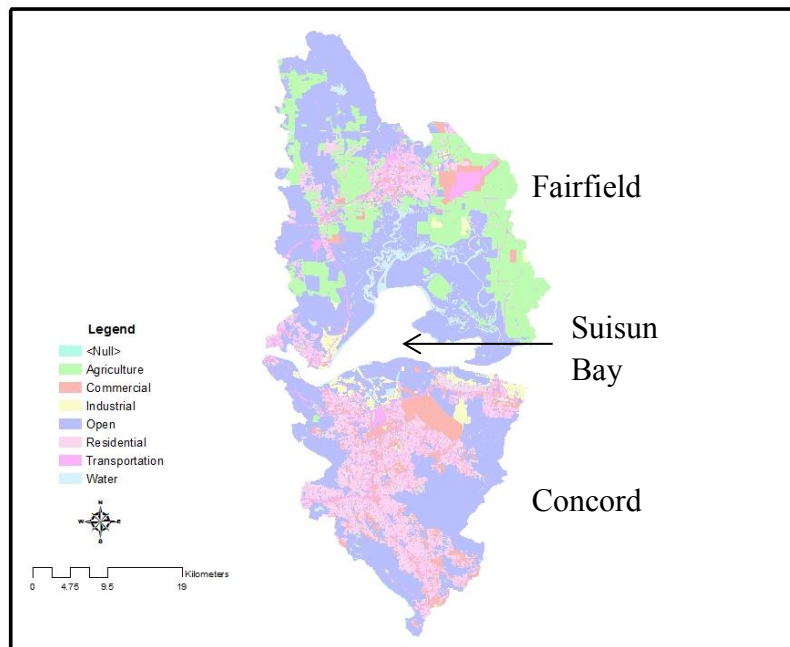


Figure A.6.3.1 Land-use breakdown for two watersheds that drain in Suisun Bay: Fairfield (north) and Concord (south)

Appendix 6.4: 1-box model for Suisun Bay

In order to evaluate the role of Suisun Bay in transforming incoming NH_4 loads, we performed a 1-box mass balance using a well-mixed Suisun Bay as the control volume. We first performed a salinity balance in order to quantify tidal flows, and then performed a NH_4 balance to evaluate the residual transformation/loss term. Data analysis with the box model focused on 2006-2011, when data from all load sources was most certain, and also on the months April-October, when residence time in Suisun Bay tends to be longest and when phytoplankton blooms have been historically observed. For these months, we assumed steady-state. Evaluation of assumptions is included in the description of each model.

Estimates of loads in and out were made using advective flow estimates from DWR DAYFLOW, tidal flow estimates from the salinity balance performed below, and concentration measurements from DWR/IEP and USGS monitoring stations. DAYFLOW measurements were extracted for the exact dates of DWR/IEP concentration measurements. The location of the flow and concentrations monitoring stations is shown in Figure A.6.4.1

Salinity Balance

To simplify our 1-box model, we made the following assumptions:

1. Treated Suisun as a well-mixed control volume
2. Steady state
3. Tidal dispersion on upstream side (exchange with D19, 657) considered negligible

The terms used in our mass balance were the following, and we solved for Q_{tide} :

1. S_{river} = flow-weighted average of S_{D19} and S_{657}
2. S_{su} = average(S_{D6} , S_{D7} , S_{D8})
3. $S_{sp} = S_{D41}$
4. $Q_{adv} = Q_{west} + Q_{rio}$
5. V_{su} = volume of Suisun Bay, 6.54×10^{11} L

Further explanation of the terms and schematic for the salinity balance are given in Fig. A.6.4.2.

Evaluation of assumptions

Assumption #1 may introduce the greatest amount of uncertainty, since Suisun Bay is not particularly well-mixed with respect to salinity (Fig. A.6.4.3). In future modeling efforts, a multi-box model, using smaller well-mixed volumes, could improve estimates of Q_{tide} . With regards to Assumption #2, although salinity is not truly steady state during April-October, the most rapid changes in salinity occur outside of these months and including non-steadiness in our model only changed the final k values by less than 7%. Assumption #3 appears to be the most valid. Salinity in the Sacramento and San Joaquin rivers is negligible and can be considered outside of tidal influence.

NH4 Balance

We used the resulting value of Q_{tide} in aNH4 mass balance, where the made the following assumptions:

1. Treated Suisun as a well-mixed control volume
2. Steady state
3. Tidal dispersion on upstream side (exchange with D19, 657) considered negligible
4. Assume loading from CCCSD mixes uniformly into Suisun Bay

We used the following terms on our model, and solved for $V_{su}k_{loss}C_{su}$ (total losses, kg-d⁻¹) and k_{loss} (loss rate, d⁻¹):

1. C_{river} = flow-weighted average of C_{D19} and C_{657}
2. C_{su} = average(C_{D6} , C_{D7} , C_{D8})
3. $C_{sp} = C_{D41}$
4. $Q_{adv} = Q_{west} + Q_{rio}$
5. V_{su} = volume of Suisun Bay, 6.54e11 L
6. $\dot{M}_{discharge} = \dot{M}_{CCCSD} + \dot{M}_{DDSD}$
7. Q_{tide} was solved for using the salinity balance

Further explanation of the terms and schematic for the NH4 balance are given in Fig. A.6.4.4.

Evaluation of Assumptions

NH4 concentrations at D6, D7 and D8 appear similar, supporting assumption #1 (Fig. A.6.4.5). However, this might be masking the influence of multiple NH4 sources into Suisun Bay. We hypothesize that NH4 concentrations actually decrease seaward from the Delta due to transformations/losses, but that CCCSD outfall just prior to D6 elevates concentrations to levels similar to those from Delta efflux. While the result corroborates our assumption of well-mixed Suisun, additional modeling on a finer spatial scale would likely reveal concentration gradients not captured by current monitoring. Regarding assumption #2, summertime NH4 concentrations are less variable than they are at other times of the year. On average, concentrations between April and October vary by a factor of roughly 2, while concentrations on the entire year vary by a factor of 4. Assumption #3 has the potential to, if anything, underestimate the loading of NH4 into Suisun Bay. If we included a tidal dispersion term on the upstream end, this would bring high-NH4 waters from the Sacramento and San Joaquin rivers and would only increase the magnitude of observed losses in Suisun Bay. Lastly, assumption #4 may be overestimating the magnitude of NH4 loads from CCCSD. In order to evaluate the importance of this assumption, we performed our calculations assuming 100%, 75%, 50% and 25% of CCCSD plume mixing in Suisun Bay prior to advection downstream (see Figure 25)

Loads in exceeded loads out for all months analyzed (Figure A.4.6). On average, 75% of loads in are transformed or lost prior to flow out of Suisun Bay (either by advection or tidal flow)

Results

Loads in exceeded loads out for all months analyzed (Figure A.6.4.6). On average, 75% of loads in are transformed or lost prior to flow out of Suisun Bay (either by advection or tidal flow) (Figure 6.20). First order loss rates were estimated at 0.1-0.3 d⁻¹, even when some of CCCSD effluent is considered lost downstream to advection prior to mixing into Suisun Bay (Figure A.6.4.7).

We performed sensitivity analyses in order to evaluate the validity of some of our key assumptions. First, based on small variation of NH₄ concentrations in April-October (Figure A.6.4.5), we assumed steady state conditions. As a comparison, we did a non-steady model and our resulting values for k vary by less than 7%, indicating that our steady-state assumption is valid. Secondly, the most uncertain term in our mass balance is the tidal flow, which we calculated using a salinity balance that itself contained simplifying assumption. We performed a sensitivity analysis in order to evaluate the effect of this parameter on our overall results. We found that if our value for tidal flow was off by a factor of 5, the contribution of transformations/losses to the overall fate of NH₄ dropped from 75% to 60%, which would still be a significant contribution.

Additional discussion of results are summarized in the main body of the report (Section 6.4.3)

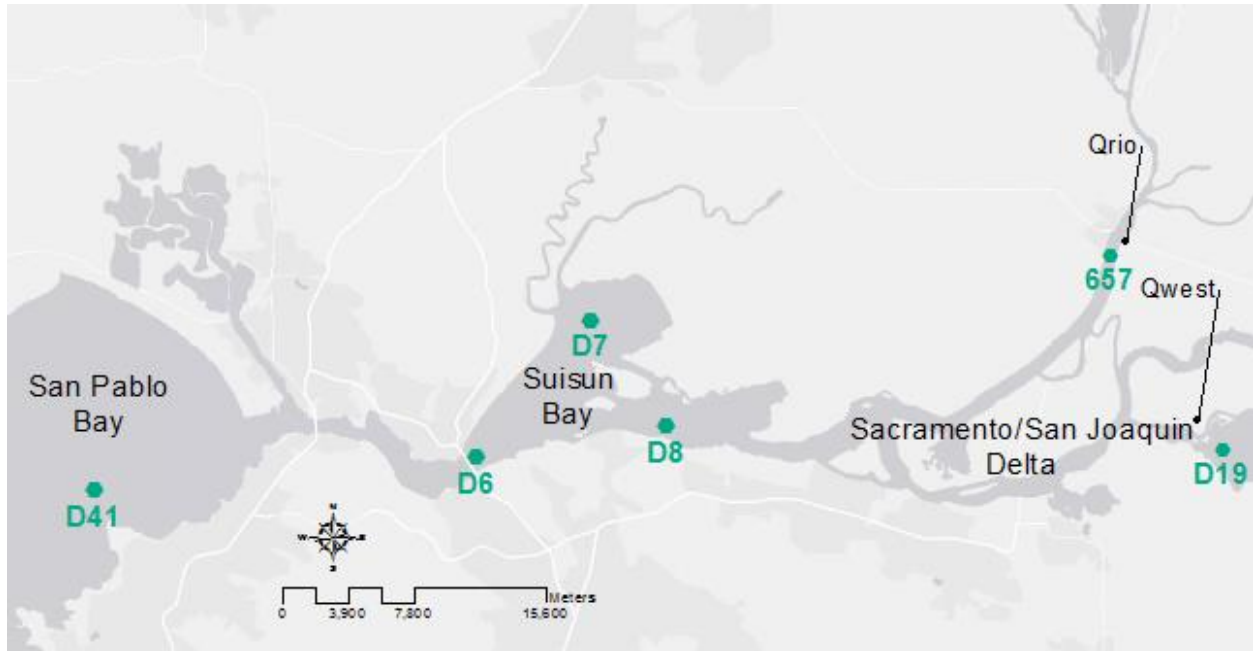


Figure A.6.4.1 Location of DWR/IEP and USGS monitoring stations (used as concentration terms) and DWR DAYFLOW stations (used as flow terms) in 1-box model for Suisun Bay. Tidal flows were estimated from a salinity balance (Fig. A.6.4.2).

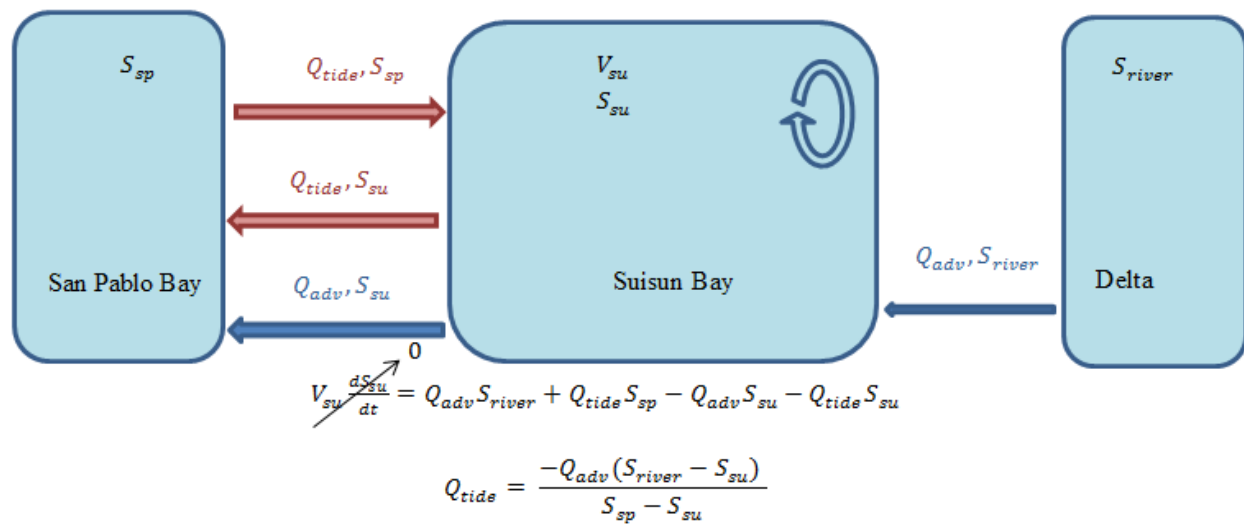


Figure A.6.4.2 Salinity mass balance schematic used to approximate the magnitude of Q_{tide} .

1. S_{river} = flow-weighted average of S_{D19} and S_{657}
2. S_{su} = average(S_{D6} , S_{D7} , S_{D8})
3. S_{sp} = S_{D41}
4. Q_{adv} = Q_{west} + Q_{rio}
5. V_{su} = volume of Suisun Bay, 6.54×10^{11} L

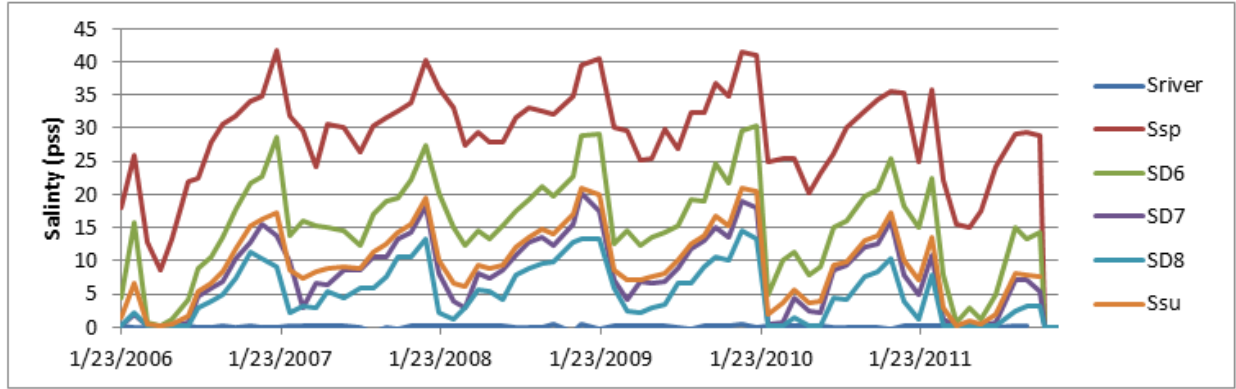


Figure A.6.4.3 Times series of salinity at locations used in mass balance (Only April-October were considered for the mode). S_{river} is the flow weighted average of salinity at DWR/IEP D19 (San Joaquin River dominated) and USGS 657 (Sacramento River dominated), S_{sp} is salinity at DWR/IEP D41 and S_{su} is the average of salinity at DWR/IEP D6, D7 and D8. This figure shows that Suisun Bay is not particularly well mixed with respect to salinity and making a well-mixed assumption may introduce uncertainty. S_{river} was negligible and therefore we neglected tidal dispersion on the upstream end of Suisun Bay

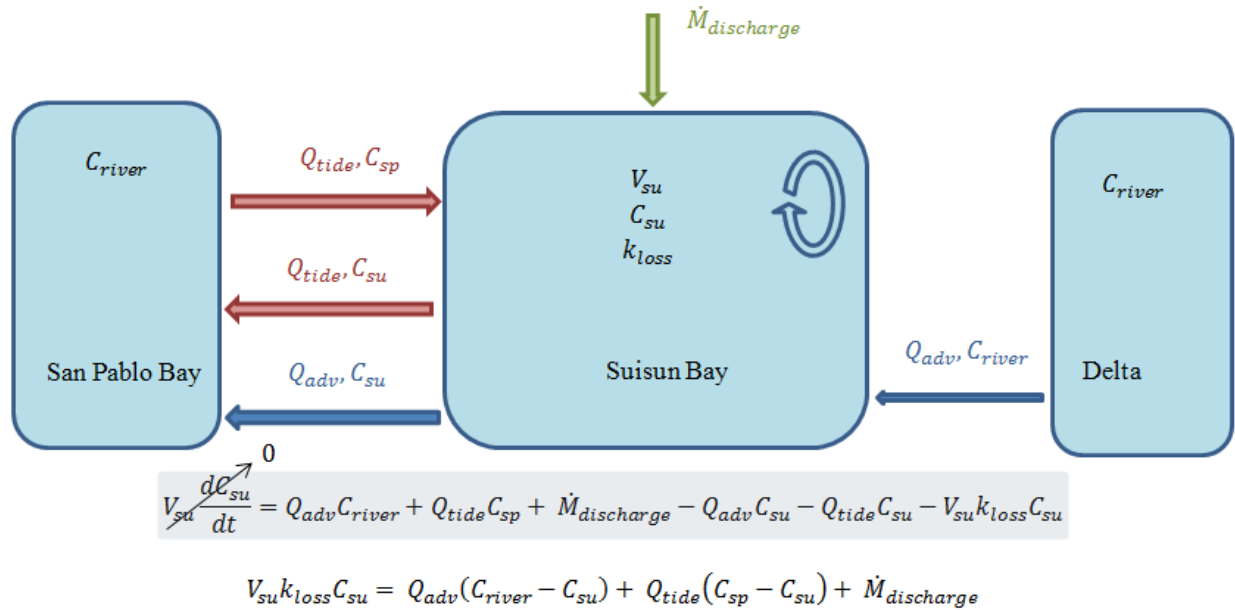


Figure A.6.4.4 Salinity mass balance schematic used to approximate the magnitude of NH_4 losses in Suisun Bay.

1. C_{river} = flow-weighted average of C_{D19} and C_{657}
2. C_{su} = average(C_{D6} , C_{D7} , C_{D8})
3. $C_{sp} = C_{D41}$
4. $Q_{adv} = Q_{west} + Q_{rio}$
5. V_{su} = volume of Suisun Bay, 6.54×10^{11} L
6. $\dot{M}_{discharge} = \dot{M}_{CCCS} + \dot{M}_{DDSD}$
7. Q_{tide} was solved for using the salinity balance

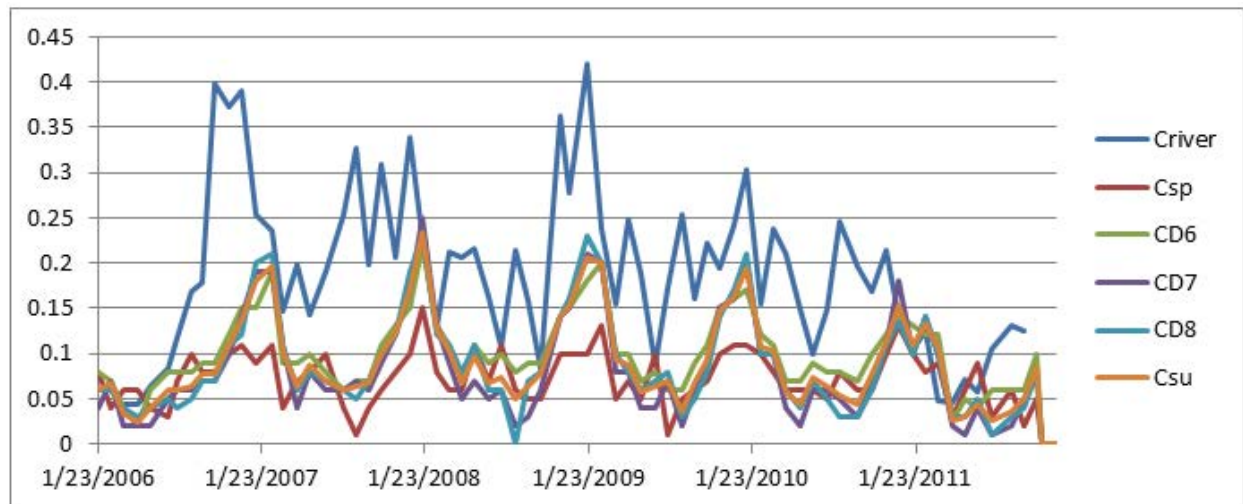


Figure A.6.4.5 NH₄ concentrations at locations used in mass balance. C_{river} is the flow weighted average of NH₄ at DWR/IEP D19 (San Joaquin River dominated) and USGS 657 (Sacramento River dominated), C_{sp} is NH₄ at DWR/IEP D41 and C_{su} is the average of NH₄ at DWR/IEP D6, D7 and D8. NH₄ is reasonably well-mixed with respect to salinity. In our calculation, we neglected upstream dispersion in Suisun Bay (see Figure A.6.4.3), however given the high concentrations of NH₄ in the rivers, if anything this omission underestimates NH₄ loads to Suisun Bay and therefore underestimates the magnitude of NH₄ losses.

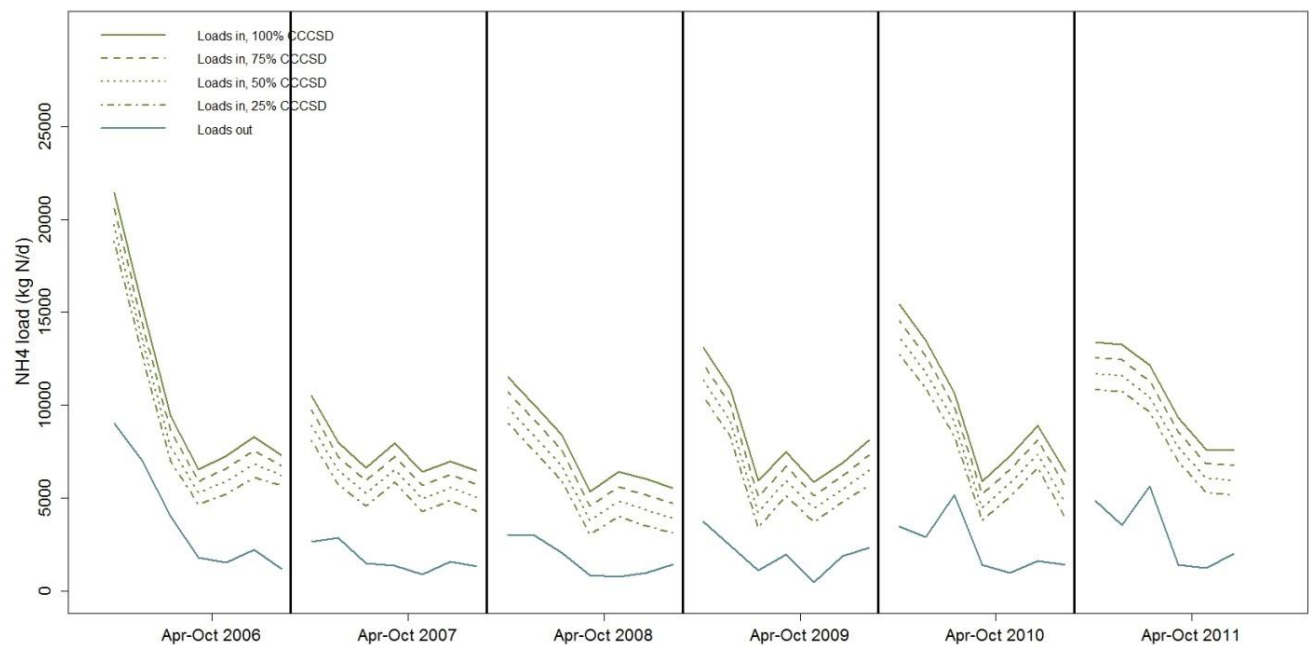


Figure A.6.4.6 Differences between NH₄ loads into Suisun Bay (including advective loads, tidal downstream tidal loads and discharger loads assuming various amounts of CCCSD effluent mixing; green line) and NH₄ loads out of Suisun Bay (including advective loads and downstream tidal loads). The difference between loads in and loads is an estimate of the magnitude of NH₄ losses in Suisun Bay (kg d⁻¹). Even when only 25% of CCCSD plume was allowed to mix into Suisun Bay prior to advecting downstream, loads in always exceeded loads out by as much as 2-3 times. First-order loss rates are presented in Fig. A.6.4.7.

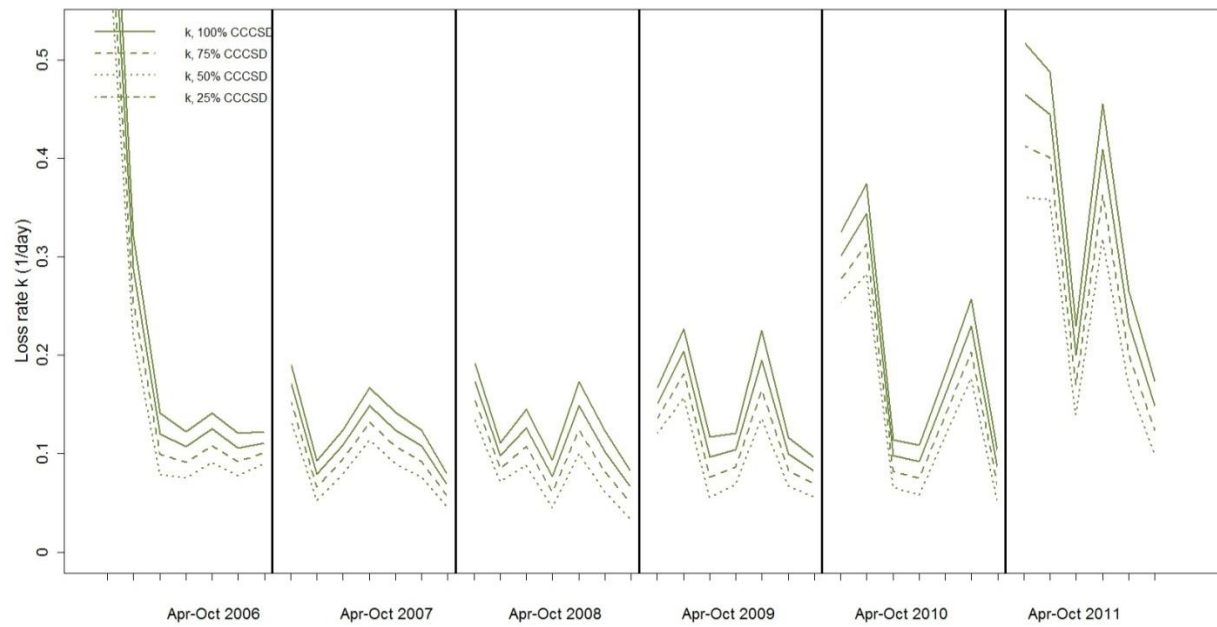


Fig A.6.4.7 Estimated first order loss rates for NH₄ in Suisun Bay during low-flow periods in Suisun Bay. Loss rates were approximated by a 1-box mass balance that considered advective loads in, downstream tidal loads in, discharger loads in, advective loads out and downstream tidal loads out of Suisun Bay. Due to the location of CCCSD discharge, it is likely that some of the effluent plume may be advected downstream prior to mixing into Suisun Bay, so the model was run assuming a range of CCCSD effluent mixing into Suisun Bay.