Suisun Bay Ammonium Synthesis Report

Document Background/Status (as of March 4 2013):

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- Work began on the report in August 2012. A draft outline of the report was shared with the Suisun Workgroup in August 2012 and discussed at a Suisun Workgroup meeting in August 2012.
- A first draft of the report was completed on October 26 2012. With the exception of several minor edits made in early November, that remains the current version (attached here).
- The draft was distributed to Region 2 staff, BACWA, researchers at SFSU-RTC, and members of SFEI's nutrient technical team. In January 2013 the draft was shared with the Suisun Workgroup.
- Comments are being accepted on the current draft and will be addressed in a revised version. The requested deadline for comments is April 5 2013.
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Suisun Bay Ammonium Synthesis Report

Draft

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Executive Summary

The ecosystem health of Suisun Bay and the Sacramento/San Joaquin Delta has experienced substantial degradation over the past several decades. The Interagency Ecological Program's (IEP) conceptual model for the Pelagic Organism Decline (POD) recognizes that multiple factors may be acting in concert to degrade habitat and contribute to the sudden decline in native and non-native pelagic fish species in the northern San Francisco Estuary, including Suisun Bay and the Delta (Baxter et al., 2010). Factors considered in the IEP conceptual model include changes in flow regime, physical alternations 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 a broad and integrated analysis of all potential drivers, and an assessment of their relative importance. This report focuses one of these high priority issues: elevated loadings and concentrations of ammonium (NH4) to Suisun Bay and their potential impacts on beneficial uses. It is expected that this report will be one of multiple reports (including those already developed, e.g., Baxter et al., 2010) that describe the state of the science and identify outstanding scientific questions that need to be addressed, and that combined offer a holistic view of habitat impacts in Suisun Bay and the Delta to inform management decisions.

Recent studies have suggested that increases in anthropogenic nutrient loads over the past few decades, in particular NH4, may be exerting adverse pressure in multiple ways. Elevated NH4 concentrations are hypothesized to be inhibiting primary productivity in Suisun Bay, San Pablo Bay, and along the Sacramento River (Section 3; Dugdale et al., 2007; Parker et al., 2012), and indirectly contributing to POD through decreased food supply. Higher NH4 levels may also be contributing to the increased frequency of *Microcystis* blooms in the Delta (Lehman et al., 2008). Changes in nutrient ratios and forms of N have been hypothesized to be exerting additional bottom-up pressures on Delta and Suisun food webs (e.g., Glibert et al., 2011). Finally, NH4 may have chronic toxicity effects on an important copepod species (*Pseudodiaptomus forbesi*) at concentrations that are observed in some areas of the Delta and the Sacramento River (Teh et al., 2011). While other aspects of nutrient cycling in Suisun Bay – e.g., changes in loads and concentrations of nutrients in their various forms, changes in NH4:NO3 and N:P and their potential food web effects – also ultimately need detailed analysis, this report focuses primarily on NH4.

In order to inform important, near-term, and potentially costly management decisions related to regulating nutrient loads, a better understanding is needed of the current state of the science related to potential impairment due to NH4, and of NH4 concentrations, sources, fate, and long-term trends in Suisun Bay. The overarching goal of this report is to provide an overview of the state of the science to inform managers about science gaps, and to serve as an initial synthesis step on this topic that should be followed by a scientific workshop that more specifically identifies: areas of agreement in the scientific community; outstanding science questions; and experiments that can target those questions. The specific goals of this report are:

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.

2. Through the perspective of the broader scientific literature, evaluate the results and interpretations of recent studies that hypothesize that elevated NH4 levels inhibit primary production rates.
3. Summarize the scientific literature related to NH4 toxicity to copepods.
4. Synthesize the scientific literature on copepod ecology and changes in community composition and abundance in Suisun Bay
5. Quantify NH4 loads to Suisun Bay, evaluate long-term changes and seasonal variations in ambient NH4 concentrations, and characterize NH4 fate.
6. Summarize key findings and identify next steps.

The report is organized with individual sections that address each of the six main goals. Overall findings are summarized below.

NH4 inhibition of primary production

The NH4 inhibition hypothesis has evolved out of extensive field transect and enclosure 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 uM) 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 NO_3 (the largest pool of N in the San Francisco Estuary) is necessary for phytoplankton bloom development (the stimulation of larger cells, i.e. diatoms). Under this model, bloom initiation is dependent on lower NH4 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 that multiple species of phytoplankton exhibit either a strong preference for NH4 or that NO3 uptake is actively inhibited by elevated NH4 concentrations. RTC studies also offer convincing support for P1, with NO3 uptake by phytoplankton strongly inhibited when NH4 exceeds 1-4 μmol L^{-1}.

P.2 is not currently well-supported by the broader scientific literature (Section 2). Few well-controlled studies have actually investigated N uptake rates during experiments in which both NO3 and NH4 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 NO3 vs. NH4 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 NO3 and NH4 are similar. The RTC studies provide some support for P2 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 NH4 uptake rates may be comparable to or even greater than NO3 uptake rates under certain conditions. In addition, in some cases where evidence from San Francisco Bay studies is either consistent or inconsistent with P2, uncertainty remains about whether experimental artifacts or other reasonable explanations could explain the observations. While P2 remains a plausible hypothesis, additional research is needed to more rigorously establish NO3 and NH4 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 currently 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 NH4 or NO3 as their nitrogen source. Multiple studies have found similar growth rates (rates of carbon fixation) across a range of taxa when using NH4 or NO3. 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 NO3 or NH4 was provided. As with P2, few studies have done growth experiments in which phytoplankton have the choice between NH4 and NO3, so there also remains a critical gap in the literature on this related topic. RTC field and enclosure experiments provide some strong evidence that primary production rates (using rates of C uptake) are slower at high NH4 levels, and that growth rates increase when NH4 is depleted and phytoplankton begin utilizing NO3 (Parker et al., 2012a, 2012b). In other studies, primary production rates are inferred from changes in chl-a or assumed to be proportional to 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 P2 and P3 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. However, their conclusions about Suisun Bay do not sufficiently address this nuance, or the extent to which the NH4-based explanations can be readily applied in Suisun Bay. Finally, NH4 levels are present at comparable levels throughout San Francisco Bay, and examples of NH4 inhibition of primary production rates have not been documented elsewhere in the system.

Similar to P2, P3 remains an entirely plausible hypothesis, in particular at the phytoplankton community level under field conditions. Inhibition of primary production rates has been proposed in other NH4-rich estuaries (e.g., Delaware Bay), and in other systems with relatively high sources of NH4 from treated wastewater effluent. 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 community provide an important and necessary perspective on how processes manifest at the field scale. However, the complexity introduced by field conditions or simulated-field conditions, during which time multiple underlying factors are changing (e.g., phytoplankton community composition, 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 NH4 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 NO3 and NH4 uptake kinetics and phytoplankton growth kinetics under a range of conditions (e.g., varying temperature and light levels, varying proportions of NO3 and NH4), 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
- If there is a difference between primary production rates, continue studies to determine its ecological significance at the ecosystem scale, including understanding the mechanisms and the conditions under which differences in growth rates will be occur, and the magnitude of the effect, in order to inform management decisions.
- 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 (e.g., Glibert et al, funded by Delta Science Program: matrix of N and P manipulations and their effect on community composition and growth; Parker et al., funded by Delta Science Program: Field observations and manipulation experiments to explore factors contributing to *Microcystis* blooms and production of microcystin; Kudela et al., submitted to IEP: Monoculture growth experiments using species cultured from Suisun Bay). Any preliminary results from those studies have not been discussed or analyzed for this report; therefore, this assessment should be revisited as that data becomes available.

Independent of whether the set of processes laid out in the NH4-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 limitiation) or biomass accumulation (clam grazing, residence time) in Suisun Bay. The RTC studies clearly acknowledge the roles of light limitation and clam grazing; they point out that NH4 inhibition of primary production may be one additional factor that limits production when conditions might otherwise allow for blooms to occur. However, this important point sometimes gets lost when the NH4-inhibition conceptual model is discussed in the context of its management implications. A quantitative analysis of the ecosystem-scale importance of the NH4-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 NH4 hypothesis, in that they will provide simultaneously provide a tool for quantitatively synthesizing existing nutrient and phytoplankton data in Suisun Bay and other embayments (e.g., Lower South Bay), identifying data and monitoring needs, and informing the broader modeling strategy for the Bay.

Finally, the form of nitrogen available to phytoplankton, e.g., NH4 vs. NO3, and changes in N:P have been hypothesized to be influencing phytoplankton assemblages in Suisun Bay and the Delta. (Wilkerson et al. 2006; Glibert et al., 2010; Parker et al., 2012b), 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, and warrants its own full investigation. This is beyond the scope of this report, and should be addressed in a subsequent report.

NH4 toxicity to copepods

There has been limited research on NH4 toxicity to copepods to date. The unionized form, ammonia (NH3), is the form that has most commonly been considered to be the form of ammonia/ammonium that is toxic to aquatic organisms. However, Teh et al. (2011) recently reported on chronic toxicity effects on the copepod *Pseudodiaptomus forbesi* at NH4 concentrations as fairly low levels. *P. forbesi* is an important prey item for multiple pelagic fish in Suisun Bay, including Delta smelt and other POD species. Teh et al. (2011) found that the survival of *P. forbesi* from early life stages to adult stages was reduced at NH4 concentrations as low as 26 μmol L^{-1}. The toxicity mechanism was hypothesized to be related to the fact that copepods excrete N waste as NH4, and that elevated NH4 levels in the ambient surrounding water could interfere with net NH4 excretion. Since NH4 levels exceed 26 μmol 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 NH4 loads to the system.

While the study by Teh et al. (2011) is a reasonably well-designed study, it has neither been replicated nor peer reviewed. Both peer review and replication would be worthwhile, in particular considering the low sample size and other methodological and statistical critiques that have been submitted as part of recent regulatory processes. Although the toxicity mechanism is plausible and there is some support in the literature, there have been only a handful of published studies on NH4 toxicity to copepods. In addition, Teh et al. (2011) found its LOEL at the lowest dosed samples, and treatments at lower levels are needed to establish a no observed effect level (NOEL). Finally, for any copepod studies, it will be important for them to be carried out at salinity ranges relevant to Suisun Bay, in particular because toxicity is thought to be exerted through the Na/K transporter, Na^+ are K^+ levels vary linearly with salinity, and copepod sensitivity to NH4 may differ at different salinities.

Copepod ecology in Suisun Bay

Copepods are key links in the San Francisco Estuary 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 *Potamocorbula 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, the unpublished report by Teh et al. (2011) of direct toxicity of ammonia to copepods has not yet been peer reviewed and remains controversial. High ammonium may have a negative effect on diatom production, which could affect copepod growth and development, and a positive effect on growth of the toxic cyanobacteria *Microcystis*, but so far no clear evidence for either of these effects has been found.

NH4 loads, ambient concentrations, and fate

Given the hypothesized impacts of elevated NH4 levels on primary production rates, and both directly and indirectly on copepod populations, a better understanding of NH4 concentrations, sources, fate, and long-term trends in Suisun Bay is necessary in order to inform important, near-term, and potentially costly management decisions to regulating nutrient loads. To do this, we compiled and analyzed data from long-term monitoring programs over the period 1975-2011, and recent studies that collected samples at higher spatial and temporal resolution. NH4 data was compared with thresholds relevant to hypothesized NH4-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 NH4 and NO3 concentrations within Suisun Bay.

NH4 concentrations in Suisun Bay exhibited pronounced seasonality and a gradual increase in baseline levels between 1975-2011. Over this period, NH4 concentrations increased at all three long-term monitoring sites in nearly all months, with statistically significant increases observed during Oct-Dec during May-June at D6 and D7. NH4 concentrations tended to be 2-4 times lower in low flow months (May-October)

The major anthropogenic NH4 loads to Suisun Bay came from the Delta and from treated wastewater effluent discharged directly to Suisun Bay. Since 1975, NH4 loads from the Delta to

Suisun increased substantially 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 ± 1840 kg N d^{-1} from 2006-2011, and 4060 ± 2660 kg N d^{-1} from 1975-1995. NH4 loads exhibited strong seasonality, as did the magnitude in the increase between pre-1995 and post-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). Since most of the NH4 transported along the lower Sacramento River has been shown to originate at Sacramento Regional Wastewater Treatment Plant (SRWTP), loads from SRWTP were presumably responsible for most of this increase. 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). 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. During the months most relevant for spring phytoplankton blooms (i.e., April and May), mean NH4 loads increased by 5000-6000 kg d^{-1} between 1975-1980 and 1998-2011, which is comparable to the NH4 load increase at SRWTP. As has been demonstrated in other studies (Foe 2010; Parker et al., 2012), much of SRWTP's NH4 load undergoes nitrification en route to Suisun Bay, and a substantial loss of NH4 is consistent with our estimated loads entering Suisun Bay. Loads from Central Contra Costa Sanitation District also increased by ~800 kg d^{-1} between the early 1990s and 2011 (mean 1990-1995 = 2620; mean 2008-2011 = 3380 kg d^{-1}). 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 1000s of kg d^{-1}, and thus are a quantitatively important unknown.

Box model mass balance estimates, calculated during May-Oct over the period 2006-2011, suggest that NH4 exhibits strong non-conservative behavior. If NH4 behaved conservatively, monthly load estimates suggest that concentrations should be on the order of 20 µmol L^{-1}. Instead, spring, summer and fall concentrations are typically 3-6 µmol L^{-1}. This large difference between predicted and measured concentration, and this specific concentration range, are highly relevant considering the levels at which NH4 is hypothesized to inhibit primary production (>2-4 µmol L^{-1}) and have toxic effects on copepods (26 µmol L^{-1}). The model results demonstrate that, on average, only 25% of the NH4 that was added to the system was actually transported out of Suisun Bay through the Carquinez Straits. The remaining ~75% of the NH4 must have been lost by transformation (e.g., nitrification) or uptake (e.g., by phytoplankton). The first order rate constants required to explain the loss of NH4 during low-flow periods was in the range of 0.1-0.3 d^{-1}, which is similar in magnitude to nitrification rates used in more advanced water quality models. This mass balance analysis did not include NH4 loads due to flux from the sediments, indicating that, if those loads were at all substantial, the calculated loss rates and first order rate constants are lower bound estimates.

Ambient NH4 concentrations in Suisun Bay frequently exceeded threshold levels for NH4 inhibition. According to the conceptual proposed by RTC researchers, at NH4 concentrations above 4 µmol L^{-1} the uptake of NO3 by phytoplankton is substantially inhibited, resulting in lower primary production rates. Although this conceptual model also indicates that 4 µmol L^{-1} is not a "bright-line" threshold, and that NO3 uptake and phytoplankton productivity are also inhibited at lower levels of NH4 (down to ~1 µmol L^{-1}), the 4 µmol L^{-1} value is used here because it is the most widely cited value; there remains considerable uncertainty around what would constitute a more appropriate value; and qualitatively similar conclusions are reached when ambient concentrations are compared to either 1 or 4 µmol L^{-1}. Since phytoplankton blooms have historically only been observed in spring, summer, and fall, the 4 µmol L^{-1} threshold is compared to ambient concentrations in April-October when the potential for impairment is most relevant. Between 1975-1986, NH4 levels exceeded 4 µmol L^{-1} in 44% of the monthly observations. Between 1987-1997, the 4 µmol L^{-1} threshold was exceeded in 70% of monthly observations. Most recently, from 1998-2011, ambient NH4 concentrations exceeded 4 µmol L^{-1} the vast majority of the time (87%). Thus, the frequency with which a 4 µmol L^{-1} threshold has been exceeded between April-October has approximately doubled over the past 35 years.

Teh et al (2011) found that the lowest observed effect level (LOEL) for chronic toxicity to copepods was 26 µmol L^{-1}. Ambient NH4 concentrations at D6, D7, and D8 were also compared to this value. Since copepods have complex life-cycles and are present year round, albeit in varying abundance, the 26 µmol L^{-1} LOEL was compared with concentrations over the entire year from 1975-2011. The value of LOEL was only exceeded two times, once at each D6 and D7, and both exceedances occurred in 1977.

The above comparisons of ambient concentrations with thresholds should be interpreted with caution for two main reasons. First, none of these thresholds has been rigorously established. The NH4-inhibition hypothesis still requires further testing. In addition, if it is found to be an important mechanism that limits primary production rates, the actual threshold value needs to be further evaluated and may in fact be lower. The copepod toxicity study by Teh et al. (2011) has neither been replicated nor peer reviewed, and both would be worthwhile, in particular considering the low sample size and other methodological and statistical critiques. In addition, Teh et al. (2011) found the LOEL at the lowest dosed samples, and treatments at lower levels are needed to establish a no observed effect level (NOEL). For any copepod studies, it will be important for them to be carried out at salinity ranges relevant to Suisun Bay, in particular because toxicity is thought to be exerted through the Na/K transporter, Na^+ are K^+ levels vary linearly with salinity, and copepod sensitivity to NH4 may differ at different salinities.

Second, while NH4 levels at the stations sampled in long-term time series may be representative of the range of average conditions observed in Suisun Bay, they may not be the highest concentrations. Data from any near-field sampling around POTW discharges have not been included in this analysis. If such data exists, they should also be compared with thresholds.

Recommended Next Steps

The recommended next steps identified here are not intended to be comprehensive, but rather communicate some broad suggestions that became clear during the development of the initial draft of this document.

1. General: A coordinated nutrient science program needs to 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, a Delta-Suisun nutrient research program could be readily coordinated with the Bay-wide nutrient strategy and with IEP. Developing such a coordinated nutrient science program is consistent with recent recommendations in the Delta Plan V6.0.

2. NH4 inhibition hypothesis*:*

2.a To develop the scientific questions and the specific studies (and study designs) that are needed to address these questions, a scientific panel should be convened. This panel should consist of regional scientists working on phytoplankton ecology and nutrient issues in the Bay, as well as outside experts. The panel should be challenged to explore the detailed evidence from studies in San Francisco Bay and literature from other systems and identify: scientific 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 consensus document summarizing their observations and recommendations, and that document can serve as the final chapter to a revised version of this report.

2.b. Whether or not NH4 inhibition is a viable mechanism, its potential importance at the ecosystem scale, relative to other factors known to play important roles in limiting primary production rates (e.g., light limitiation) or biomass accumulation (clam grazing, residence time) in Suisun Bay, has not been adequately investigated. Such an analysis could be carried with relatively basic biogeochemical models and existing data, and using parameterizations of the proposed mechanisms. These modeling efforts have benefits far beyond testing the NH4 hypothesis, in that they will provide simultaneously provide a tool for quantitatively synthesizing existing nutrient and phytoplankton data in Suisun Bay and other embayments (e.g., Lower South Bay), identifying data and monitoring needs, and informing the broader modeling strategy for the Bay.

3. NH4 toxicity to copepods: The chronic toxicity test of Teh et al. (2011) should be replicated. Recognizing that this study has drawn criticism in the past, 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). While other more nuanced questions and complex study designs may eventually be warranted (e.g., effect of food limitation and NH4), replicating the chronic toxicity experiment first, and determining if similar or different thresholds are observed, is a logical next step. The revised study design should include lower NH4 concentrations to establish a no observed effect level (NOEL). The need for carrying out the experiment at different salinities relevant to Suisun Bay also deserves consideration.

1. Introduction and Background

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1.1 Introduction

[to be developed further]

The specific goals of this report are:

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.
2. Through the perspective of the broader scientific literature, evaluate the results and interpretations of recent studies that hypothesize that elevated NH4 levels inhibit primary production rates.
3. Summarize the scientific literature related to NH4 toxicity to copepods.
4. Synthesize the scientific literature on copepod ecology and changes in community composition and abundance in Suisun Bay
5. Quantify NH4 loads to Suisun Bay, evaluate long-term changes and seasonal variations in ambient NH4 concentrations, and characterize NH4 fate.
6. Summarize key findings and identify next steps.

The report is organized with individual sections that address each of the six main goals.

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

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26 October 2012

2.1 Introduction

The goal of this section is to synthesize the scientific literature on phytoplankton growth and 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. The section begins with a brief overview of some key issues related to photosynthesis and carbon fixation. Next factors that regulate uptake and assimilation of N are described from a molecular and biochemical perspectives. Subsequently, the discussion moves to experimental data on preference for different N forms, factors regulating kinetics of N uptake, effects of N substrate on growth rates, and interactions in mixed phytoplankton communities. The section closes with an overview conceptual model of N uptake and utilization based on the current literature

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 the cells. Both ATP and electrons (via NADPH) are needed to drive C-fixation. The process providing ATP and electrons for C-fixation is called photosynthesis.

2.2.1 Photosynthesis

Photosynthesis proceeds in the same manner in all photosynthetic organisms because the components of the photosynthetic apparatus are very well conserved (Appendix A.2). 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. 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 photoinhibiton, the loss of photosynthetic function due 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 CO_2 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). 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 *Synechochoccus* 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..

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 use by marine phytoplankton

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). Marine and estuarine phytoplankton utilize 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 N substrates, and therefore pathways, are important for marine phytoplankton N demand but will not be discussed here.

2.3.1 Molecular perspective on nitrogen uptake and assimilation

In order for phytoplankton to use N they have to transport it into the cell. By examining the expression of the various transporters phytoplankton have available in their genomes it is possible to characterize the propensity to utilize different forms of N. The higher the expression (mRNA abundance), the more of that transporter is being made in the cell (Fig. 2.3), providing clues about the N sources that phytoplankton are using.

2.3.1.1 NO3- uptake and reduction

Nitrate is actively transported into marine phytoplankton via the high-affinity transporter Nrt2 (also called NAT) 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 ≅60 µmoles NO_3^- L, 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 weisflogii* 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) 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 uptake 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 ($CO(NH_2)_2$) 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^+ uptake 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*:** Elevated external NH_4^+ levels are toxic to photosynthetic organisms because the build-up of a charged molecule on one side of the cell membrane results in the establishment of a high cross-membrane potential. While NH_4^+ is mostly transported into the cell via active transport (as are nearly all charged molecules) mediated by the AMT1 transporter, it can also passively diffuse into the cell via channels (facilitated diffusion) and aquaporins (Loque et al. 2009 and references therein). When external concentrations are elevated, these channels will allow a large influx of NH_4^+ down its concentration gradient. The influx initiates active pumping to rid the cytosol of NH_4^+ and to prevent an intracellular pH disturbance (Bligny et al. 1997). However, the efflux of NH_4^+ maintains the cross-membrane gradient, and thereby the channel influx, and necessitates continued, active efflux pumping at a great energetic cost to the cell, culminating in the cessation of growth and sometimes death of the organism (Britto et al. 2001, Szczerba et al. 2008).

Some plant species have adapted to high external NH_4^+ concentrations by preventing the establishment of a cross-membrane potential, eliminating the futile NH_4^+ cycling and high respiratory cost of efflux pumping (Britto et al. 2001). Because the susceptibility to the establishment of a cross-membrane potential varies from organism to organism depending on their transport mechanisms, susceptibility to NH_4^+ toxicity also varies greatly. For example, susceptibility to NH_4^+ toxicity is known to vary by orders of magnitude in aquatic plant species and in unicellular algae. Freshwater unicellular algae such as *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). Among marine and estuarine species, diatoms 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 very high affinities for NH_4^+ can be susceptible to toxicity at concentrations as low as 100 µmol NH_4^+ L^{-1} (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 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, Huppe and Turpin 1994, Mock et al. 2008, Brown et al. 2009, Hockin et al. 2012).

2.3.1.4 Expression of N transporters 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^- uptake 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, uptake can proceed rapidly. In cells where expression is low or non-existent under starvation, up-regulation of 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 varies with phytoplankton taxa, so does expression of other transporters. A surprising finding in the HAB pelagophyte *Aureococcus anophagefferens* is that the most expressed transporter in its genome is specific to purines, most likely guanine (Fig. 2.4). In this organism, growth on organic N suppresses expression of transporters for inorganic N sources almost completely. In contrast, expression of transporters 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 uptake strategy from taking up 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.

In contrast with the eukaryotic phytoplankton discussed above, cyanobacteria appear to be NH_4^+ specialists. For one, expression of the *AMT1* NH_4^+ transporter 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 uptake 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, uptake of N must keep pace with C fixation and growth. If a cell is taking up 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.

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 µmol m^{-2} s^{-1}) and non-limiting (170 µmol m^{-2} 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 µmoles photons m^{-2} s^{-1} there is a 0.6 d^{-1} increase in growth rate with every 100 µmol m^{-2} 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).

2.3.2.3 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 NO_3^-

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

2.3.3 Phytoplankton N uptake and preferences 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 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

Uptake of N as a function of its external concentration takes the shape of a hyperbola (Fig. 2.9) where the half-saturation constant, K_s, can be calculated using the Michaelis-Menten equation:

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

where V is the uptake velocity, V_{max} is the maximum uptake velocity, and S is the substrate concentration (i.e. concentration of N) (see also Figure 3.1). The affinity for a particular substrate is inversely proportional to the half-saturation constant, K_s, i.e. a low K_s denotes a high affinity for a particular N substrate. When competing for a limiting resource, having a low K_s is an advantage in low-nutrient marine environments. Because a cell's N demand is ultimately determined by its growth rate (i.e. the faster the organism is growing, the faster it will take up N), V_{max} was hypothesized to reflect the organism's growth rate (Eppley et al. 1967). Under high nutrient conditions, defined as S being much greater than K_s, the ratio $S/(K_s+S)$ will approach a value of 1, and the actual uptake velocity will equal V_{max}.

In theory, each organism will have different hyperbolas for each N source that it utilizes. In practice, phytoplankton ecologists tend to generalize K_s and V_{max} depending on the size of the organism so that large-celled phytoplankton are expected to have high K_s and high V_{max} values (for all N substrates), and dominate high nutrient environments, while smaller cells with low K_s and low V_{max} values are expected to dominate oligotrophic, nutrient-poor environments (Eppley et al. 1969, Eppley and Renger 1974).

Early investigations by Eppley and coworkers used short-term uptake measurements of N-depleted cultures to determine kinetic parameters. They made predictions regarding the environmental conditions that would favor growth of one species over the other based on the kinetic parameters that they measured. When they tested these predictions they found that they did not hold true. They concluded that short-term uptake measurements and kinetic parameters do not accurately reflect total N uptake over a diel cycle, or phytoplankton growth rates, because they do not take into account N uptake and growth that occurs during the dark period (Eppley et al. 1969, Eppley and Sournia 1971, Eppley and Renger 1974). It turns out that high rates of N transport and assimilation are achieved in the dark using stored energy (carbon) which “smooths out” C:N assimilation. It also decouples N uptake from irradiance to some extent, resulting in minimal difference between NO_3^- and NH_4^+ (Clark et al. 2002).

2.3.3.2 ^{15}N tracer technique and uptake kinetics

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 communities (McCarthy et al. 1972, McCarthy and Goldman 1979, Wheeler et al. 1982). McCarthy soon noticed 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).

2.3.3.3 Nitrogen preferences and the Relative Preference Index

The discovery that NH_4^+ uptake rates were very high compared with their ambient concentrations prompted McCarthy to compare uptake of a particular N substrate (as a fraction of total N uptake) to the fractional contribution of that same N substrate to the total ambient N pool:

$$\frac{\text{Uptake of } NO_3^- / (\text{uptake of } NO_3^- + NH_4^+ + \text{Urea})}{[NO_3^-]/[NO_3^- + NH_4^+ + \text{urea}]}$$

Coined the Relative Preference Index (RPI), this index helped illustrate that there was “a universally high phytoplankton preference for NH_4^+ and urea over NO_3^-“ in coastal phytoplankton communities (McCarthy et al. 1977). McCarthy also noted that NH_4^+ concentrations in excess of 0.5-2 µmoles L^{-1} almost completely suppressed NO_3^- utilization (Fig. 2.9, McCarthy et al. 1975, 1977). This corroborated culture investigations (section 2.3.2) demonstrating that phytoplankton preferred NH_4^+ as a N source (Fig. 2.10). Note that in this discussion, preference for NH_4^+ means the same as suppression of NO_3^- uptake. Hereafter, preference will be defined by the degree of suppression in uptake by another substrate. For a more nuanced discussion of terms used in the past to define preference and inhibition see Dortch (1990).

The fact that NH_4^+ and urea were preferred to NO_3^- in natural, mixed populations led to intense efforts to characterize 1) whether this order of preference held true for individual phytoplankton species and 2) the time it would take for phytoplankton to switch sources, from NO_3^- to NH_4^+, for growth. Whereas some studies indicated that urea was preferred to NO_3 after NH_4^+, others indicated that NO_3^- was preferred to 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 the number of sources and whether they were reduced or oxidized. 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". Each individual phytoplankton species tested differed in the 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 were the molecular mechanisms were not clear. Today these terms are supplanted by "induction" and "repression", which reflect the turning on and off, respectively, of the genes or proteins.

2.3.3.4 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. 2.11).

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. 2.11). 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. 2.11b). 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 important 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. 2.11 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).

2.3.3.5 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 to either 1) cover more of your surface area with proteins to capture the limiting nutrient or 2) tap into alternative N source comes into play and can impact growth rates. Investigators 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. 2.12). 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, proportion of N uptake does not only reflect genetic capabilities of the dominant phytoplankton group, it also reflects 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. 2.12 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.

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 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.

2.3.3.6 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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2.6 Figures and Tables

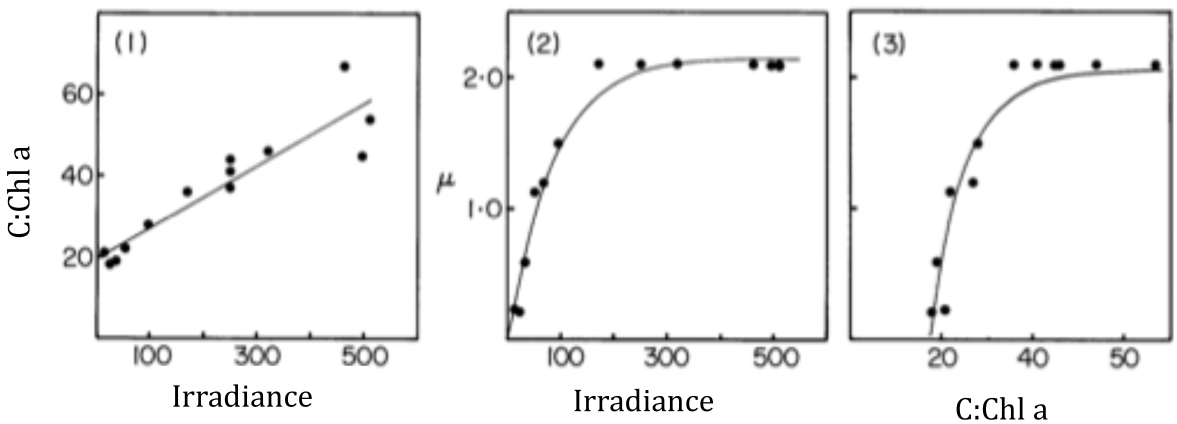

Figure 2.1 Reproduced from Geider 1987; Light dependence of C:Chl a for the diatom *Thalassiosira pseudonana*.. (1) C:Chl *a* versus Irradiance (μmol m^{-2} s^{-1}) and (2) is growth rate (d^{-1}) versus irradiance and (3) is growth rate versus C:Chl *a.*

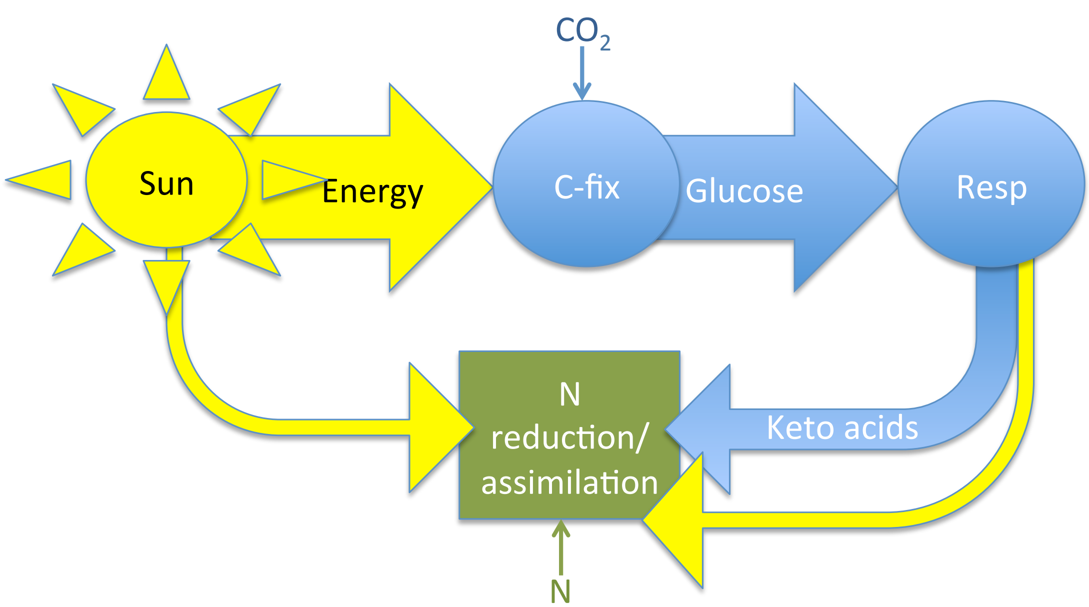

Figure 2.2. Flow of energy in the form of reductant and ATP (yellow arrows) and carbon (blue arrows) to N assimilation. C-fix=carbon fixation, Resp=respiration

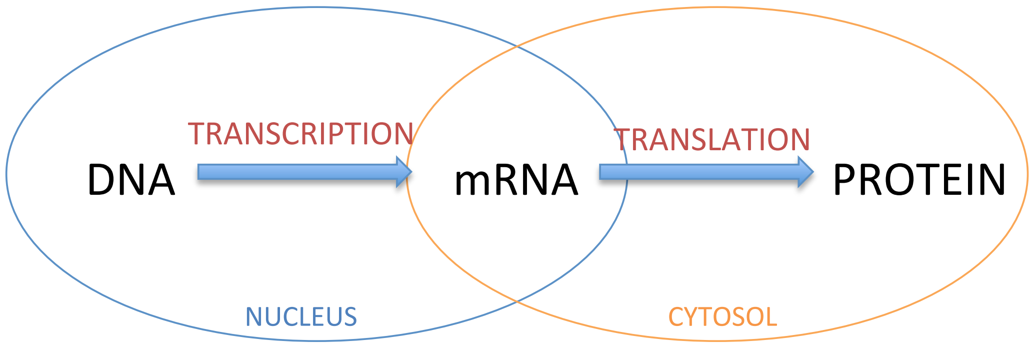

Figure 2.3. Information contained in an organism's genome is transcribed into mRNA before it's translated into protein. The amount of mRNA corresponds with the amount of protein that will be synthesized and is called "expression"

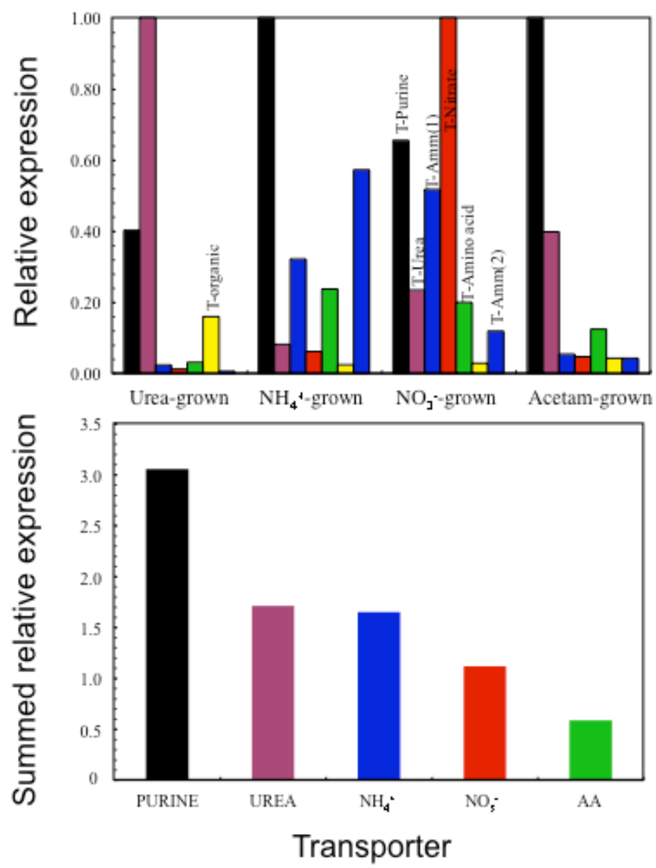

Figure 2.4. Top panel: relative expression (normalized to the most expressed transporter at each growth source) of N transporters in *A. anophagefferens* cultures grown on urea, NH_4^+, NO_3^-, and acetamide. Black bars represent mRNA abundance of the purine transporter URA; pink bars represent the urea transporter *DUR3*, blue bars represent NH_4^+ transporters *AMT1* and *ABC*; red bars represent the NO_3^- transporter *Nrt2*, green bars represent a putative amino acid transporter AA, and green 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.

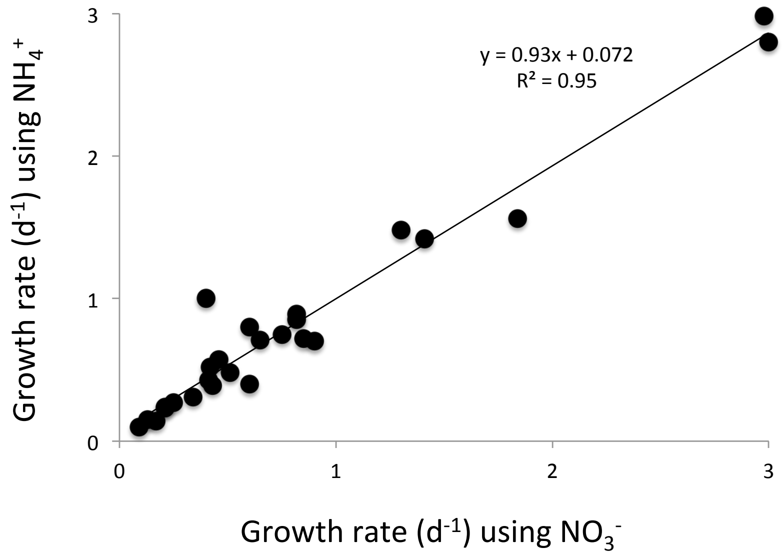

Figure 2.5 Growth rates of phytoplankton using NH_4^+ plotted as a function of growth rates using NO_3^- as the sole source of nitrogen. Data compiled from Ferguson et al. 1976, Dortch and Conway 1984, Levasseur et al. 1993, Berman and Chava 1999, Herndon and Cochlan 2007, Berg et al. 2008, Solomon and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

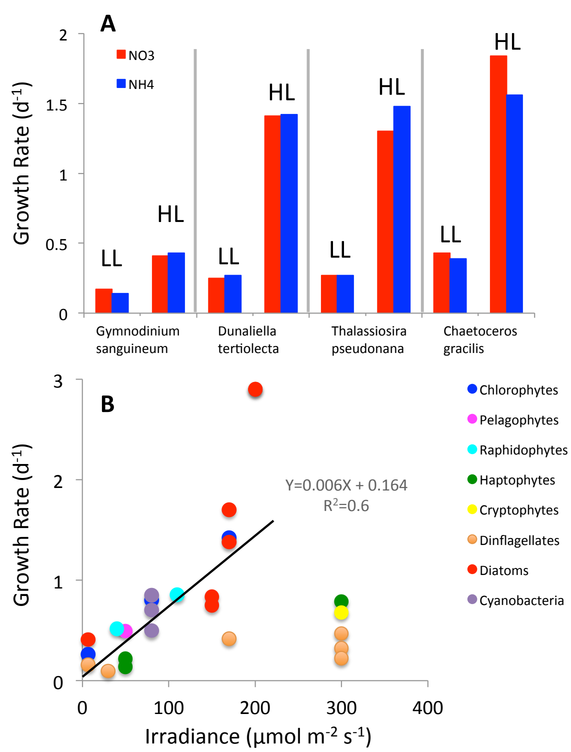

Figure 2.6 Growth rates of A) the dinoflagellate *Gymnodinium*, the chlorophyte *Dunaliella*, and the diatoms *Thalassiosira* and *Chaetoceros* using ammonium (blue bar) or nitrate (red bar) as the sole source of nitrogen at low light (LL; 7 µmol $m^{-2}s^{-1}$) and high light (HL; 170 µmol $m^{-2}s^{-1}$). 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 5.

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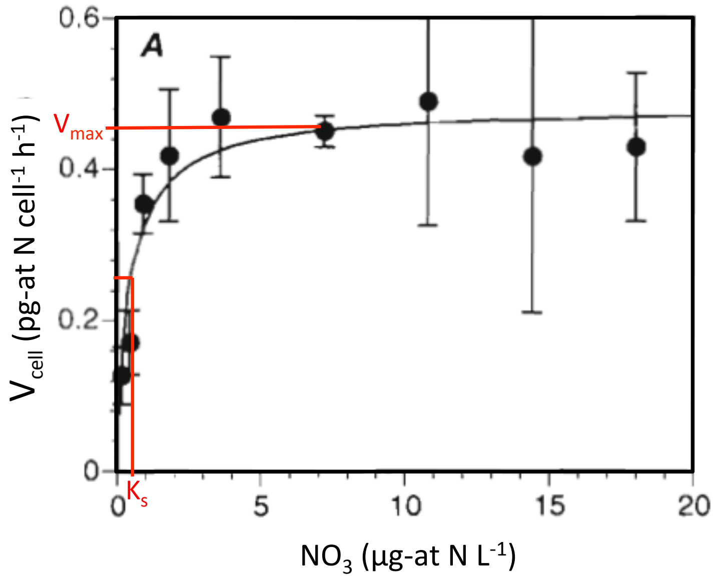

Figure 2.7 A) Rubisco expression as a function of phytoplankton taxa and B) carbon fixation as a function of diatom-specific Rubisco expression. Data are from John et al. 2007b.

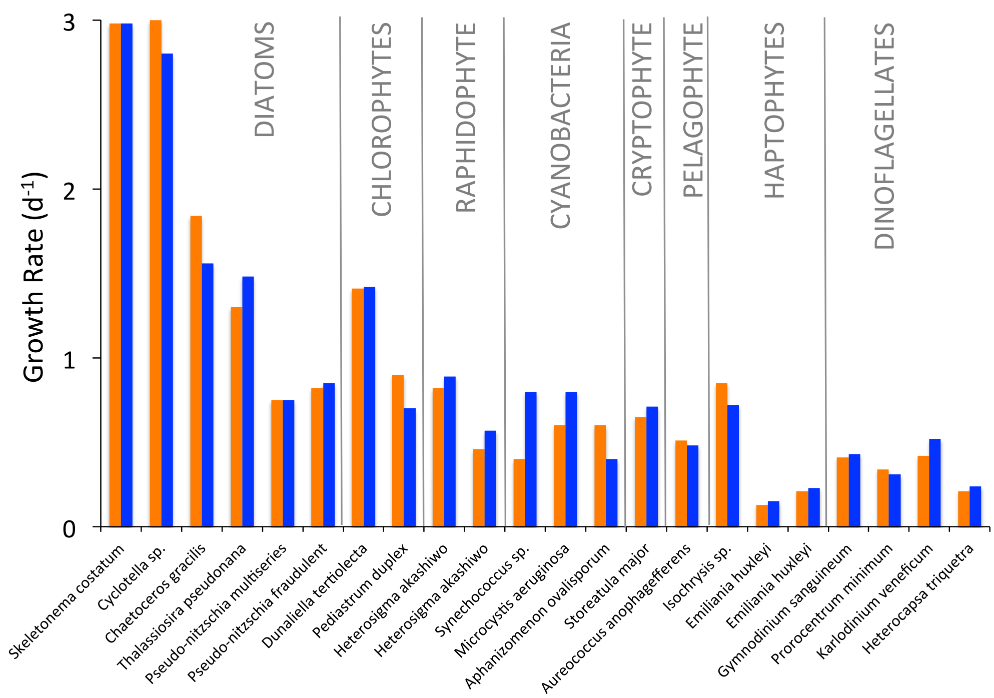

Figure 2.8 Growth rates of 8 major phytoplankton taxa. Red bars are cultures grown on nitrate and blue bars cultures grown on ammonium. Data sources the same as for Figure 5.

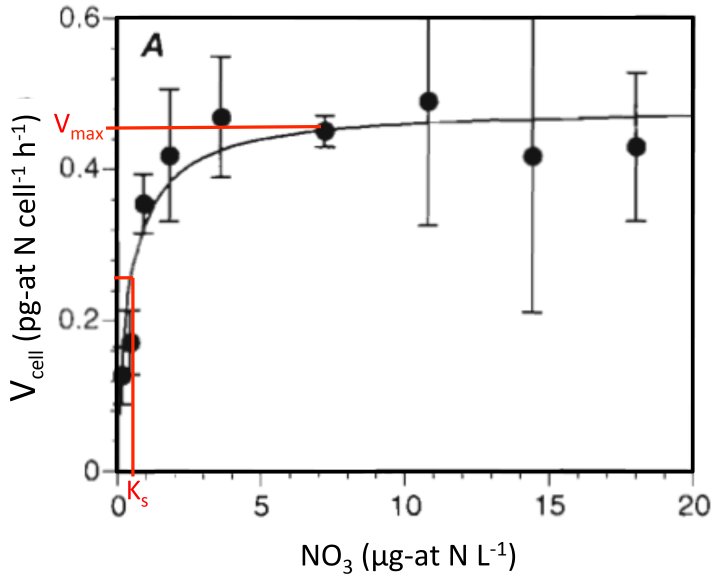

Figure 2.9. Uptake of NO_3^- (on a per cell basis) as a function of NO_3^- concentration. Figure from Kudela and Cochlan 2000.

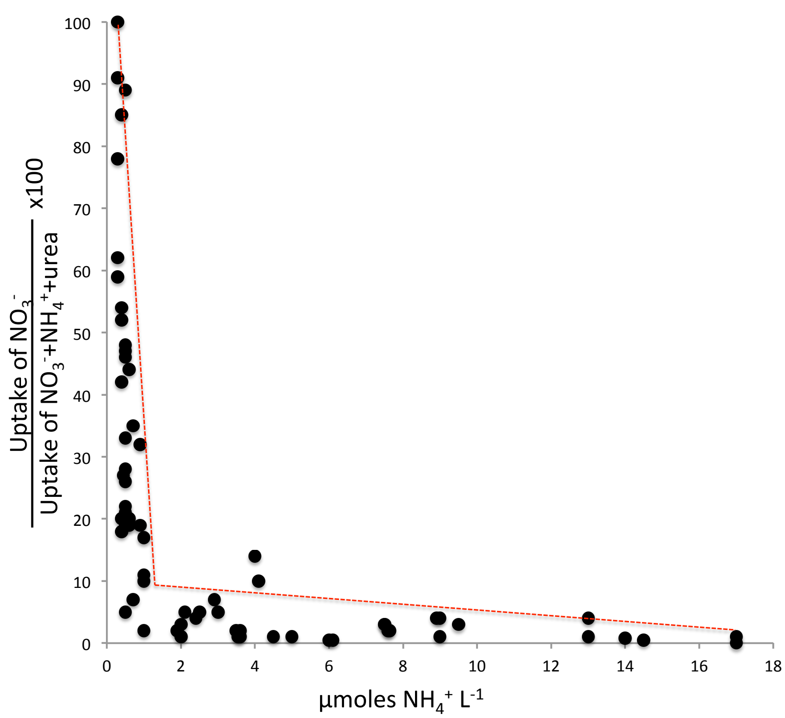

Figure 2.10. Percent NO_3^- uptake as a function of ambient NH_4^+ concentration in natural phytoplankton assemblages. Adapted from McCarthy et al. 1975

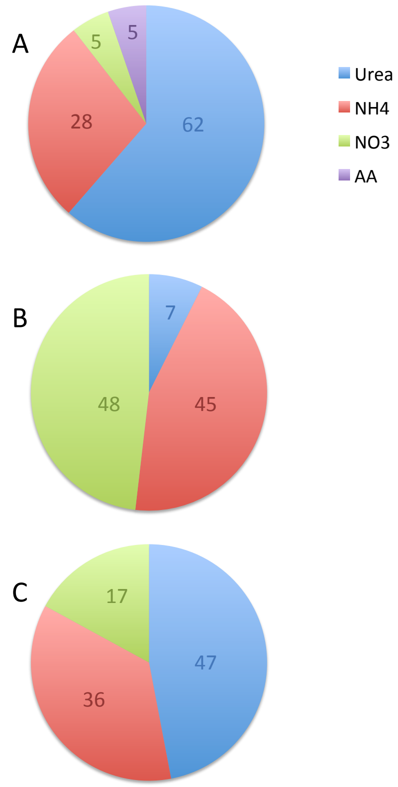

Figure 2.11. Percent contribution (number in each slice) of urea (blue), NH_4^+ (red), NO_3^- (green) and amino acids (AA, purple) to total N uptake during near-monospecific blooms (>90% community composition) of phytoplankton. A) The Brown Tide former *Aureococcus anophagefferens* (Berg et al. 1997), B) The spring bloom diatom *Thalassiosira baltica* (Berg et al. 2001), and C) the Red Tide dinoflagellate *Lingulodinium polyedrum* (Kudela and Cochlan 2000).

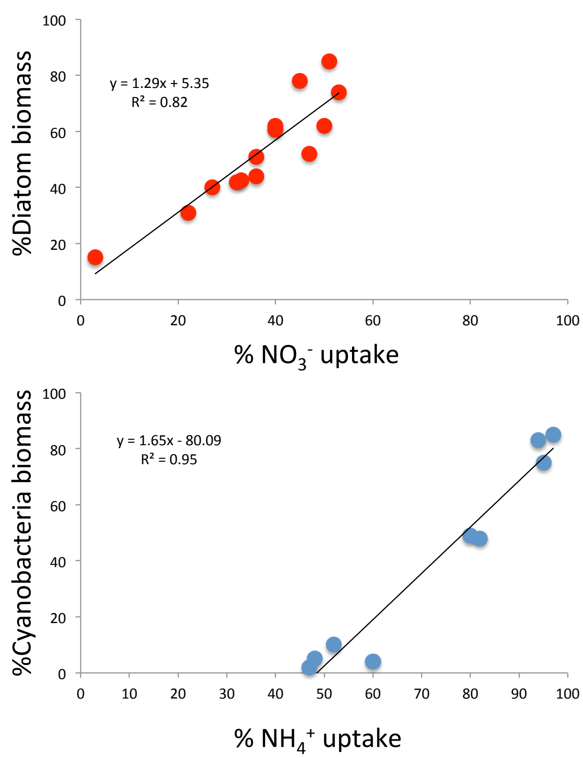

Figure 2.12. Percent diatom community composition as a function percent NO_3^- uptake (top panel). Percent cyanobacteria community composition as a function of percent NH_4^+ uptake. Figure from Glibert and Berg 2009.

3. Research on NH4 inhibition of primary production in Suisun Bay

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3.1 Introduction

The Pelagic Organism Decline (POD) in the early 2000s triggered investigations into the role of ammonia as one of several potential factors that contribute to the demise of fisheries in the Delta and Suisun Bay. These studies are beginning to address important questions regarding the potential impact of ammonium (NH_4) on aquatic species in the Delta-Suisun Bay ecosystem. Front and center of the investigations are questions and concerns about the effects of ammonium on the Delta-Suisun foodweb. These have been sparked, for example, by observations of statistically significant inverse correlations between ammonium concentrations, chlorophyll concentrations, and the abundance of two copepod species considered as important food sources in Suisun Bay (Ballard et al. 2009, Foe 2010, Fullerton 2010). Several recent studies suggest that elevated ammonium levels in Suisun Bay are inhibiting phytoplankton production in Suisun Bay (Wilkerson et al. 2006, Dugdale et al. 2007, Dugdale et al. 2012, Parker et. al 2012a, Parker et al., 2012b,).

The goal of this section is to synthesize the current state of the science related to the role of NH4 in controlling phytoplankton primary production in Suisun Bay. The effect of elevated NH_4^+ (and other nutrient-related issues, e.g., changes in N:P and overall higher N) on phytoplankton community composition is another potential issue of concern; however, that issue is beyond the scope of this report, and will be addressed in a subsequent report.

The section begins with an overall description of the conceptual model that has evolved from RTC studies carried out over the past 10 years. The conceptual model is followed by a more detailed discussion of the RTC studies whose observations form the basis for the conceptual model that is divided into three main parts, including how their findings compare to the current conceptual model that emerges from the broader scientific literature around the topic of N utilization and growth by phytoplankton (Chapter 2). Observations in other systems are then briefly described. The section ends with a summary and important questions remaining to be addressed.

3.2 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 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 uM) 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.

When NH_4 levels are relatively high in Suisun Bay, the NH4-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 NH4 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. Given that NH4 levels in Suisun Bay frequently exceed 1-4 µmol L^{-1} (Section 6), when this conceptual model is applied to interpret conditions in Suisun Bay, it suggests that NH4-inhibition of primary production has contributed to the current rarity of spring phytoplankton blooms in Suisun Bay. By extension, it is argued that the elevated NH4 levels, and the associated lack of algal blooms in Suisun Bay, have likely contributed to 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 µmol L^{-1}, phytoplankton nitrate uptake is enabled. With continued phytoplankton growth, ammonium concentration is further reduced to ≤ 1 µmol 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 major role in limiting primary production rates and decreasing biomass accumulation, including light limitation, benthic grazing by filter-feeding clams, and flushing (Section 1.2). 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, NH4 inhibition is considered to be an additional mechanism and impediment, in addition to these other factors, 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 NH4 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 NH4 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}NH4, ^{15}NO3, and $^{13}CO_2$; and enclosure experiments in which NO3, NH4, chl-a and tracer uptake rates were measured in mixed plankton communities over time.

The NH4-inhibition conceptual model can be visualized most straightforwardly by considering the biomass specific uptake rates of NO3, NH4, and C (with carbon uptake being a direct measure of primary production rate). In the discussion below, these are abbreviated as V_{NO3}, V_{NH4}, and V_C. The sum of all dissolved inorganic nitrogen (DIN) is abbreviated here as $V_{DIN.}$ While the conceptual description below, and the reference to experimental observation of these rates in Section 3.3, are detailed and technical, these concepts are critical to understanding and evaluating the NH4-inhibition conceptual model.

V_{NO3} is a rate, determined by measuring the amount of "labeled" NO3 taken up by phytoplankton over the course of an incubation experiment. In essence, V_{NO3} represents the rate at which NO3 is taken up by the overall phytoplankton community per unit mass of phytoplankton. In that sense it is diagnostic of the "average" physiological or biochemical state of the phytoplankton in terms of their ability or need to take up NO3. Thus, changes in V_{NO3}, or differences in V_{NO3} as a function of space or time, signal a change in either their need for NO3 (e.g., more or less light causing changes in primary production rates) or their ability to take up NO3 (e.g., inhibition by NH4). V_{NH4} is calculated in an analogous way. Similarly, 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.

In laboratory experiments, when a pure culture of phytoplankton is grown under constant light with varying levels of NO3, V_{NO3} commonly varies as shown in Figure 3.1B (Michaelis Menten kinetics; See also Section 2). V_{NO3} increases almost linearly as a function of NO3 concentration, and asymptotically approaches $V_{NO3,MAX}$ for those light conditions. V_{NH4} behaves similarly (Figure 3.1B).

When phytoplankton are given both NH4 and NO3 simultaneously, P1 of the NH4-conceptual model says that the relationship should look quite different. When NH4 concentrations exceed 1-4 μmol L^{-1} V_{NO3} should be inhibited, and phytoplankton will only take up NH4. This is presented schematically for 3 scenarios in Figure 3.2, with varying levels of NH4 but constant levels of NO3 (similar to the case in Suisun Bay and elsewhere in San Francisco Bay prior to and during the early stages of a phytoplankton bloom). P2 of the NH4-inhibition conceptual model argues that NH4 uptake is slower than NO3 uptake. Two illustrations of how this could be the case are presented in Figure 3.2A and Figure 3.2B. Figure 3.2A illustrates how V_{NO3}, V_{NH4}, and V_{DIN} would look if the maximum rate of NO3 uptake is greater than the maximum rate of NH4 uptake. As NH4 concentrations increase, the total rate at which DIN can be taken up by phytoplankton (pure cultures, or mixed communities) will decrease. Figure 3.2B illustrates the case when $V_{NO3,MAX}$ and $V_{NH4,MAX}$ are equal, but when phytoplankton are not very efficient at using NH4 at relatively low concentrations. At intermediate NH4 concentrations (but still greater than 1-4 μmol L^{-1}), V_{DIN} will be less than the $V_{DIN,MAX}$. Figure 3.3 illustrates the case in which $V_{NO3,MAX}$ and $V_{NH4,MAX}$ are equal, and phytoplankton are efficient at using NH4 at low levels.

It stands to reason that, in general, if some factor decreases the rate at which phytoplankton can take up DIN – and their rate of DIN uptake is the condition that is limiting growth – the rate of primary production will decrease. Under these conditions, an experimental or field observation that $V_{DIN} < V_{DIN,MAX}$ should indicate that ultimately primary production rates will be lower. A direct measurement that would be consistent with this effect would be seeing a reduction in V_C co-occurring in space or time with elevated NH4 and decreased V_{DIN}. This is essentially P3 of the NH4 conceptual model.

3.3 State of the science

3.3.1 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 µmol 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 (NO3) 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 NO3 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 NO3 uptake by phytoplankton occurred, and that NO3 uptake only occurred when NH4 concentrations dropped below 4 µmol L^{-1}. In Suisun Bay, a bloom (chl-a ~ 30 ug 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 (8-17 ug 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 umol L^{-1}) NH4 concentrations. Increases in NO3 transport rates (V_{NO3} x PON) 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 measured in Dugdale et al. (2007) or Wilkerson et al. (2006), but were inferred from changes in chl-a or variations in N uptake rates.

In all three sub-embayments, there was a clear relationship between ambient NH4 concentration and V_{NO3} (Figure 3.3A). The authors note that V_{NO3} began to increase when NH4 decreased below 4 µmol L^{-1}; V_{NO3} increased rapidly as ammonium approached concentrations of ~1 µmol L^{-1} and lower. The observed exponentially-increasing NO3 transport rate when NH4 decreased below 4 µmol L^{-1}, and the nearly uninhibited uptake when NO3 was below 1 µmol L^{-1}, strongly support P1 of the NH4-inhibition conceptual model. As discussed in Section 2, the concept of NH4 preference, or NH4 inhibition of NO3 uptake, is well-supported in the phytoplankton research literature (e.g., Dortch et al., 1990), and the results of Dugdale et al. (2007) are consistent with those findings.

While V_{NO3} decreased sharply as NH4 increased, V_{NH4}, actually increased with increasing NH4 in San Pablo and Central Bays (Figure 3.3B). This increase is consistent with classical Michelis-Menten-like kinetics of phytoplankton nutrient uptake (e.g., Eppley et al., 1967), in which V_{NH4} is proportional to NH4 concentration at non-saturating levels, until some $V_{NH4,MAX}$ is reached.

The highest observed values for V_{NH4}, which occurred at the highest observed NH4 concentrations (Figure 3.3B) were comparable to those for V_{NO3} at low NH4 concentrations (Figure 3.3A). Interestingly, this suggests that the phytoplankton community was able to utilize

NH4 at similar rates as NO3 at the extreme ends of the observed NH4 levels. The overall rate of DIN uptake, $V_{DIN} = V_{NO3} + V_{NH4}$’ is ultimately the amount of N being taken up by phytoplankton (assuming uptake of organic N is negligible). To a first approximation, $V_{NO3,MAX}$ and $V_{NH4,MAX}$ do not appear substantially different; i.e., it is not obvious that the conceptualized illustration of P1 as presented in Figure 3.2A is consistent with this set field observations. From Figures 3.3A and 3.3B, the relationship between V_{DIN} and NH4 concentration at intermediate NH4 concentrations is unclear. Does V_{DIN} vary as a function of NH4, or is it more or less constant? Understanding this point is critical to evaluating P2 of the NH4-inhibition conceptual model.

The relationship between V_{NH4} and NH4 concentration in Suisun Bay differed considerably from that observed in San Pablo Bay and Central Bay. Across the range of observed NH4 concentrations in Suisun, V_{NH4} remained low and relatively constant (although with considerable variability), and was not correlated with NH4 concentration (Figure 3.3C). V_{NO3} (Figure 3.3A) was also low in Suisun, even at the lowest NH4 concentrations, although there were limited data in this concentration range because of generally higher NH4 concentrations in Suisun. Thus, although V_{NH4} and V_{NO3} 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 NH4. Dugdale et al (2007) note that the “relationship for V_{NH4} versus NH4 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; Wilkerson 2009), as described below.

A time-series of N uptake rates in San Pablo was also presented (Figure 3.4). In the San Pablo time series, the highest measured rate of N uptake was actually a V_{NH4} around April 1, when NH4 was approximately 10 umol L^{-1}. V_{NO3} was low, and did increase when NH4 concentrations decreased. However, $V_{DIN} = V_{NO3} + V_{NH4}$ was actually greater around April 1 compared to subsequent months, arguing that, although NH4 concentrations decreased and V_{NO3} increased, the overall rate of N uptake did not increase when NH4 was low. The 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_{NO3}, V_{NH4}, and V_{DIN} in San Pablo and Central Bays are not necessarily consistent with P2 of the NH4-inhibition conceptual model; on the contrary they might be interpreted as suggesting that NH4 and NO3 are actually utilized comparably well by the phytoplankton community. Since environmental conditions play a strong role in shaping the physiological state of phytoplankton (e.g., they 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), the variation in the total size of the summed bars in Figure 3.4 complicates these interpretations.

3.3.2 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 NH4 concentrations decreased below a few micromolar, V_{NO3} increased substantially, consistent with field observations and with an inhibition by or preference for NH4 (see Figure 7, Dugdale et al. 2007). In addition, in some incubations, maximum observed values for V_{NO3} exceeded maximum values for $V_{NH4,}$ consistent with P2 of the NH4-inhibition conceptual model. Primary production rates were not measured, but changes in chlorophyll were monitored. While chlorophyll levels did increase more rapidly once NH4 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 NH4 additions of 0, 5, 10, 20, and 30 umol L^{-1}. These incubations demonstrate clearly that V_{NO3} remained low until NH4 concentrations decreased to below ~4 umol L^{-1}, consistent with P1 of the NH4 inhibition conceptual model (see Figure 8 of Dugdale et al., 2007). No data were presented for how V_{NH4} varied with NH4 concentrations, which would be valuable information for interpreting how $V_{DIN} = V_{NO3} + V_{NH4}$ varied as a function of NH4 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 NO3, such that higher C uptake and biomass accumulation are linked with NO3 uptake" compared to NH4 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_{NO3} and V_{NH4}, a main enhancement of the study design was measuring 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 (2012) to attribute increases in chlorophyll to larger-celled species (>5 μm, e.g., diatoms) and smaller-celled species (<5 μm).

The Parker et al (2012a) enclosure experiments from San Pablo and Central Bay behaved similar to each other with respect to their uptake of N and C (Figure 3.5). The presence of NH4 above ~1 umol L^{-1} resulted in suppressed V_{NO3} (Figure 3.5A and 3.5C). This result is consistent with the P1 of the NH4-inhibition conceptual model, with field and enclosure results of Dugdale et al. (2007), and with the broader literature that NH4 inhibition is a common phenomenon among marine and estuarine phytoplankton (Section 2). V_{NH4} was approximately 2-fold greater than V_{NO3} at the beginning of incubations (Figure 3.5C and 3.5D). Both V_{NH4} and V_{NO3} increased substantially over the first 24 hours, although NH4 was still present at ~3 μmol L^{-1}, and V_{NH4} remained 2-3 times greater than V_{no3}. By 36 hours, nearly all NH4 had been consumed, and V_{NO3} increased to 0.05-0.06 h^{-1}, which was approximately 2-fold greater than the maximum values for V_{nh4} measured during the experiments. These observations are consistent with P2 of the NH4-inhibition conceptual model that maximum NO3 uptake rates – once NH4 has decreased below ~1 μmol L^{-1} – are greater than maximum NH4 uptake rates, and that overall inorganic N uptake ($V_{nh4+no3}$) is greater when NH4 concentrations are low. Parker et al. (2012a) suggest that the mechanism underlying the higher rate of NO3 uptake relates to the concept of acceleration of uptake -"shift-up" - such that the maximal NO3 uptake ($V_{NO3,MAX}$) is variable and proportional to the NO_3 concentration (Zimmerman et al., 1987; Wilkerson et al., 1987; Dugdale et al., 1990).

Although both V_{NH4} and V_{NO3} 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_{NO3} after NH4 levels decreased to near-zero values. These observations are consistent with P3 of the NH4-inhibition conceptual model that primary production is greater when NH4 levels are low and phytoplankton begin utilizing NO3 for growth. Plots for July and September incubations are not presented but maximum V_{NH4} and V_{NO3} 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 μm size fraction, suggesting that much of the new production was due to larger-celled phytoplankton, and likely diatoms.

Enclosure experiments from Suisun Bay behaved differently from those in San Pablo Bay and Central Bay (Figure 3.5). Initial values (t = 0) of V_{NH4} were lower in Suisun than the other embayments, and gradually increased over the experiment, eventually (after 72 h) equaling the maximum V_{NH4} values observed in the other sub-embayments (which occurred after only 24 h; Figure 3.5D). After 72 h, NH4 decreased to approximately zero, and V_{NO3} was higher at 96 h than the maximum V_{NH4} 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 $V_{no3.}$ V_{NH4} was ~3-fold lower at t = 0 and t = 24 h than V_{NH4} in the San Pablo and Central Bay enclosures, and ~40% less than their maximum even after 48 h. Although the starting NH4 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_{NHH} actually increased linearly with increasing NH4 concentration up to ~10 μmol L^{-1} in Central and San Pablo Bays, so elevated NH4 in Suisun cannot be readily invoked as the cause for suppressed V_{NH4}. In fact, the suppressed V_{NH4} in Suisun enclosures in Parker et al (2012) are qualitatively consistent with the low V_{NH4} measurements in Suisun observed by Dugdale et al. (2007) (Figure 3.3C). 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 NH4 condition, the result of wastewater loading to the northern SFE (Jassby, 2008), is potentially exacerbated by some additional stress that results in low NH4 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 may be a valid explanation, in particular given the magnitude of anthropogenic contaminants loaded to the system (agriculture, wastewater). However, the fact that V_{NH4} and V_C gradually increase over the incubation would require that bioavailable levels of the toxic substance(s) decreased.

Parker et al. (2012a) also quantified maximum uptake rates for NH4 and NO3 using natural phytoplankton assemblages from Central Bay, incubated at 50% incident light. The maximum uptake rate for NO3, $V_{NO3,MAX}$ was 0.044 h^{-1} while $V_{NH4,MAX}$ was 0.033 h^{-1} (Figure 3.6). This finding is consistent with P2 of the NH4-inhibition conceptual model that phytoplankton in SFE can take up NO3 more rapidly than NH4. Based on visual inspection, the rates do appear consistently different. However, there is currently limited data, and it is not stated whether the difference is statistically significant. The relationship in Figure 3.6, and the near saturation of uptake rate shown (i.e., V_{NH4} reaching a relatively constant value) at NH4 concentrations of ~3-4 μmol L^{-1}, differs from the results of Dugdale et al. (2007) (Figure 3.3B), who observed that V_{nh4} increased linearly with NH4 up to ~10 μmol L^{-1}.

While a graph for V_{NO3} vs. NH4 was presented for the entire set of incubations in Parker et al (2012) (Figure 3.6B), no similar plot is available for V_{nh4} vs. NH4. If, however, the V_{NH4} vs. NH4 relationship in Figure 3.6A is more or less relevant for the enclosure experiments, and that relationship was superposed on Figure 3.6B, it appears that $V_{DIN} = V_{NH4}+V_{NO3}$ at NH4 concentrations above 2-3 μmol L^{-1} are comparable to or greater than all but several of the V_{NO3} values at low NH4. A similar observation was made above regarding the Dugdale et al. (2007) findings. This observation does not detract from the main findings of Parker et al. (2012), in particular that substantial increases in V_c accompanied a shift to primarily NO3 uptake by phytoplankton. However it does indicate that the variation in V_{DIN} with NH4 concentration may actually be fairly small and that carefully designed experiments are needed to test P2 of the NH4-inhibition conceptual model. Parker et al. (2012) note that few studies exist showing faster phytoplankton growth on NO3 than NH4. The literature review in Section 2 is consistent with that assessment.

Parker et al (2012a) make two broad comments on the overall potential impact of elevated NH4 in the northern SFE that deserve some discussion. First, "An ammonium based system will likely exhibit a primary production of <20% of that where NO3 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 NH4 (i.e., pesticides or other toxic compounds), there may be comparisons that would be better suited for exploring this issue, and that may lead to a different estimate. Second, a related point: "…enabling NO3 utilization by phytoplankton will increase the rate of carbon uptake (i.e., primary production), and chl-a, whereas contaminant levels of NH4 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 (NH4 toxicity to phytoplankton) is not necessarily well-supported by data from this study (i.e., Figure X; V_{nh4} increases with increasing NH4), conflicts with data reported in Dugdale et al. (2007) (Figure X; V_{nh4} increased with increasing NH4), and is not consistent with the literature (Section X) based on NH4 concentrations in SFE.

Finally, there remains the possibility that experimental artifacts could explain some of the observations in Figure 3.5. Water samples were collected from a relatively low-light conditions to which the phytoplankton were acclimated, and they were in a physiological state that was optimized for growth at those light levels. The incubations were carried out at 50% natural light, which could be 2-3 higher light levels than they experienced in situ. 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 ($\leq$ 24 hr), some portion of the low V_C and low $V_{DIN} = V_{NH4} + V_{NO3}$ could be an experimental design artifact related to phytoplankton populations not yet having fully adjusted to growth at high light levels. Some of the acceleration of V_C and V_{NO3} after 24 hr could conceivably be related to an overall increase in growth due to phytoplankton finally acclimating, as opposed 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 (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 intensies.

3.3.3 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 3.7).

Field and incubation data identified sharp declines in NO3 uptake (V_{NO3}) and C uptake (V_C) downstream of SRWTP, co-occurring with sharp increases in NH4 concentrations. The authors conclude that the high NH4 levels along the Sacramento River and through Suisun Bay prevented phytoplankton from accessing the large NO3 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.

Upsteam of SRWTP, the majority of inorganic nitrogen was present as NO3, with higher concentrations in March (~15 µmol L^{-1}) than April (~2 µmol L^{-1}), likely due to larger contributions from agricultural runoff in March (Figure 3.8). During both months NH4 concentrations increased from low levels (< 1 µmol L^{-1}) upstream of SRWTP to 30-40 µmol L^{-1} immediately downstream of SRWTP. NH4 concentrations decreased by a factor of 2 over the subsequent 50-70 km (travel time ~ 4-6 days), due primarily to nitrification, and, as expected, was accompanied by increases in NO3. NH4 concentrations continued to decrease as water traveled through Suisun and San Pablo Bays, due to further nitrification and from tidal mixing with saltier lower-NH4 waters.

Introduction of treated effluent from SRTWP had a substantial influence on the form of N taken up by phytoplankton. Upstream of the SRWTP, V_{NO3} was relatively high and V_{NH4} was low (Figure 3.9). The relative magnitudes of V_{NO3} and V_{NH4} shifted sharply downstream of SRWTP. V_{NO3} decreased by more than one order of magnitude, and V_{NH4} increased by approximately one order of magnitude. This sharp decline in V_{no3} is consistent with P.1 of the NH4-inhibition conceptual model that at elevated NH4 levels NO3 uptake is inhibited, or NH4 uptake is preferred. In March 2009, $V_{DIN} = V_{NH4}+V_{NO3}$ measured at stations upstream of SRWTP were larger than all $V_{DIN} = V_{NH4}+V_{NO3}$ measurements at riverine stations downstream of SRWTP, and those in Suisun Bay. A similar pattern of sharp increase of V_{NH4} and decrease of V_{NO3} also occurred downstream of SRWTP in April 2009. However, over the first 40 km downstream of SRWTP, $V_{NH4,}$ actually exceeded V_{NO3} upstream of SRTWP. Although there was substantial variation, V_{NH4} 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.

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.C and 3.D). The decreases in V_C, coinciding with high NH4 concentrations and uptake of primarily NH4, are consistent with P.3 of the NH-inhibition conceptual model that the phytoplankton community grows more slowly when primarily utilizing primarily NH4.

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 3.7). In both cases, a substantial portion of the chl-a decrease occurred upstream of

SRWTP (30-50%). Much of the decrease upstream of SRWTP could have resulted from a deepening of the water column (depth ~ 1.5m at I-80 and > 6m near SRWTP) and lower resulting light availability and productivity (and gradual loss of phytoplankton via settling), although other factors cannot be ruled out (e.g., other pollutants). Unlike chl-a, V_c did not exhibit a pronounced decrease upstream of SRWTP (Figure 3.9), consistent with the notion that light limitation may have been a major driver, as opposed to other potential explanations (e.g., a unknown contaminant). It should be noted, though, that C and N uptake data was available for only a limited number of upstream stations (4 in March, 3 in April).

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_{NH4} 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_{NH4}, and V_c is interesting in that light penetration was 2-3 times lower in these areas due to higher turbidity. It seems likely 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 NH4 utilization downstream of SRWTP are indeed consistent with P3 of the NH4-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 10-50k 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 likely accounts for some of the increases in V_c, $V_{DIN} = V_{NH4} + V_{NO3}$, 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 NH4 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 with the NH4-inhibition conceptual model, or open questions, 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_{nh4}, with V_{nh4} downstream of SRWTP exceeding V_{NO3} upstream of SRWTP ($V_{nh4+no3}$ values were comparable). In addition, V_{NH4} measured at the San Pablo station in April was greater than V_{NO3} upstream of SRWTP in April. Furthermore, the highest measured value for V_c in April was observed at the San Pablo Bay site, and the majority of inorganic N uptake was NH4 (80%). The comparable magnitudes in April of V_{NO3} and V_{NH4} upstream and downstream of SRWTP, respectively, are not necessarily supportive of P.1 of the NH4-inhibition conceptual model, that uptake of V_{NO3} is greater than V_{NH4}. Some of these observations may themselves be related to variations in light.

Parker et al. (2012b) also argue that at elevated NH4 levels (e.g., above 20 µmol L^{-1}), NH4 uptake was itself inhibited. Although a statistically significant negative correlation between V_{nh4} and NH4 concentration was found for a subset of samples, the number of data were limited, and more investigation would be needed to confirm this mechanism. Finally, the potential role that light availability played in the observations may require additional quantitative evaluation to rule out this possibility, given that water and phytoplankton traveled through a wide range of light-levels, that travel time along the river was several days from I-80 to the entrance to Suisun Bay (and much longer to San Pablo Bay), and that the response time of phytoplankton communities to adjust their growth rates to increased light availability can be on the time scale of days.

3.3.4 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 crystalline 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 clearly demonstrated that strong inhibition of nitrate NO_3 uptake occurred at NH4 concentrations above 1 µmol L^{-1}. In one set of experiments, V_{nh4} appeared to decrease at higher NH4 levels (50 µmol L^{-1}). In a second experiment, NH4 uptake followed classical Michaelis-Menten uptake kinetics, and no decrease in V_{nh4} was observed in experiments with NH4 concentrations up to 100 µmol L^{-1}. Although inhibition of NO3 uptake was observed, no change in primary production rates (i.e. V_c) were discernible.

In the experiments carried out using treated wastewater effluent, NO3 uptake was suppressed at higher proportions of effluent and higher NH4 concentrations, consistent with the 'clean' experiments. However, in the effluent addition experiments, NH4 uptake rates actually decreased at effluent proportions that yielded NH4 concentrations greater than 8 µmol L^{-1}. Similarly, in contrast to the "clean" additions, there was a discernable decrease in V_c with increasing effluent additions yielding ammonium concentrations greater than 8 µmol 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 µmol 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.).

3.3.5 Field Observations (2010) and criteria for bloom occurrence

[still under construction]

Dugdale et al. (2012) propose three criteria to evaluate when conditions are favorable for phytoplankton blooms in Suisun Bay:

1) *Loading Criterion:* Ammonium loading must not exceed the capacity of the phytoplankton to assimilate the inflow of ammonium.
2) *Concentration Criterion:* the ammonium concentration must be ≤ 4 µmol L^{-1} to enable phytoplankton nitrate uptake.
3) *Washout Criterion*: the dilution rate of phytoplankton biomass set by river flow must not exceed the phytoplankton growth rate to avoid "washout".

3.4 Summary

The NH4 inhibition hypothesis has evolved out of extensive 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 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 uM) 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.

There is strong support in the scientific literature for P.1, with numerous studies demonstrating that multiple species of phytoplankton exhibit either a strong preference for NH4 or that NO3 uptake is actively inhibited by elevated NH4 concentrations. RTC studies also offer convincing support for P1, with NO3 uptake by phytoplankton strongly inhibited when NH4 exceeds 1-4 µmol L^{-1}.

P2 is not currently well-supported by the broader scientific literature (Section 2). Few well-controlled studies have actually investigated N uptake rates during experiments in which both NO3 and NH4 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 NO3 vs. NH4 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 NO3 and NH4 are similar. The RTC studies provide some support for P2 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 NH4 uptake rates may be comparable to or even greater than NO3 uptake rates under certain conditions. In addition, in some cases where evidence from San Francisco Bay studies is either consistent or inconsistent with P2, uncertainty remains about whether experimental artifacts or other reasonable explanations could explain the observations. While P2 remains a plausible hypothesis, additional research is needed to more rigorously establish the NO3 and NH4 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 currently 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 NH4 or NO3 as their nitrogen source. Multiple studies have found similar growth rates (rates of carbon fixation) across a range of taxa when using NH4 or NO3. 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 NO3 or NH4 was provided. As with P2, few studies have done growth experiments in which phytoplankton have the choice between NH4 and NO3, so there also remains a critical gap in the literature on this related topic. RTC field and enclosure experiments provide some strong evidence that primary production rates (using rates of C uptake) are slower at high NH4 levels, and that growth rates increase when NH4 is depleted and phytoplankton begin utilizing NO3 (Parker et al., 2012a, 2012b). In other studies, primary production rates are inferred from changes in chl-a or assumed to be proportional to 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 P2 and P3 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. However, their conclusions about Suisun Bay do not sufficiently address this nuance, or the extent to which the NH4-based explanations can be readily applied in Suisun Bay. Finally, NH4 levels are present at comparable levels in South San Francisco Bay, and examples of NH4 inhibition of primary production rates have not been documented there.

Similar to P2, P3 remains an entirely plausible hypothesis, in particular at the phytoplankton community level under field conditions. Inhibition of primary production rates has been proposed in other NH4-rich estuaries (e.g., Delaware Bay), and in other systems with relatively high sources of NH4 from treated wastewater effluent (ref). 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 and necessary perspective on how processes manifest at the field scale. However, the complexity introduced by field conditions or simulated-field conditions, during which time multiple underlying factors are changing (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 NH4 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 NO3 and NH4 uptake kinetics and phytoplankton growth kinetics under a range of conditions (e.g., varying temperature and light levels, varying proportions of NO3 and NH4), 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.
- If there is a difference between primary production rates, continue studies to determine its ecological significance 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, in order to inform management decisions.
- 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 (e.g., Glibert et al, funded by Delta Science Program: matrix of N and P manipulations and their effect on community composition and growth; Parker et al., funded by Delta Science Program: Field observations and manipulation experiments to explore factors contributing to *Microcystis* blooms and production of microcystin; Kudela et al., submitted to IEP: Monoculture growth experiments using species cultured from Suisun Bay). Any preliminary results from those studies have not been discussed or analyzed for this report; therefore, this assessment should be revisited as that data becomes available.

Independent of whether the set of processes laid out in the NH4-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 clearly acknowledge the roles of light limitation and clam grazing; they point out that NH4 inhibition of primary production may be one additional factor that limits production when conditions might otherwise allow for blooms to occur. However, this important point sometimes gets lost when the NH4-inhibition conceptual model is discussed in the context of its management implications. A quantitative analysis of the ecosystem-scale importance of the NH4-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 NH4 hypothesis, in that they will provide simultaneously provide a tool for quantitatively synthesizing existing nutrient and phytoplankton data in Suisun Bay and other embayments (e.g., Lower South Bay), identifying data and monitoring needs, and informing the broader modeling strategy for the Bay.

Finally, changes in the form of nitrogen available to phytoplankton, e.g., NH4 vs. NO3, and changes in N:P have been hypothesized to be influencing phytoplankton assemblages in Suisun Bay and the Delta (e.g, Wilkerson et al, 2006; Glibert et al., 2011), 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, and warrants its own full investigation. This is beyond the scope of this report, and should be addressed in a subsequent report.

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3.6 Tables and Figures

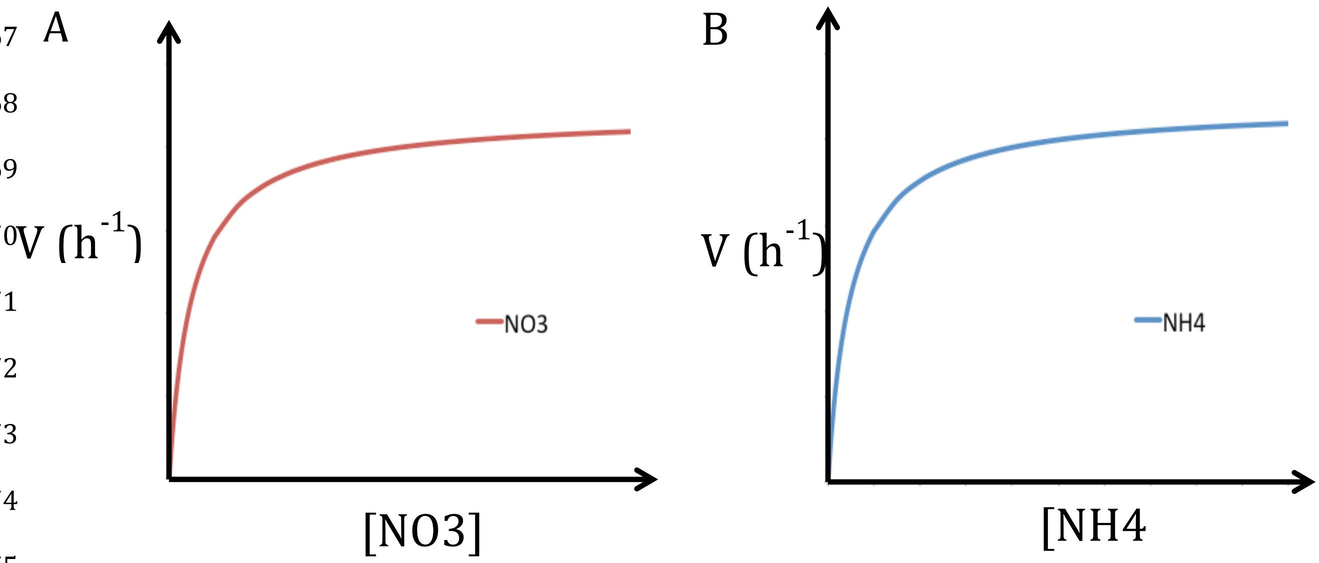

Figure 3.1 Conceptualization of the uptake kinetics of **A.** NO3 and **B.** NH4, under constant light conditions.

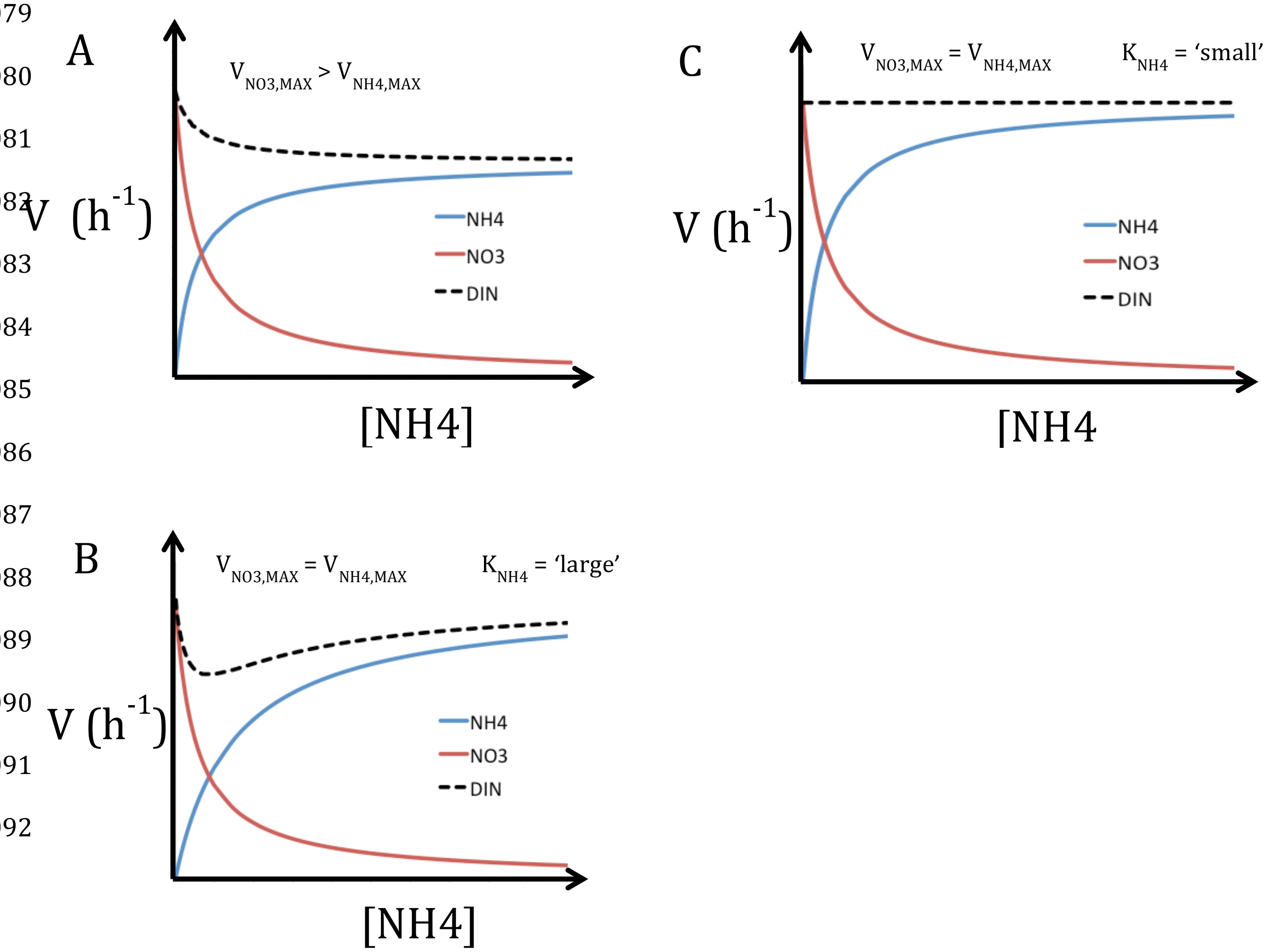

Figure 3.2 Conceptualization of N uptake kinetics in the presence of both NH4 (variable) and NO3 (constant) under three scenarios:

A. $V_{NO3,MAX} > V_{NH4,MAX}$

B. $V_{NO3,MAX} = V_{NH4,MAX}$ but inefficient NH4 uptake at low NH4

C. $V_{NO3,MAX} = V_{NH4,MAX}$ and efficient NH4 uptake at low NH4

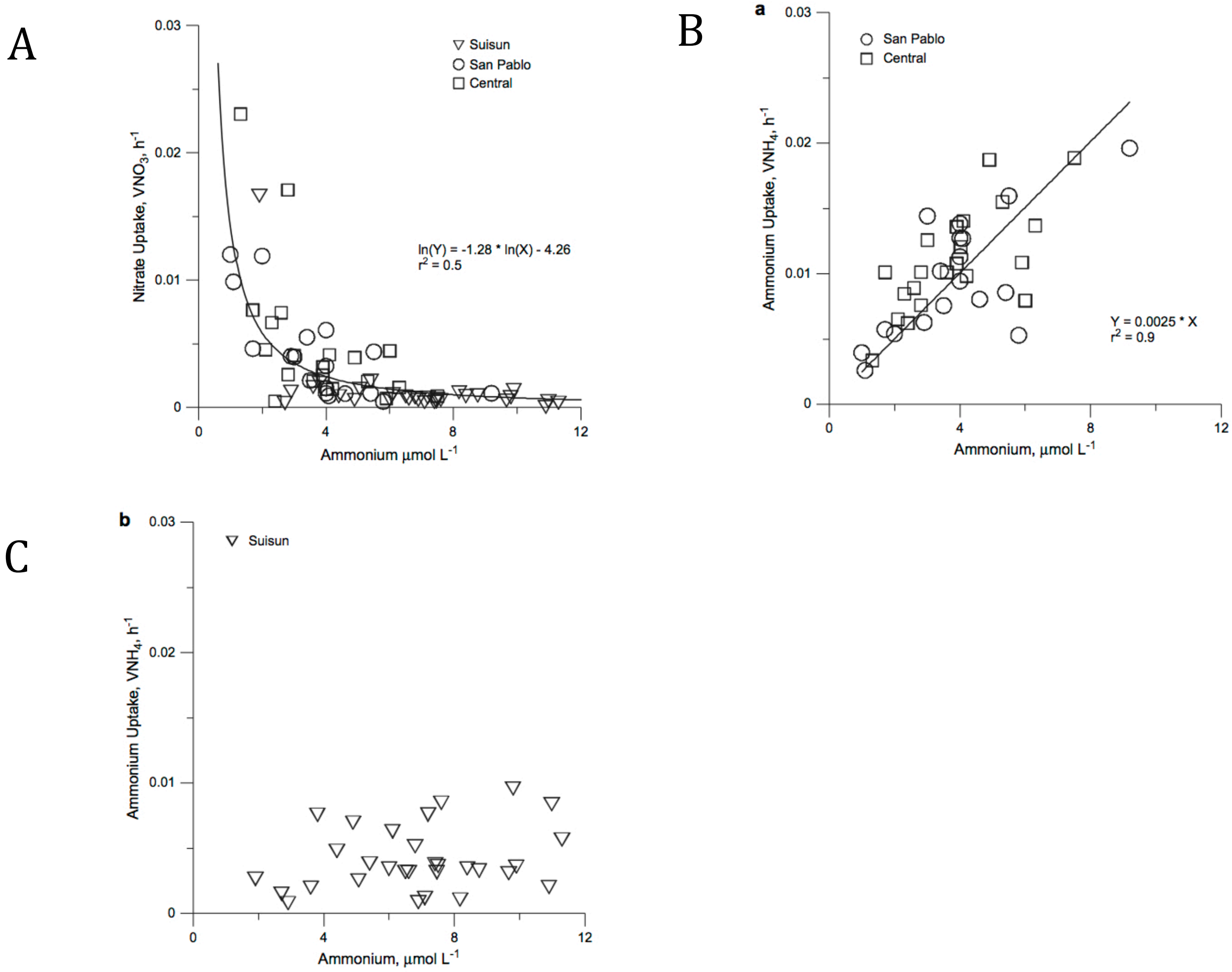

Fig. 4. Trace $V\text{NH}_4$, h^{-1} versus NH_4 concentration, µmol L^{-1} for the spring bloom periods in (a) San Pablo and Central Bays, (b) Suisun Bay.

Figure 3.3 From Dugdale et al. (2007) **A**. V_{NO3} versus NH4 concentration in Suisun, San Pablo, and Central Bays. **B**. V_{NH4} vs. NH4 in San Pablo and Central Bays. **C**. V_{NH4} vs. NH4 in Suisun Bay.

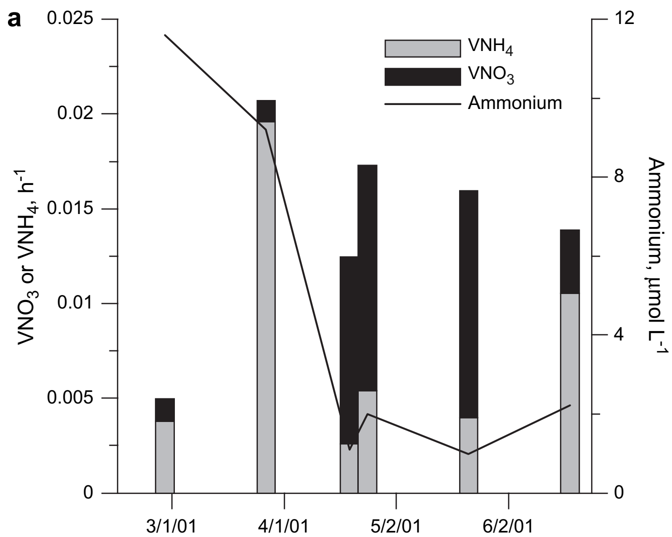

Figure 3.4 From Dugdale et al. 2007. Time series of uptake rates and NH4 concentrations in San Pablo Bay (field investigation). Chl-a concentrations (not shown here) increased steadily from 2 mg/L at end of February to 12 mg/L toward the end of April. Chl-a levels decreased linearly to 1 mg/L by late June. (chl data not shown)

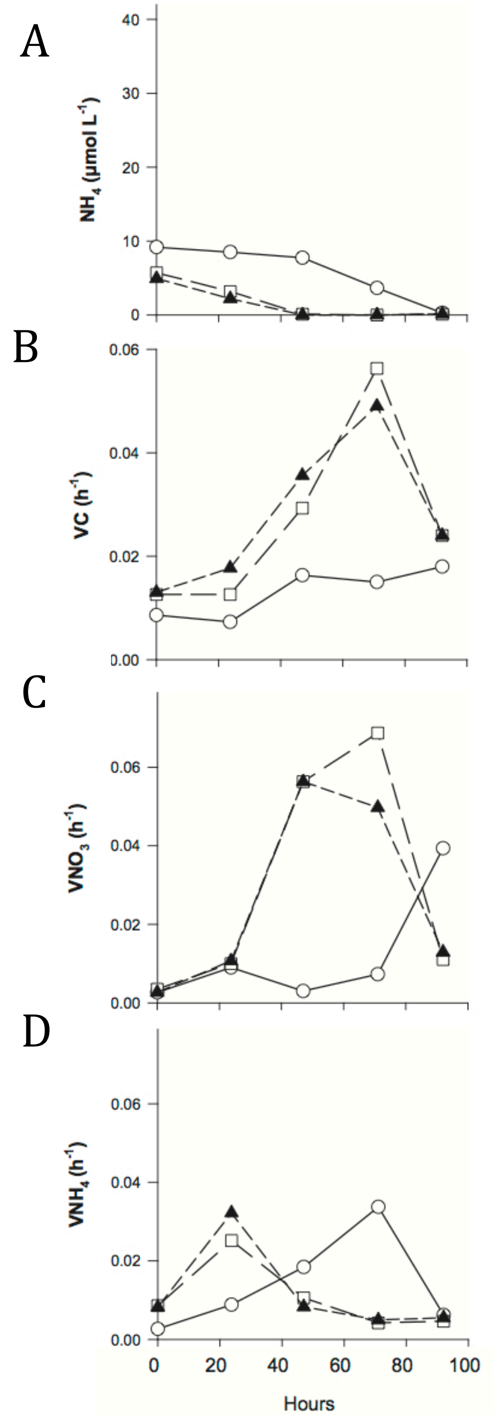

Figure 3.5 From Parker et al. 2012a. Time series of NH4 and NO3 concentrations and specific uptake during March enclosure experiments conducted in Suisun Bay (open circles), San Pablo Bay (open squares) and Central Bay (closed triangles).**A.** NH4 **B,** specific C uptake, VC, **C,** specific NO3 uptake, VNO3, **D.** specific NH4 uptake rate, VNH4.

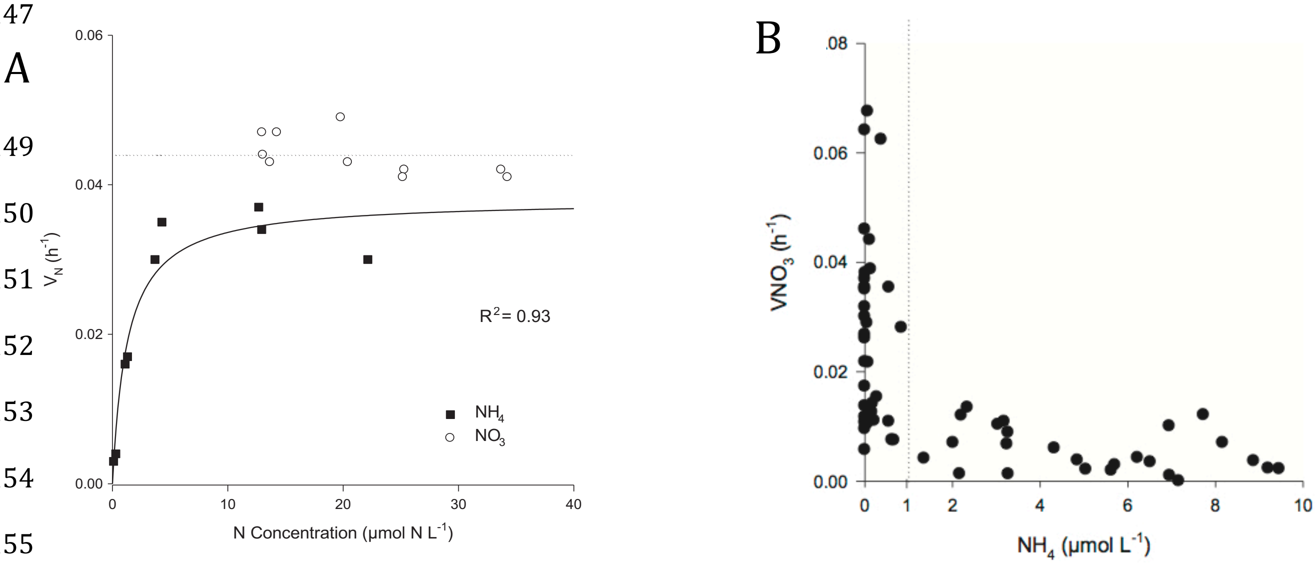

Figure 3.6 A. Michaelis-Menten kinetic curves for NO3 (open circles) and NH4 (closed squares) in central San Francisco Bay in April 2005. Data for VNH4 vs. [NH4] were fit to a hyperbolic function. Dotted line is average VNO3. **B**. Biomass-specific NO3 uptake versus NH4 concentration. Results from enclosure experiments conducted in March, July and September (n =120).

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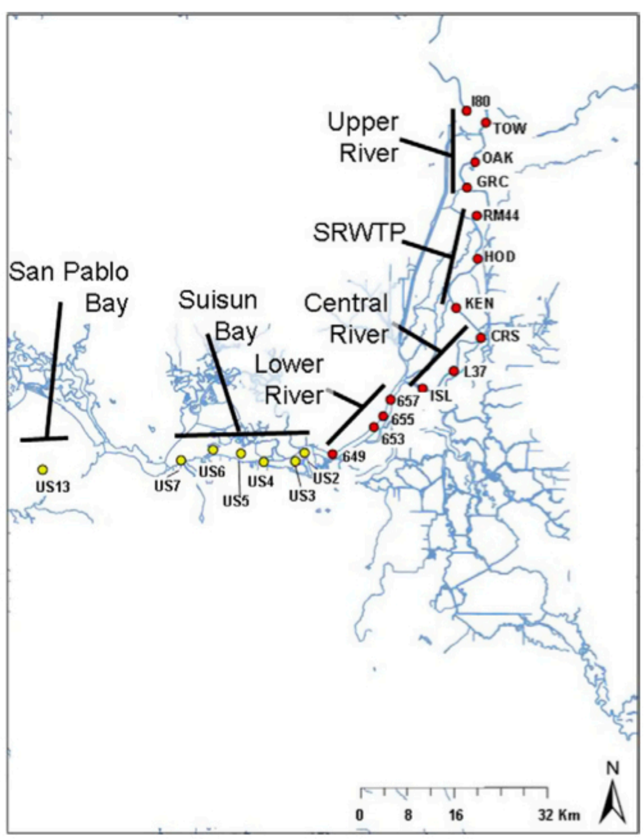

Fig. 1. Study region of the Sacramento River and San Francisco Estuary, CA showing sampling stations and river and Northern estuary transect regions.

Figure 3.7 From Parker et al. 2012b

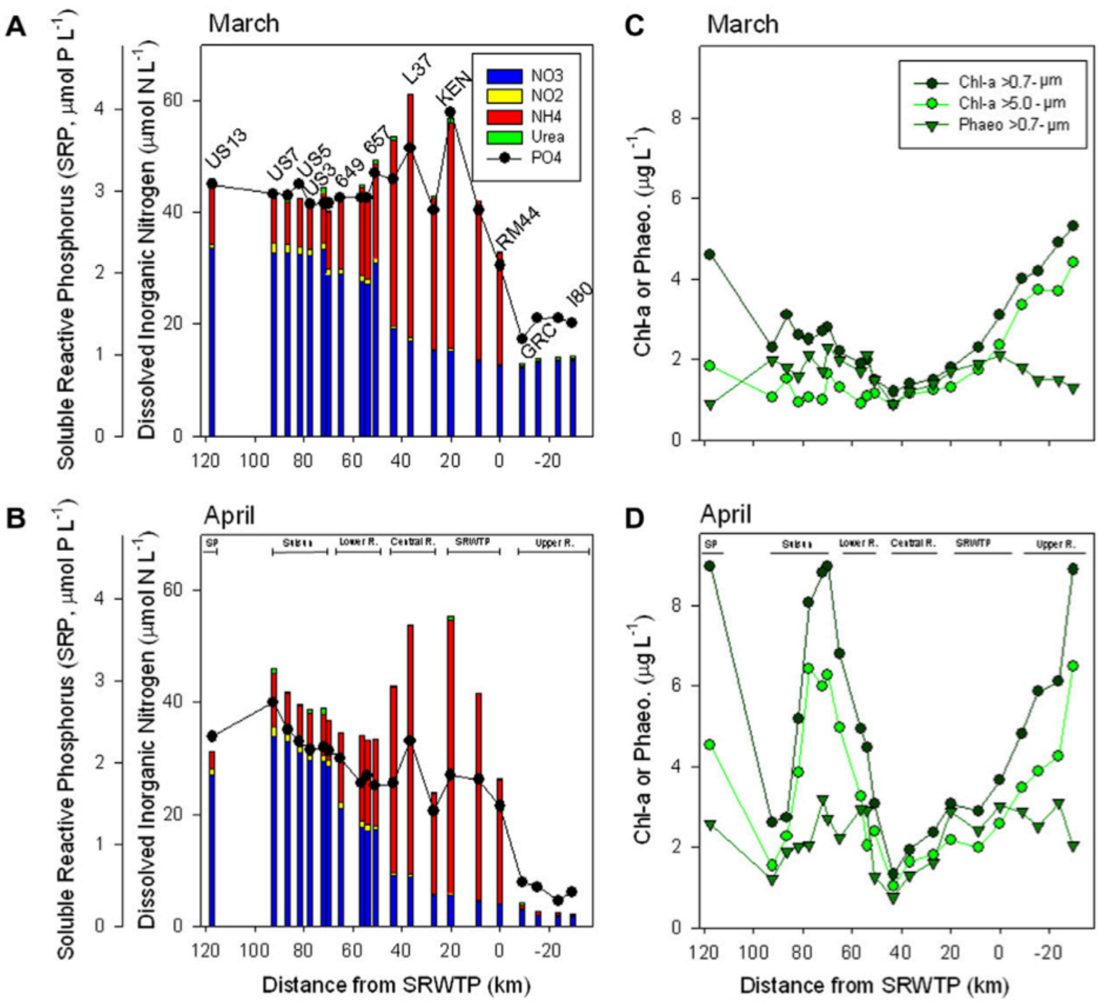

Fig. 4. Inorganic nutrient concentrations measured in the Sacramento River and Northern SFE in (A) March and (B) April 2009 (NO_3; blue, NO_2; yellow, NH_4; red, urea–N; green, SRP; black). Concentrations of chlorophyll-a in cells >0.7-μm diameter (closed circle) and >5.0-μm (open circles) and phaeophytin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

Figure 3.8 From Parker et al. 2012b

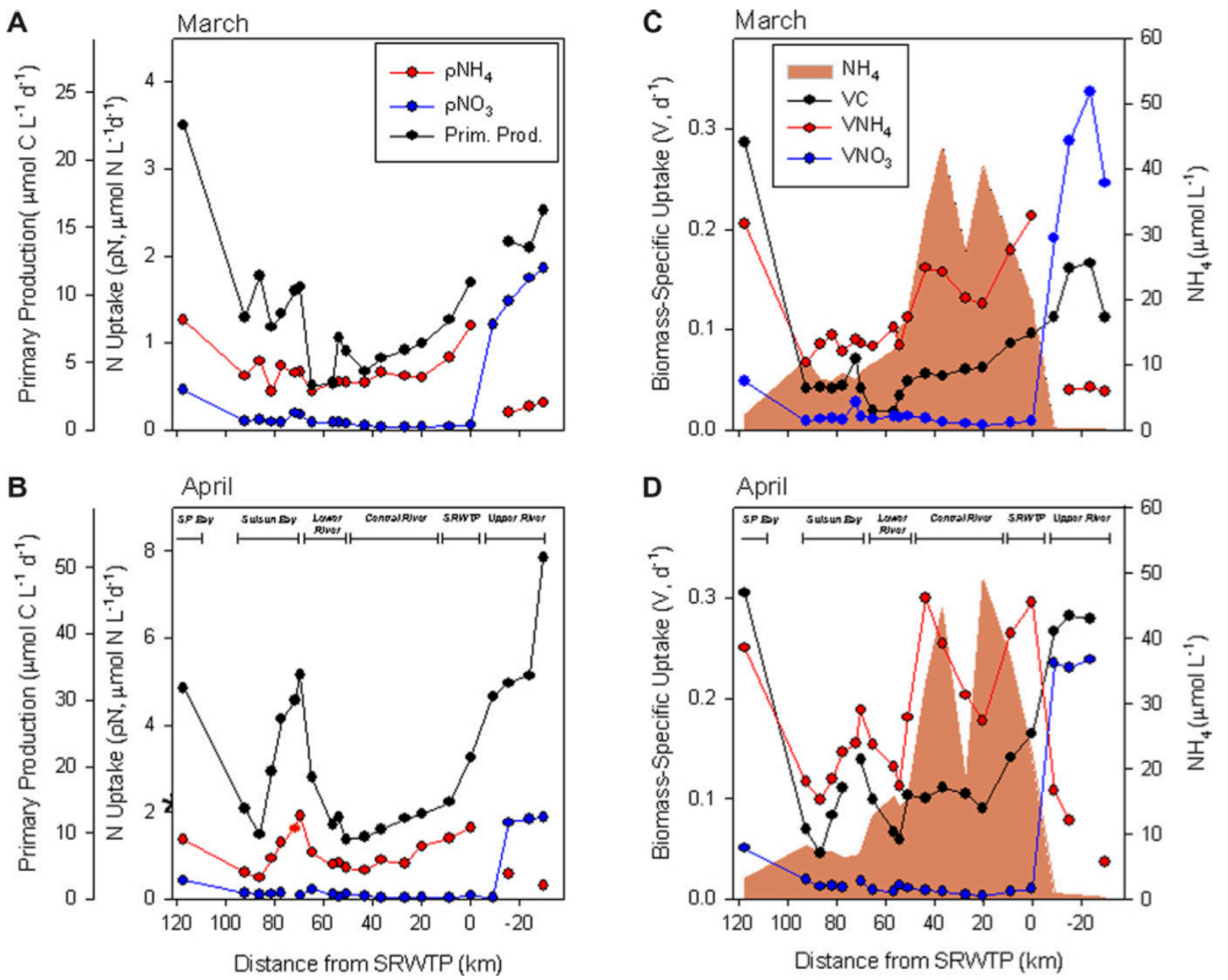

Fig. 5. Primary production and phytoplankton nitrogen uptake in the Sacramento River and Northern SFE during (A) March and (B) April 2009. Biomass-specific carbon uptake and phytoplankton nitrogen uptake and NH_4 concentrations (shaded area) during (C) March and (D) April 2009. *Y*-axes for phytoplankton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

Figure 3.9 From Parker et al. 2012b

4. Evidence for toxicity of ammonium to copepods and other aquatic species

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[this may expand in the next draft]

4.1 Introduction

Changes in quality and availability of food for pelagic fishes has been identified as one potential factor that could be contributing to the recent Pelagic Organism Decline (POD) in the Delta and Suisun Bay. According to Baxter et. al. (2010), overall zooplankton abundance and size has decreased over the last four decades, which could be exerting bottom-up pressure on the food web since is zooplankton are the primary prey for Delta smelt and other pelagic fishes. Grazing, low prey abundance and direct toxicity of contaminants are hypothesized to be acting in concert to keep zooplankton populations low.. In this section, we briefly summarize the results of several studies of the toxic effects of ammonia or ammonium on copepods, and explore the mechanism of toxicity. A comparison of these thresholds against ambient concentrations in Suisun Bay can be found in Section 6.

4.2 NH4+ toxicity on Pseudodiaptomus forbesi

In a 2011 study funded by the Central Valley Regional Water Quality Control Board, Teh et. al. (2011) studied the acute and chronic effects of ammonia on *Pseudodiaptomus forbesi,* and the results are summarized in Table 1. This species is of particular interest because according to preliminary studies by the CA Department of Fish and Game (CDFG) that examined gut contents of larval fish, during most times of the year *P. forbesi* is the dominant food source for all fish that have shown declining populations (delta smelt, longfin smelt, striped bass and threadfin shad), and changes in *P. forbesi* abundance is likely to have significant effects on the food web.

Teh et al. (2011) found that at a fixed concentration of total ammonia nitrogen (TAN; TAN=NH3 + NH4+) of 5ppm, survival of *P. forbesi* decreased to 30% as pH increased to 8.6. This was likely due to increasing fraction of TAN that is present as NH3, the form of TAN that is known to be toxic to fish and other aquatic species. NH3 is usually expelled from the cell by passive diffusion, and increasing concentrations of NH3 outside the cell reduce this efflux and cause toxic levels of NH3 to accumulate in the cell (cite). These results agree with previous studies that have found decreasing LC50 for NH3 as pH increases (cite). However, at a typical estuarine pH, the ionized form NH4+ will dominate and the above NH3 toxicity mechanism will be less important. Along these lines, Teh et al (2011) noted decreased survival of *P. forbesi* at low pH where nearly all TAN will was present as NH4+. When pH was fixed at 7.8, survival decreased to 36% as TAN increased to 8 ppm, and survival was nearly 0% when the pH dropped to 7.4 with the same TAN concentration.

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 NH4 at levels as low as 0.36 mg N L^{-1} (26 µmol L^{-1}). The lowest dose in the study was 26 µmol L^{-1}, and was the lowest observed effect level; a no observed effect level was not established.

4.3 Additional studies on ammonium toxicity

A further search of the literature revealed that there are few studies of direct ammonium toxicity to copepods. However, there are multiple studies of NH4+ toxicity to other aquatic invertebrates, such as arthropods and crustaceans. Several studies studies have documented that at certain pH values, total ammonia is a better predictor than NH3 of acute toxicity, and have suggested that joint toxicity may be exerted by both NH3 and NH4+ on a variety of aquatic invertebrates (Armstrong 1978, Erickson 1985, Borgmann 1993, .Kater 2006). Additional studies have demonstrated that toxicity of NH4+ is reduced as cation concentrations increase in the test water (Borgmann 1993, Ankley 1995, Borgmann 1996). It is worth noting that these studies reported acute effects, not chronic effects, and even the lowest reported LC50 (approximately 100 µM for *Hyalella azteca*, Borgmann 1996) is still more than an order of magnitude above ambient ammonium concentrations typical 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 specifiy pH, so the partitioning of TAN between ionized and unionized forms is unknown (Buttoni 1994). Buttoni observed an LC_{50} 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, this study did not specify pH.

4.4 Mechanism of NH4+ toxicity

The exact process of NH4+ toxicity to copepods has not been well studied, but there have been some efforts to characterize this mechanism in other crustaceans. Armstrong et al (1978) proposed a conceptual model suggesting that NH4+ may interfere with 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 NH4+ (which can easily substitute for K+) are transported out of the cell. This cycle not only brings an important nutrient (Na+) into the cell, but also expels waste nitrogen. In crustaceans, more than half of waste nitrogen is expelled as NH4+ (Regnault 1986). However, accumulation of NH4+ on the exterior of the cell is hypothesized to inhibit export of NH4+ from the cell (Teh et al. 2011). This model has also been adopted to explain observed effects in crustacean species, such 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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4.6 Figures and Tables

Study Goal	Results	Notes
6-day LC on juvenile *P. forbesi* 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 *P. forbesi* 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	*P. forbesi* are more sensitive at lower pH
4-day LC on nauplii (larval) *P. forbesi* 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 *P. forbesi* are more sensitive than juvenile *P. forbesi*
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	

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

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 *Potamocorbula 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 atrributing 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 *P. 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 A). 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.

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. 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 *Limnoithona sinensis* in freshwater in 1979 and *L. tetraspina* in brackish water in 1993 (Fig. 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). 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 *Potamocorbula amurensis* (see life history discussion below).

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-πόδί, "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. 2A and 2C) and at the station defined by salinity closest to 3.5 (Fig. 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. 2B).

Figure 1 is plotted by salinity range because zooplankton generally are arranged in the estuary according to salinity (Fig. 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 ~16ºC (L. Sullivan[*]).

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 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 >>1 inertial forces prevail and organisms move by accelerating water to overcome drag that becomes increasingly turbulent as Re becomes larger. At Re <<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 "intertial 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).

<u>Retention mechanisms</u> 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 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

[*] Names in parentheses refer to unpublished data collected by these researchers in my laboratory.

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).

<u>Feeding</u> 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 dielly, 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 *Potamocorbula 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. 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 B) 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. 1 and 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 dominant in the low-salinity regions of most north temperate estuaries, including those in Europe, North America, and Asia, although it can also invade freshwater (Lee 1999). The species in the SFE is most closely related to those from eastern North America, implying that they were introduced, probably along with striped bass in 1879. Before this introduction there was probably another member of this complex in the SFE. *E. affinis* was once the most abundant copepod in the LSZ year-round (Figs. 1, 3) but since the arrival of *Potamocorbula* it has been nearly absent in summer. It was a key prey species for delta smelt and young striped bass.

Pseudodiaptomus forbesi has largely supplanted *E. affinis* as key prey for delta smelt and other fishes by virtue of its moderately high abundance in summer in the LSZ (Fig. 1). However, its population center is in freshwater (Fig. 3), so it occurs in the LSZ through advection and dispersion. This implies that advection due to river flow may be important in resupplying the LSZ with these copepods.

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.).

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 15ºC, and development time decreases about 2-fold for every ~6ºC 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 at15ºC and 8 days at 22ºC. Food-replete development times at 15ºC 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 the 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 ~10% d^{-1}, by which their populations can double in only a week (Fig. 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.

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 *Potamocorbula amurensis* (Kimmerer et al. 1994). The high proportion of young stages of *P. forbesi* in freshwater (Fig. 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.

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. 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. 6).

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 μM, 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. 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.

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 W.6). 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 W.6), 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 occured 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 *P. 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:

<u>Effects of nutrients</u> 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.

<u>Effects of freshwater clams</u> 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.

<u>Importance of peripheral habitats to the foodweb</u> 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.

<u>Effects of restored marsh</u> 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.

<u>Monitoring needs</u> 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.

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5.11 Figures and Tables

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 subsmpled; averaged over depths	Subsampled
Taxonomic details			*Acartia* spp. not distinguished	*Acartia hudsonica* as *A. clausi*	*Acartia* spp. distinguished in 109 samples
Data avail.	No	No	Yes	Yes	Yes

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

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

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	Repro-duction	Feeding	Food for fish	Habitat/Notes	References
Acartia 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)
Eurytemora affinis	Sac	Filter-feeder; general omnivore	LSZ species (formerly abundant)	Species complex; formerly abundant ln LSZ all year; now confined to winter-early spring	Lee 2000
Pseudodiaptomus forbesi	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
P. marinus	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
Sinocalanus doerrii	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,
Tortanus dextrilobatus	Broadcast	Ambush predator	Marine to brackish	Feeds on other copepods	Orsi and Ohtsuka 1999, Hooff and Bollens 2004
Acartiella sinensis	Broadcast	Cruising predator	LSZ	Feeds on other copepods, e.g., *L. tetraspina*	Orsi and Ohtsuka 1999, York et al. in revision
Oithona davisae	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
Limnoithona tetraspina	Sac	Ambush predator on microzooplankton	LSZ; small size limits availability	Slow growth rate, chronically food-limited	Ferrari and Orsi 1984, Gould and Kimmerer 2010
Limnoithona sinensis	Sac	Infer from *L. tetraspina*	Freshwater; small size limits availability	No ecological studies	Ferrari and Orsi 1984
Acanthocyclops vernalis	Sac	Ambush predator	Freshwater and LSZ	Several other related cyclopids present, *A. vernalis* most abundant	Li and Li 1979
Harpacticoids	Sac		Various	Mostly juveniles of benthic species. Several pelagic species in more saline waters.	

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

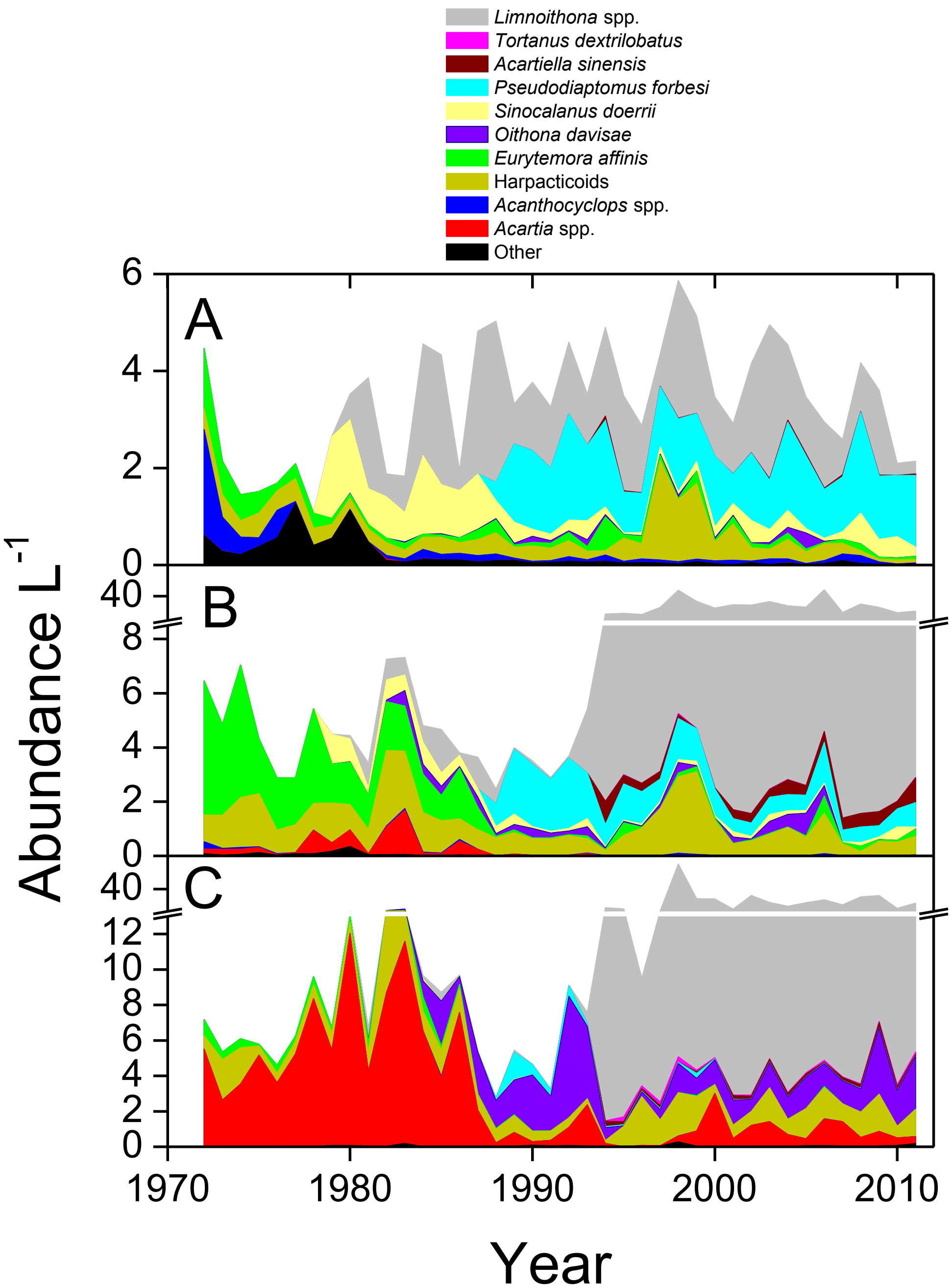

Figure 5.1 Cumulative abundance of adult copepods in three salinity ranges: A, <0.5, B, Low-Salinity Zone at 0.5 - 6, and C, >6. Immature stages have been excluded because nauplii have not been consistently identified to species, and copepodites only in some years. Copepod species are ordered vertically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

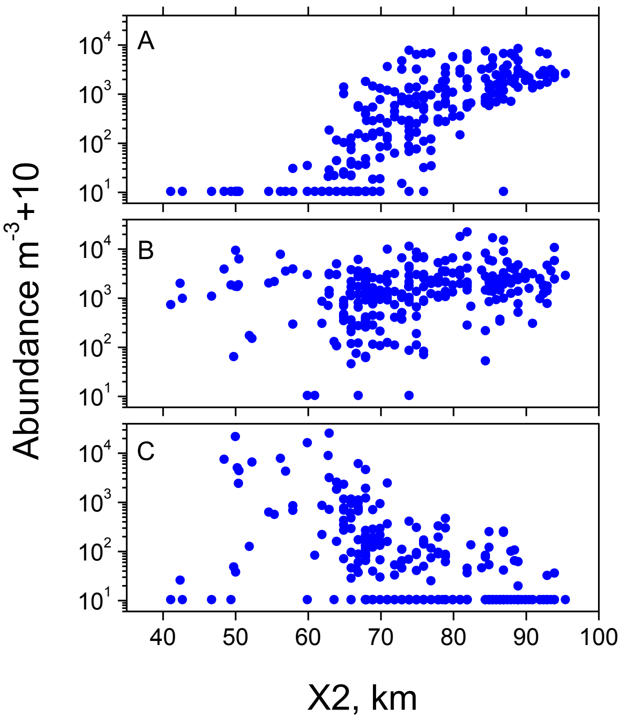

Figure 5.2 Abundance of *Eurytemora affinis* during 1972-1986 vs. X2. Data from: A, station NZ062 on the lower Sacramento River; B, the station in each survey with salinity closest to 3.5, the approximate salinity where the abundance of E. affinis was highest; C, station NZ020 in western Suisun Bay.

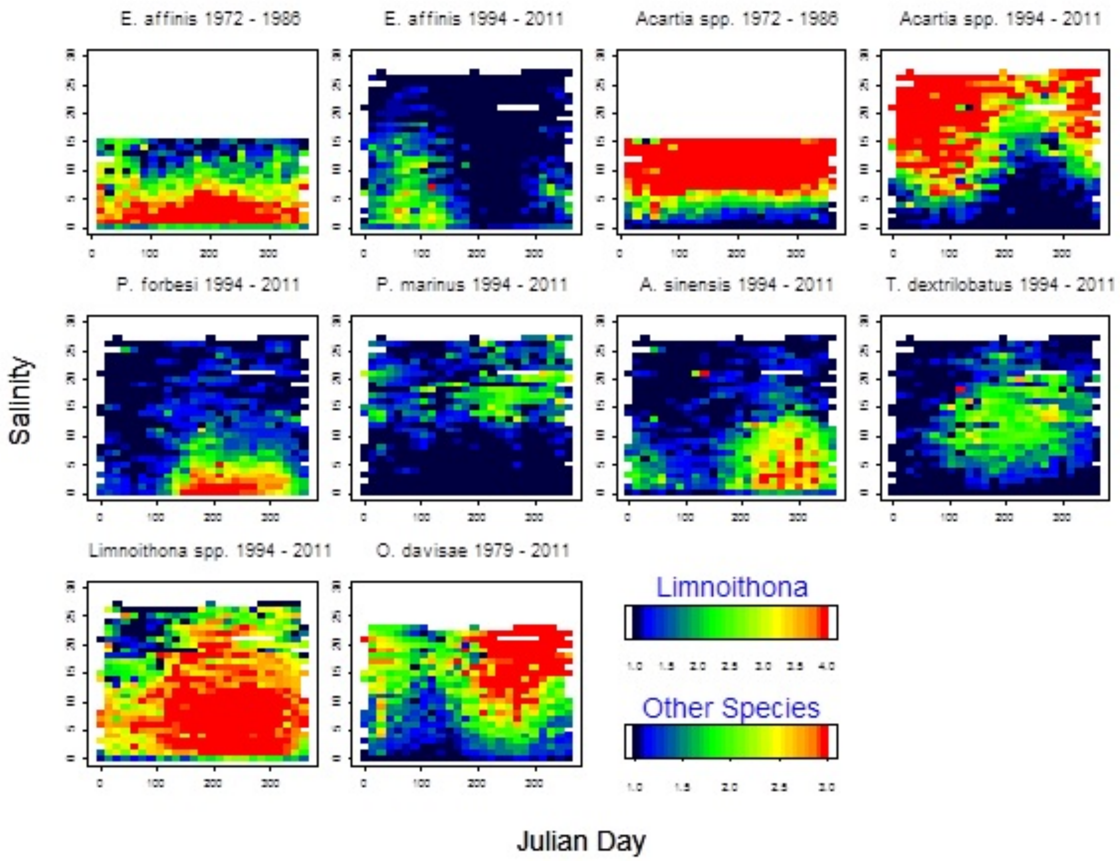

Figure 5.3 Image plots of $\log_{10}$ (abundance +10) of common zooplankton species by Julian day and salinity. Note that the lowest salinity band (0-1) includes large areas of freshwater containing few copepods. The highest salinity bands are cut off for early years because those salinities were not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

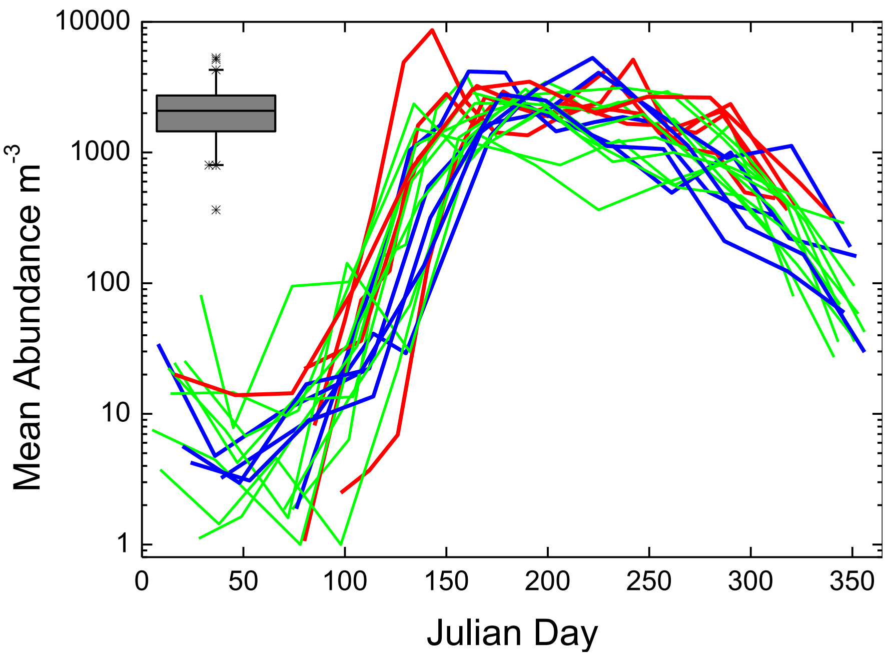

Figure 5.4 *Pseudodiaptomus forbesi* Mean abundance by day of the year for all samples in freshwater. Each line represents a single year from 1989 to 2008. Colors indicate wet (blue, 1993, 1995, 1998, 2005, 2006) and dry (red, 1990-1992, 1994, 2008) years and those between (green).

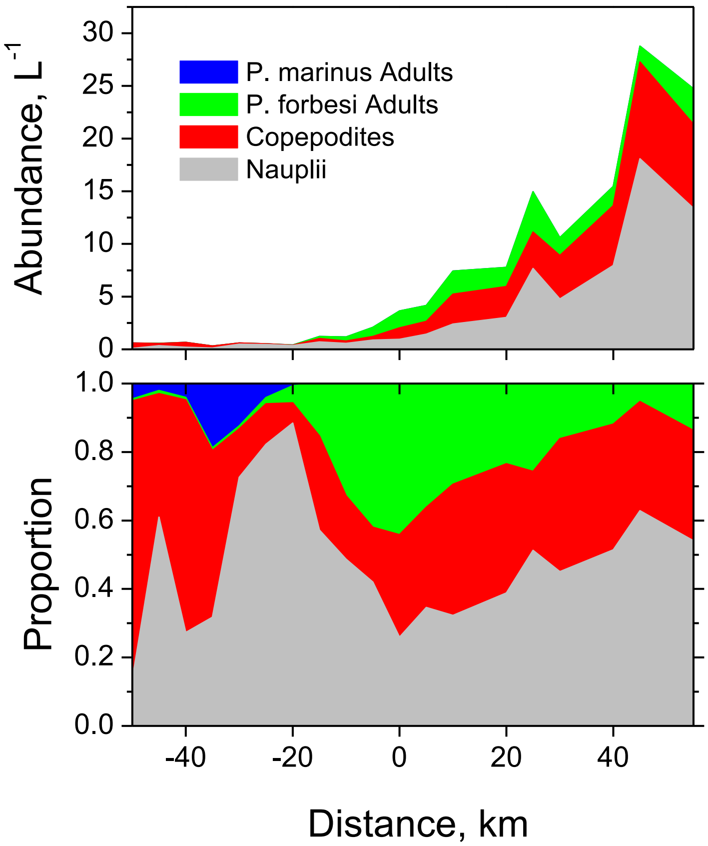

Figure 5.5 Distributions of gross life stages of *Pseudodiaptomus* species by distance from the 2 psu isohaline. A distance of 0 is essentially X2. Positive distances are based on station locations, and negative ones on salinity corrected to distance by the mean relationship of salinity to distance from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

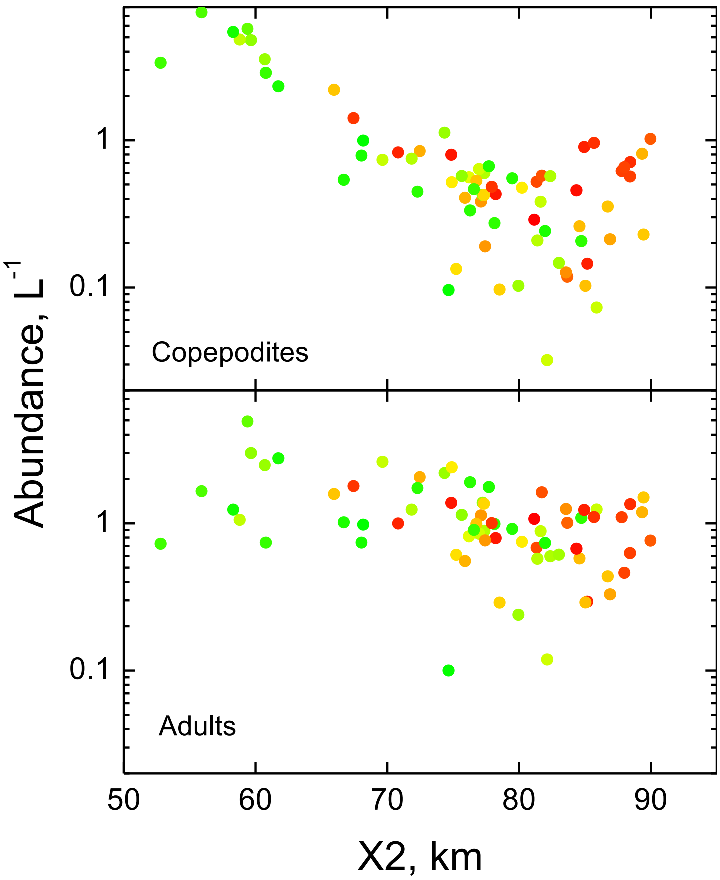

Figure 5.6 *Pseudodiaptomus forbesi*. Abundance in the LSZ for copepodites (including a small fraction of *P. marinus*) and adults as a function of X2.

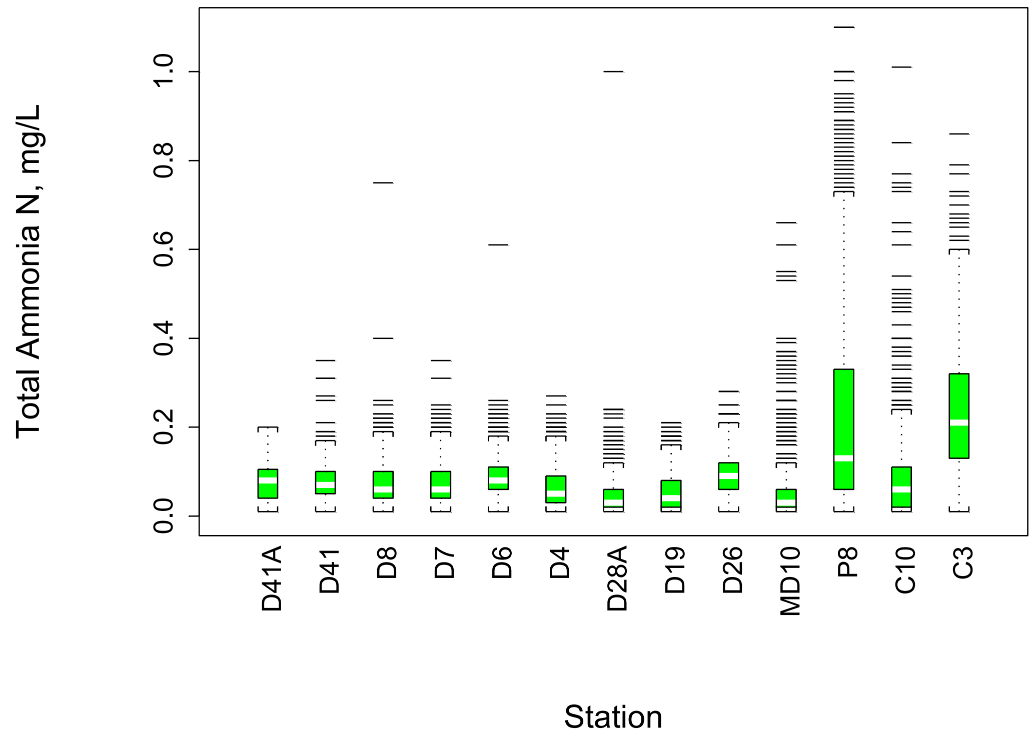

Figure 5.7 Total ammonia nitrogen in stations visited frequently in the IEP Environmental Monitoring Program. The stations have been ordered roughly from the San Pablo Bay to the eastern delta (http://www.water.ca.gov/bdma/images/Metadata-DiscreteWQ_stations.jpg). Station C3 is at Hood below the Sacramento 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).

6. Synthesis of ambient water quality 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 sudden decline in native and non-native pelagic fish species in the northern San Francisco Estuary, including Suisun Bay and the Delta (Baxter et al., 2010). Factors considered in the IEP conceptual model include changes in flow regime, physical alternations to habitat, land use changes, invasive clams and copepods, and contaminants, including nutrients. Recent studies have suggested that increases in anthropogenic nutrient loads over the past few decades, in particular ammonium (NH4) may be exerting adverse pressure in multiple ways. Elevated NH4 concentrations are hypothesized to be inhibiting primary productivity in Suisun Bay, San Pablo Bay, and along the Sacramento River (Section 3; Dugdale et al., 2007; Parker et al., 2012), and indirectly contributing to POD through decreased food supply. Higher NH4 levels may also be contributing to the increased frequency of *Microcystis* blooms in the Delta (Lehman et al., 2008). *Microcystis* are poor quality food for primary consumers, and also release the toxin microcystin. Changes in nutrient ratios and forms of N have been hypothesized to be exerting additional bottom-up pressures on Delta and Suisun food webs (e.g., Glibert et al., 2011). Finally, NH4 may have chronic toxicity effects on an important copepod species (*Pseudodiaptomus forbesi*) at concentrations that are observed in some areas of the Delta and the Sacramento River (Section 4 and 5; Teh et al., 2011).

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. Among the numerous science priorities, a better understanding of NH4 concentrations, sources, fate, and long-term trends in Suisun Bay is necessary in order to inform important, near-term, and potentially costly management decisions to regulate nutrient loads. The goals of Section 6 are to:

- Synthesize existing data on ambient NH4 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 NH4 sources, and assess their relative importance
- Explore the underlying causes of spatial, seasonal, or temporal variations in NH4 concentrations
- Explore how ambient NH4 concentrations compare with various thresholds or guidance levels that studies have suggested may impair beneficial uses.

To do this, we compiled and analyzed data from USGS and DWR/IEP long-term monitoring programs over the period 1975-2011, and recent studies that collected samples at higher spatial and temporal resolution. NH4 data was compared with thresholds relevant to hypothesized NH4-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 NH4 and NO3 concentrations within Suisun Bay. While other aspects of nutrient cycling in Suisun Bay – e.g., changes in loads and concentrations of nutrients in their various forms, changes in NH4:NO3 and N:P and their potential food web effects – also ultimately need detailed analysis, this section focuses primarily on NH4. Nitrate and chl-a data are also presented, but are not the focus.

6.2 Methods:

6.2.1 Ambient water quality data

Nutrient concentration data were obtained from multiple sources (Table 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)[2] and U.S. Geological Survey (USGS)[3]. 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 also carries out a monthly sampling campaign along a transect through Suisun Bay to Rio Vista. The USGS collects discrete water samples for nutrients at only 3 stations in this region, and sampling for nutrients was sporadic prior to 2006. Time series of available NH4, NO3 and PO4 data from all DWR/IEP and USGS stations in Suisun Bay are shown in Figs A.6.1.1 – A.6.1.3. Available chlorophyll-a data, which were generally more abundant than nutrient data, are shown in Fig. A.6.1.4. Stations D6, D7 and D8 had the most complete long-term records available data record (Figs. 6.1 and 6.2).

In addition to long-term sampling programs mentioned above, special studies provide additional data at different spatial or temporal resolution. Studies conducted by San Francisco State University's Romberg Tiburon Center (RTC) collected nutrient and chlorophyll data for 7-9 locations within Suisun Bay on a near-weekly basis for April-September 2010 and April-July 2011. Location of all DWR/IEP, USGS and RTC sampling sites is shown in Fig. 6.3.

While DWR/IEP, USGS and RTC (and also wastewater dischargers mentioned below) measurements are truly quantifying total ammonia, and not just the ionized form NH4, at pH values typical of Suisun Bay (average of 7.7 between 1975-2011), more than 95% of total ammonia will be NH4. For this report, total ammonia will be simply referred to as the dominant form, NH4.

[2] http://www.water.ca.gov/bdma/meta/Discrete/data.cfm

[3] http://sfbay.wr.usgs.gov/access/wqdata/

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 that Jassby and Cloern (2000) used 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 to 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 NH4 and NO3 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 limited uncertainty to the overall load magnitude, and estimates are reasonably well constrained for 1975-1995 and for 2006-2011. Further details of this method are given in Appendix 6.2, which also includes a discussion of uncertainties associated with this approach.

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 Costa County Sanitation District (CCCSD), Delta Diablo Sanitation District (DDSD) and Fairfield-Suisun Sewer District (FSSD). The period of record and frequency of data samples varied by discharger. In general, flow data was ample, while nutrient data was generally available on a less frequent basis. Most dischargers measured NH4 concentration in effluent on a monthly basis; CCCSD was the exception in that they measured NH4 on a daily basis for the past few 20 years. When flow data was available, but nutrient concentration data was not, we used best estimates for NH4 and NO3 concentration from the literature in order to estimate nutrient load. For plants that do not nitrify, NH4 was assumed to be 25 mg L^{-1} and NO3 was assumed to be 1 mg L^{-1}. For plants that do nitrify, NH4 was assumed <1 mg L^{-1} and NO3 was assumed to be 23 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 undertook trial periods of nitrification (1977-1982, 1987-1988), during which time NH4 and NO3 loads differed from the majority of loads over the period of record. Time series of NH4 and NO3 loads including these periods are presented in Figs. A.6.2.1.5 and A.6.2.1.6, and data included below focus on 1990-2011.

Currently there exists limited data or model results on stormwater flows and nutrient loads in the Bay Area. In the absence of existing estimates for stormwater loads to Suisun Bay, we aimed to obtain order of magnitude monthly load estimates that could be compared with other sources. Approximate 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 for these watersheds were computed using ArcGIS (Fig. A.6.3.1). Using this information and 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 Beneicia 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 small compared to Delta and POTW loads that were considered. Therefore, their omission is not expected to substantially influence total estimates. The magnitude of internal nutrient loads from sediment flux is currently poorly constrained, and order of magnitude estimates are discussed in Section 6.4.2.

6.2.3 Data Analysis

We evaluated seasonal, temporal and spatial variations of ambient nutrient concentrations in Suisun Bay, with a primary focus on NH4, but also including NO3 and chl-a. We focused on DWR/IEP stations D6, D7 and D8 because of both the completeness of their data record and their distribution throughout Suisun Bay, which were used to investigate spatial variation in nutrient trends. 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, we averaged available nutrient concentration data by month. Organizing the observations by monthly averages allowed for seasonal variations within a given era to be readily visualized, and how these seasonal trends evolved from one era to the next. 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, and the median slope of these lines is the Theil slope (Jassby 2002). Statistical significance of these trends was evaluated based on the Kendall tau test, where any trend with $p < 0.05$ is considered significant. Additionally, by comparing nutrient concentrations between stations, we assessed whether there were local variations in nutrient concentrations. Because DWR/IEP data sites were only sampled monthly, some events in Suisun Bay may have been missed (i.e. short-lived algal blooms, abrupt fluctuations in nutrient concentrations). Therefore we supplemented our analysis with the near-weekly RTC data in Spring 2010 and 2011. Not only does this dataset have greater temporal resolution, but more sites were sampled at one time as well, which may provide a more comprehensive snapshot of ambient conditions in Suisun Bay. Lastly, we compared observed NH4 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). Frequency and duration of episodes of concentrations near these thresholds would have important implications for management discussions on the ability of nutrients to exert bottom-up effects on pelagic organism populations.

Seasonal and temporal variations in nutrient loads into Suisun Bay were also assessed. Loads were analyzed in a similar manner as nutrient concentration data, utilizing long-term 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 are different than those for ambient nutrient concentration analysis, but result in part from the changes in data availability at 1995 and 2006. We maintained these same eras for the analysis of loads from wastewater dischargers as much as possible, but were limited by the period of operation for some of the plants. For example, DDSD data was not reported prior to 1991, and FSSD data prior to 2004 was not available.

To characterize the fate of NH4 within Suisun Bay and factors influencing seasonal variations in NH4 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 water quality monitoring of nutrient concentrations 1975-2011

Analysis of long-term trends in NH4 concentrations focused primarily on DWR/IEP stations D6, D7 and D8 because of the continuous record of data from 1975-2011 (Figs 6.1 and 6.2, Table 6.2). Although the emphasis of this report is on NH4, long-term data for NO3 and chlorophyll-*a* are also included here.

As evident in time series plots at D6, D7, and D8 (Fig. 6.1), NH4 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. 6.4, where monthly-average NH4 concentrations at each station are presented for three eras. Over the period of 1975-2011, NH4 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 NH4 between low-flow (May-October) and high-flow months (November - April) was consistently observed at D6, D7, and D8. NH4 concentrations tended to be 25-75% higher at D6 than at both D7 and D8 during multiple months (Fig. A.6.1.9).

While, in general, NO3 concentrations increased in similar proportions as NH4 between 1975-2011, seasonal NO3 patterns differed considerably from seasonal NH4 patterns (Fig. 6.5). NO3 concentrations increased between 1975-2011 at all stations in nearly all months, with statistically significant increases in Oct-Dec and in at least one summer month at all stations. Although there was a substantial overall NO3 increase over the entire period of record (Fig. 6.5, Fig. A.6.1.2), comparing values in 1987-1997 against those from 1998-2011 suggest that NO3 levels may actually be declining in certain months (Fig. 6.5), in particular over the last ~5 years (Fig. A.6.1.2). Seasonal variations in NO3 concentrations were quite pronounced from 1975-1986, with concentrations varying by a factor of 2-3 between summer and winter (Fig. 6.5). However, in the latter two eras, seasonal variations were relatively muted. Nitrate concentrations did not differ substantially between D6, D7, and D8 (Fig. A.6.1.10).

Chlorophyll-*a* concentrations were dramatically impacted by the well-documented *Corbula amurensis* clam invasion in 1986 (Jassby 2008; Fig. 6.2). Peaks decreased from 20-30 $\mu g\ L^{-1}$ prior to 1986 to rarely more than 10 $\mu g\ L^{-1}$ in the subsequent 25 years. Analysis of monthly trends over the entire record of 1975-2011 show that, as expected (and as shown elsewhere; e.g., Jassby 2008), chl-a concentrations exhibited statistically significant decreases in May-Dec, and in February at station D7 and D8 (Fig. 6.6). To evaluate post-*Corbula* trends, we also analyzed chl-a concentrations over the period 1987-2011 (Fig. 6.7). Chl-a concentrations have remained low over during all months, with statistically significant but modest increases only observed during April at D7 and D8, and in September at D6. Consistent seasonal variations were evident in all eras (Fig. 6.2, Fig. 6.7). Chl-a levels peaked in spring and early summer, and declined from late summer through winter. Chl-a levels did not differ substantially between among D6, D7, and D8 after 1986 (Fig. A.6.1.11).

6.3.2: RTC Special Studies 2010 and 2011

Researchers from RTC carried field studies in Suisun Bay in Spring 2010 and 2011 to investigate nutrient uptake rates and phytoplankton growth rates in Suisun Bay, exploring the hypothesis that elevated NH4 concentrations lead to decreased primary production rates. An overview of their interpretation of 2010 results (Dugdale et al. 2012) with respect to NH4 inhibition is presented in Section 3. Here spatial and temporal trends in NH4, NO3, and chl-a concentrations are discussed.

The RTC field studies produced valuable data at higher spatial and temporal resolutions that complement the long-term DWR/IEP and USGS datasets. Contour plots of NH4 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 NH4 concentrations (< 4 $\mu mol\ L^{-1}$) along stretches of 20-30 km. These zones of low NH4 coincided with phytoplankton blooms of greater than 20 µg chl-a L^{-1}. Low NH4 concentrations (< 2 µmol L^{-1}) were also observed at D7, and persisted at ≤ 2 µmol L^{-1} during most of April and May 2010 (Fig. 6.11; full time series presented in Fig. A.6.1.12). During this time, chl-a ranged from 10-30 µg L^{-1} at D7 and water residence times in Suisun Bay in April and May 2011 were 5-35 days. Dugdale et al. (2012) argued that these residence times were sufficient to allow phytoplankton blooms to develop, but that flows were sufficiently high to dilute NH4 concentrations to levels that would ultimately allow phytoplankton to access NH4 and grow more rapidly. While depressed NH4 levels (3-4 µmol 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. 6.1), the nearly-depleted NH4 levels (1-2 µmol L^{-1}) were not necessarily evident, nor do they give a clear impression of the size and duration of this zone. While somewhat elevated chl-a levels were seen during monthly monitoring at D7 (Fig. 6.2), peak values were closer to 10 µg L^{-1}, and little if any chl-a increase was observed at D6 and D8. A substantial bloom (30 µg L^{-1} chl-a) was observed at USGS 3 (Fig. A.6.1.4), but not the other two major USGS stations. Thus, monthly measurements at the major monitoring stations in Suisun Bay have the potential to miss substantial but relatively short-lived blooms that actually occur over fairly large areas of the system.

The RTC data for 2011 have not yet been published in a peer reviewed paper, but were provided by RTC for this report. NH4 concentrations were less than 4 µmol L^{-1} for most of April and May 2011 along the main east-west channel (Fig. 6.10a). Chl-a levels remained low throughout the entire time period along this transect (Fig. 6.10c). At D7, NH4 concentrations were ≤2 µmol L^{-1} throughout April and May 2011. Two short-lived (<1 week) and modest peaks in chl-a peak were observed at D7 in mid and late May (15 and 10 µg L^{-1}, respectively), but otherwise chl-a levels remained low there. High flows were occurring in Spring 2011 which resulted in short residence times of 6 days or less. Thus, despite the low NH4 concentrations, the conceptual model laid out by Dugdale et al. (2012) would suggest that phytoplankton would have been flushed out of Suisun Bay at a rate faster than their growth rate, thus preventing a bloom from occurring.

6.3.3: Load estimates

Delta efflux NH4 loads to Suisun Bay exhibited strong seasonal and interannual variability (Fig. 6.12). Delta NH4 loads to Suisun Bay were highest during high flow months (Jan-Mar; Fig. 6.12, Fig. 6.13). The vast majority of the Delta load came from Sacramento River inputs flowing past Rio Vista, as opposed to flows coming from the central or southern Delta (Fig. A.6.1.13). Interannual variability in river flows had a strong influence on NH4 loads (Fig. 6.12), presumably because NH4 loaded to the Sacramento at SRWTP was was more rapidly transported to Suisun Bay, allowing less time for nitrification.

Overall, between 1975 and 2011, statistically significant increases in NH4 loads occurred in 7 months (Fig. 6.13b), although this increase was not uniform during the entire period (Fig. 6.13a). Between 1975-1995, there was no evidence for increased NH4 loads; in fact, loads decreased in many months over this period (Fig. 6.13a). However, daily NH4 loads increased in all months from 1987-1995 to 1996-2005, with the largest increases during high flow-months, and increases continued in some months from 1996-2005 to 2006-2011. There was a 3-4x difference in NH4 loads between summer and winter, and this seasonality was observed over all eras from 1975-2011.

NO3 loads from the Delta to Suisun also showed strong seasonality and interannual variability (Fig. 6.12, Fig. 6.13c). However long term trends in NO3 loads were less pronounced and consistent than those for NH4. Over 1975-2011, loads did not change significantly, except during June (Fig. 6.13d). Most of this increase occurred between era 2 (1987-1995) and era 3 (1996-2005). Seasonal variations in NO3 loads remained fairly consistent over time, with NO3 loads from the Delta more than tripling between low-flow and high-flow months (Fig. 6.13c). Similar to NH4, NO3 loads into Suisun Bay were dominated by those flowing past Rio Vista, due to larger flow past this point, despite higher NO3 concentration in flows past Twitchell Island (Fig. A.6.1.14).

For quantification of internal discharge loads to Suisun Bay, we considered 3 POTWs. As noted in Section 6.2.2, there are other point source N loads to Suisun Bay; however we currently have limited data on their loads, and it is expected that they are relatively minor sources, although this will be confirmed in an on-going study on nutrient loads to the entire Bay. Two POTWs, CCCSD and DDSD, contribute most of the wastewater NH4 loads to Suisun Bay. The third, FSSD performs nitrification and their NH4 loads are minimal compared to CCCSD and DDSD, on average less than 1% of the other two combined, and are therefore not presented here. CCCSD's load was the largest due to its 3-fold higher flow rate. 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, 6.14). 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.6.1.15b). The load estimates for CCCSD represent its total discharge. Given the proximity of CCCSD's discharge to Carquinez Straits, some uncertainty remains about the proportion of CCCSD's load that is mixed into Suisun Bay, as opposed to being disproportionately advected downstream. This is discussed further in Section 6.4.2.

DDSD's average NH4 load was 1080 kg N d^{-1},for 1991-2011, with loads ranging from 560 to 1790 kg N d^{-1} (Fig. 6.14). Monthly-averaged NH4 loads from DDSD exhibited no consistent change from 1991-2011 (Fig. A.6.1.15d), and loads tended to be higher in Dec-Jun than other months (Fig. A.6.1.15c).

CCCSD, DDSD and FSSD contributed the three largest direct NO3 loads to Suisun Bay. Because the treatment process at FSSD includes denitrification, its NO3 loads were the largest of these three (Fig. 6.15). NO3 loads from FSSD ranged from 70-1920 kg N d^{-1}, with a mean of 840 kg N d^{-1}. Between 1989 and 2011, NO3 loads from CCCSD range from 10-3730 kg N d^{-1}; however, except for frequent elevated NO3 loads between 1998 and 2001, NO3 loads were typically between 150-300 kg d^{-1}, with a mean of 190 kg N d^{-1}. DDSD's NO3 loads were the smallest, ranging from 10-150 kg d^{-1} with an average of 40 kg d^{-1}. Although NO3 loads exhibited considerable variability, few statistically significant changes in NO3 loads from point sources were evident given the current data

(Fig. A.6.1.15f,h,j). The addition of nitrification to the treatment at FSSD in the 1990's would have substantially changed the form in which their N load entered the system, however we do not currently have pre and post-nitrification data that would allow this comparison.

6.3.4:Stormwater runoff

The total watershed area that drains directly to Suisun Bay had an area of 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 NH4 loads from the watersheds were essentially zero during dry periods (Fig. 6.16). During high flow periods, maximum NH4 loads were 200-600 kg N d^{-1}, which is 5-15x lower than POTW loads and more than an order of magnitude lower than Delta loads during the same months. NO3 stormwater loads were also essentially zero during dry periods, but were several times higher than NH4 loads during high-flow periods due to typically higher NO3 concentrations in stormwater runoff (Fig. 6.17). NO3 load estimates ranged from about 1000-2000 kg N d^{-1} during wet months, which are comparable to POTW loads, but 5-10-times lower than Delta loads during these same months.

6.4 Discussion

6.4.1 Comparison of NH4 concentrations with hypothesized thresholds

The IEP's conceptual model for the POD identifies multiple factors that may contribute to ecosystem decline and to the POD (Baxter et al., 2010). Among those factors, NH4 was identified as one contaminant that could potentially adversely impact the food web in Suisun Bay and the Delta in a few ways: a) inhibiting primary production and thereby limiting food supply (e.g., Dugdale et al., 2007); b) selecting for different phytoplankton species, altering phytoplankton community composition, and decreasing food quality (Glibert et al., 2011); or c) having a direct chronic toxic effect on copepods that decreases their reproductive success or survival of offspring to adult forms. NH4 loads to Suisun Bay have increased significantly over the past few decades (Fig. 6.13 and 6.14). The increased loads have been accompanied by significantly increased NH4 concentrations within Suisun Bay during certain times of the year (Fig. 6.4). In this section observed NH4 concentrations in Suisun Bay are compared with NH4 thresholds that recent studies have identified as 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. The role that changes in NH4:NO3 and N:P in Suisun Bay may have on phytoplankton community composition and related impacts on ecosystem health will be explored in a subsequent report.

According to the conceptual proposed by RTC researchers (Section 3), at NH4 concentrations above 4 µmol L^{-1} the uptake of NO3 by phytoplankton is substantially inhibited, resulting in lower primary production rates (Dugdale et al., 2007). Although this conceptual model also indicates that 4 µmol L^{-1} is not a "bright-line" threshold, and that NO3 uptake and phytoplankton productivity are also inhibited at lower levels of NH4 (down to ~1 μmol L^{-1}; Parker et al., 2012), we will use the 4 μmol L^{-1} value here because it is the most widely cited value; there remains considerable uncertainty around what would constitute a more appropriate value; and qualitatively similar conclusions are reached when ambient concentrations are compared to either 1 or 4 μmol L^{-1}. Since phytoplankton blooms have historically only been observed in spring, summer, and fall, the 4 μmol L^{-1} threshold is compared to ambient concentrations in April-October when the potential for impairment is most relevant. Between 1975-1986, NH4 levels exceeded 4 μmol L^{-1} in 44% of the monthly observations at D6, D7, and D8 (Table 6.3). Between 1987-1997, the 4 μmol L^{-1} threshold was exceeded in 70% of monthly observations. Most recently, from 1998-2011, ambient NH4 concentrations exceeded 4 μmol L^{-1} the vast majority of the time (87%). Thus, the frequency with which a 4 μmol L^{-1} threshold has been exceeded between April-October has approximately doubled over the the past 35 years (Table 6.3). In order to evaluate the NH4 inhibition hypothesis (Dugdale 2007) prior to the influence of the *Corbula* clam invasion (1987) or significant increases in NH4 loading from CCCSD and Sacramento Regional Water Treatment Plant (SRWTP) during the 1990's (Fig. 6.14, Jassby 2008), we focused on a time series of chl-*a*, NH4, NO3 and residence time data for DWR/IEP station D7 from 1975-1986 (Fig. 6.18). Frequent chl-*a* above 30 µg L^{-1} were observed, which is typical for other Suisun stations prior to the *Corbula* invasion (Fig. A.6.1.4). While a majority of these blooms occurred when NH4 was below 4μM, and the time of greatest chl-*a* increase seems to align with NH4 concentrations dipping just below this threshold, NO3 uptake proceeds bloom formation, so phytoplankton may be able to access the NO3 pool prior to NH4 dropping below 4μM.

Teh et al (2011) found that the lowest observed effect level (LOEL) for chronic toxicity to copepods was 26 μmol L^{-1} . Ambient NH4 concentrations at D6, D7, and D8 were also compared to this value. Since copepods have complex life-cycles and are present year round, albeit in varying abundance, the 26 μmol L^{-1} LOEL was compared with concentrations over the entire year from 1975-2011. The value of LOEL was only exceeded twice, once at each D6 and D8 in in 1977 (Table 6.3). There have been limited studies of NH4 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 μmol L^{-1}, although no pH was specified with this threshold to the exact partitioning between NH4 and NH3 is unknown (Buttino 1994). This value is based on only a single study, and its relevance for Suisun Bay is unknown, so the comparison should be interpreted with caution. NH4 concentrations at D6, D7, and D8 exceeded 11 μmol L^{-1} 11% of time between 1998-2011, which was approximately 2 times more frequent than between 1975-1986.

All of the above comparisons should be interpreted with caution for two main reasons. First, none of these thresholds has been rigorously established. The NH4-inhibition hypothesis still requires further testing (Section 2 and 3). In addition, if it is found to be an important mechanism that limits primary production rates, the actual threshold value needs to be further evaluated and may in fact be lower. The copepod toxicity study by Teh et al. (2011) has neither been replicated nor peer reviewed, and both would be worthwhile, in particular considering the low sample size and other critiques methodological and statistical critiques (Section 5). In addition, Teh et al. (2011) found its LOEL at the lowest dosed samples, and treatments at lower levels are needed to establish a no observed effect level (NOEL). A similar set of arguments apply to the copepod study by Buttino (1994). For any copepod studies, it will be important for them to be carried out at salinity ranges relevant to Suisun Bay, in particular because toxicity is thought to be exerted through the Na/K transporter, Na^+ are K^+ levels vary linearly with salinity, and copepod sensitivity to NH4 may differ at different salinities (ref; S. Teh, pers. comm.).

Second, while NH4 levels at the stations sampled in long-term time series and in RTC special studies may be representative of the range of average conditions observed in Suisun Bay, they may not be the highest concentrations. Data from any near-field sampling around POTW discharges have not been included in this analysis. Undiluted treated wastewater effluent that did not undergo nitrification contains NH4 at concentrations of 1700 μmol L^{-1}. Dilutions of 65-fold are needed to reduce effluent to < 26 μmol L^{-1} . This is not a particularly large dilution, and likely happens over small distances from outfalls because of high mixing energy in Suisun Bay. Nonetheless, if such data exists, it should also be compared with thresholds, along with a consideration of the importance of the area of lower dilution (e.g., its size or location).

6.4.2: Seasonal and temporal trends in NH4 concentrations and loads

NH4 concentrations in Suisun Bay have increased by approximately 50% in several months of the year between1975-2011 (Fig. 6.4). Statistically significant increases were observed during October-December at all DWR stations, and statistically significant increases were also detected in May-June at D6 and D8. NH4 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 the temporal increase in NH4 concentrations and their pronounced seasonality.

Since 1975, NH4 loads from the Delta to Suisun have increased substantially (Fig. 6.13), 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 ± 1840 kg N d^{-1} from 2006-2011, and 4060 ± 2660 kg N d^{-1} from 1975-1995. NH4 loads exhibited strong seasonality (Fig 6.12, Fig 6.13), as did the magnitude of the increase between pre-1995 and post-1995. Since most of the Delta-derived NH4 load entering Suisun came from the Sacramento River (Fig. A.6.1.13), and most of the NH4 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 this increase. SRWTP's NH4 loads increased by larger 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 NH4 loads increased by 5000-6000 kg d^{-1} between 1975-1980 and 1998-2011, which is comparable to the NH4 load increase at SRWTP. 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 (Fig. 6.13). As has been demonstrated in other studies (Foe 2010; Parker et al., 2012), much of SRWTP's NH4 load undergoes nitrification en route to Suisun Bay, and a substantial loss of NH4 is consistent with our estimated loads entering Suisun Bay (Fig. 6.13). To more thoroughly assess the Delta's role in modulating nutrient loads to Suisun Bay, we also calculated the total monthly NH4 loads that enter the Delta from all major tributaries (Sacramento, San Joaquin, and smaller eastern tributaries), and the amounts that leave the Delta (either to Suisun or 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 NH4 was removed within the Delta either by nitrification or uptake by phytoplankton. This value was up to 90% during some months (Novick et al., in preparation). Thus, the Delta acts as a substantial biogeochemical reactor, and its NH4 removal efficiency appears to vary seasonally, likely due to factors such as residence time and temperature.

Loads from CCCSD also increased by ~800 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 NH4 source to Suisun Bay, but its NH4 loads have remained relatively constant since 1990, followed by stormwater loads, which initial estimates suggest contribute less than 5% of NH4 loads during wet periods.

Identifying which sources contributed most to the observed increases in NH4 concentrations in Suisun Bay (Fig. 6.4) is not straightforward, because of the large seasonal variation in loads from the Delta. Fig. 6.19 illustrates the magnitudes of NH4 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 spring, summer, and fall months during some years (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 6.13) relative to the increase from CCCSD (several hundred kg N d^{-1}) (Fig. A.6.1.15). However, in the remaining months the increases from the two sources were more comparable. 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 Suisun. The higher NH4 concentrations observed at D6 than both D7 and D8 in long-term monitoring data, and at USGS7 and USGS 8 relative to other stations further east during RTC studies, are consistent with some incomplete mixing; however the spatial difference in concentration (a few micromolar) is a fairly modest local increase, considering that the NH4 concentration in CCCSD's effluent was approximately 1500 uM. Finally, "internal" sources of NH4, namely NH4 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 seasonal variations (e.g., due to temperature changes and delivery of fresh organic matter to sediments). A recent study of sediment nutrient fluxes in Suisun Bay and the Delta found that NH4 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 NH4 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.

6.4.3 Examining NH4 fate in Suisun Bay

NH4 concentrations in Suisun Bay varied seasonally by as much as a factor of 2-3 between low-flow and high-flow months, a pattern that has remained similar over the past 35 years (Fig. 6.4). This seasonal variation cannot be explained by seasonal variations in NH4 loads alone: when current NH4 loads from the Delta and Suisun Bay POTWs were considered along with typical flushing rates during spring, summer, and fall, the predicted NH4 concentration was on the order of 20 μmol L^{-1} (assuming conservative behavior), as compared to the observed levels 3-6 μM from May –September (Fig. 6.4). This large difference between predicted and measured concentration, and this specific concentration range, are relevant considering the levels at which NH4 is hypothesized to inhibit primary production (>2-4 μmol L^{-1}) and have toxic effects on copepds (LOEL = 26 μmol L^{-1}). To further explore the seasonal variations in NH4 concentrations and NH4 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 model results demonstrate that on average only 25% of the NH4 that was added to the system was actually transported out of Suisun Bay through the Carquinez Straits (advective transport and tidal exchange combined; Fig 6.20). By difference, ~75% of NH4 loss from Suisun Bay must have occurred by transformation (e.g., nitrification) or uptake (e.g., 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 NH4 still needs to undergo transformation/loss within Suisun Bay in order to explain the observed concentrations.. The magnitudes of the transformation/loss term and downstream transport term varied within a given year (Fig. 6.20). As expected, as flow decreased from April-October (and residence time increases), the magnitude of downstream transport decreased. It was initially somewhat surprising, however, to see that the size of transformation/loss term was actually larger in April and May than in later months when residence times were longer and temperatures warmer. We hypothesize that the higher transformations/loss rate may be due in part to phytoplankton uptake. April and May are the months in during phytoplankton growth rates have typically been greatest in Suisun Bay (Kimmerer and Thompson, submitted), 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 can be readily explained by estimated clam grazing and microzooplankton grazing, which typically exceeded or matched gross primary production rates (Kimmerer and Thompson, submitted). The first order rate constant required to explain the transformation/loss of NH4 during low-flow periods was in the range of 0.1-0.3 d^{-1} (Fig. A.6.4.7), which is similar in magnitude to nitrification rates used in more advanced water quality models (e.g., ~0.1 d^{-1}; J Fitzpatrick, HDR, pers. comm.). This mass balance analysis did not include NH4 loads due to flux from the sediments, indicating that, if those loads were at all substantial, the calculated loss rates and first order rate constants are lower bound estimates.

The simplifying assumptions made in this 1-box model undoubtedly introduced a certain degree of uncertainty. We evaluated, either qualitatively or quantitatively, the uncertainty introduced by some of the key assumptions, and this discussion can be found 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 ambient play an important role in determining NH4 concentrations during low-flow months. Characterizing these processes further, including seasonal and temporal variability, would require modeling Suisun Bay on a finer spatial and temporal scale. Model accuracy would be enhanced by refinement of nutrient loads estimates (including sources not included here because of limited data availability) and more frequent water quality monitoring with more complete coverage of Suisun Bay. Better estimation of the frequency, duration and spatial extent with which NH4 concentrations exceed various thresholds or guidance levels that studies have suggested may impair beneficial uses could shed light on the role that changing nutrient concentrations, in particular NH4, could play in the recent decline of pelagic fish populations. Given the recent significant increase in NH4 concentrations and loads (Fig. 6.4, Fig. 6.13), a better understanding of NH4 concentrations, sources, fate, and long-term trends in Suisun Bay is necessary in order to inform important, near-term, and potentially costly management decisions to regulate nutrient loads.

6.5 References

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6.6 Tables and Figures

Station Name	Source	NH_4	$NO_3 + NO_2$	TKN	Organic N	PO_4	TP	Chl-a
DWR-EMP Stations								
D6	DWR-EMP	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-EMP	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-EMP	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-EMP	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-EMP	1975 n=11	1975 n=10	1975 n=11	1975 n=11	1975 n=12	1975 n=12	1975 n=10
D10	DWR-EMP	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-EMP	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 Stations								
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

Table 6.1 Available water quality data from DWR/IEP [1] and USGS[2] stations in Suisun Bay. The number of available data points is indicated by n=

[1]http://www.water.ca.gov/bdma/meta/Discrete/data.cfm [2]http://sfbay.wr.usgs.gov/access/wqdata/

	Flow	NH4	NO3	Total P
CCCSD	1975-1978 3-4x/month 1979-2011 Daily	1975-1978 3-4x/month 1979-2011 Daily	1993-2011 3-4x/month	1975-2011 3-4x/month
DDSD	1991-2011 Daily	1992-1993 Monthly 2007-2011 Monthly	1992-1993 Monthly 5/2007-8/2007 Monthly	1992-1993 Monthly
FSSD	2004-2012 Daily	2004-2012 3-4x/month	2004-2012 3-4x/month	2004-2012 3-4x/month

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)

		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

Table 6.3 Comparison of ambient NH4 concentrations in Suisun Bay in three eras (1975-1986, 1987-1997 and 1998-2011) to relevant environmental thresholds. Dugdale et al (2007) believe that NH4 concentrations above 4 μM inhibit NO3 uptake and limit primary production (See section 3). Comparisons to this threshold are limited to months when phytoplankton blooms are known to occur in Suisun Bay. Buttoni et al (1994) observed decrease a LOEL of approximately 11 μM to the copepod *Arcatis*. Teh et al (2011) observed a LOEL of approximately 26 μM to the copepod species *Pseudodiaptomus*. Comparisons to these two LOEL thresholds included all months of the year, since copepods are present in some life stage year-round.

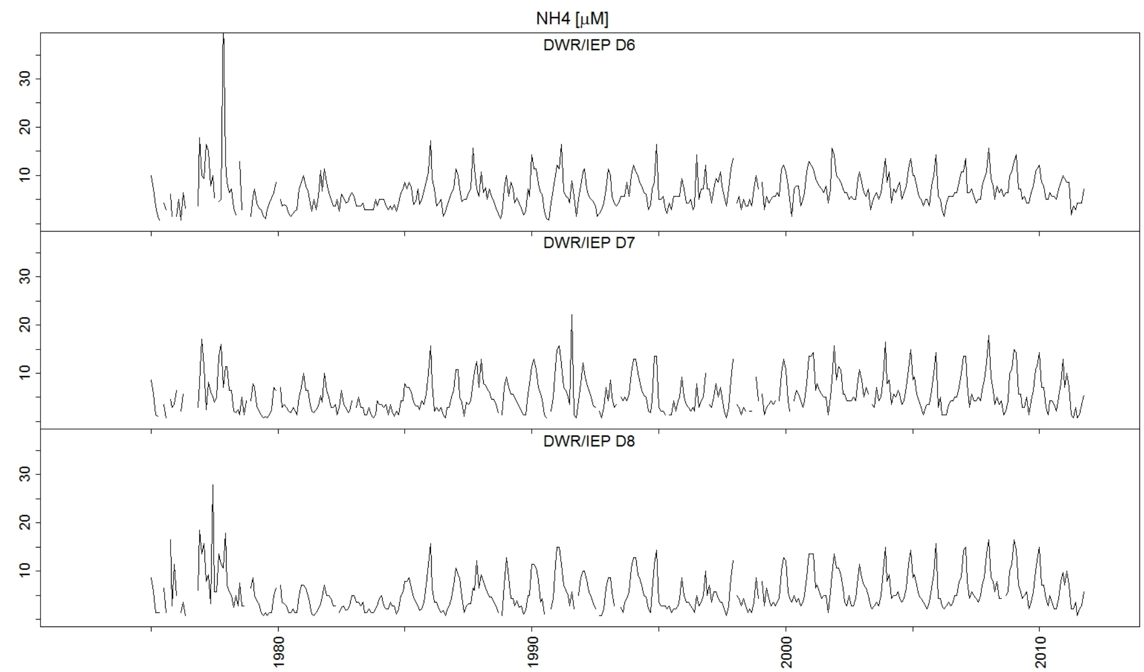

Figure 6.1 Time-series of available NH4 data in µM at key Suisun Bay DWR/IEP stations. Stations D6, D7 and D8 had the most continuous record of data of all DWR/IEP or USGS stations in Suisun Bay (see Fig. A.6.1.1)

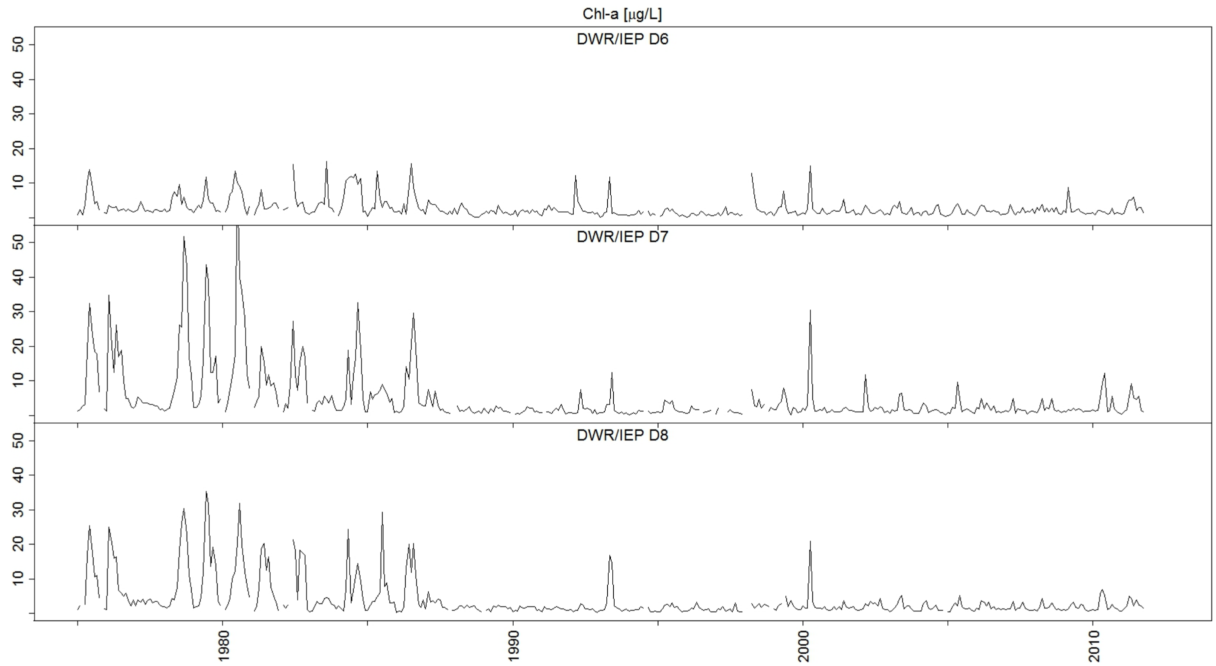

Figure 6.2 Time-series of available chlorphyll-*a* data in µg L^{-1} at key Suisun Bay DWR/IEP stations.

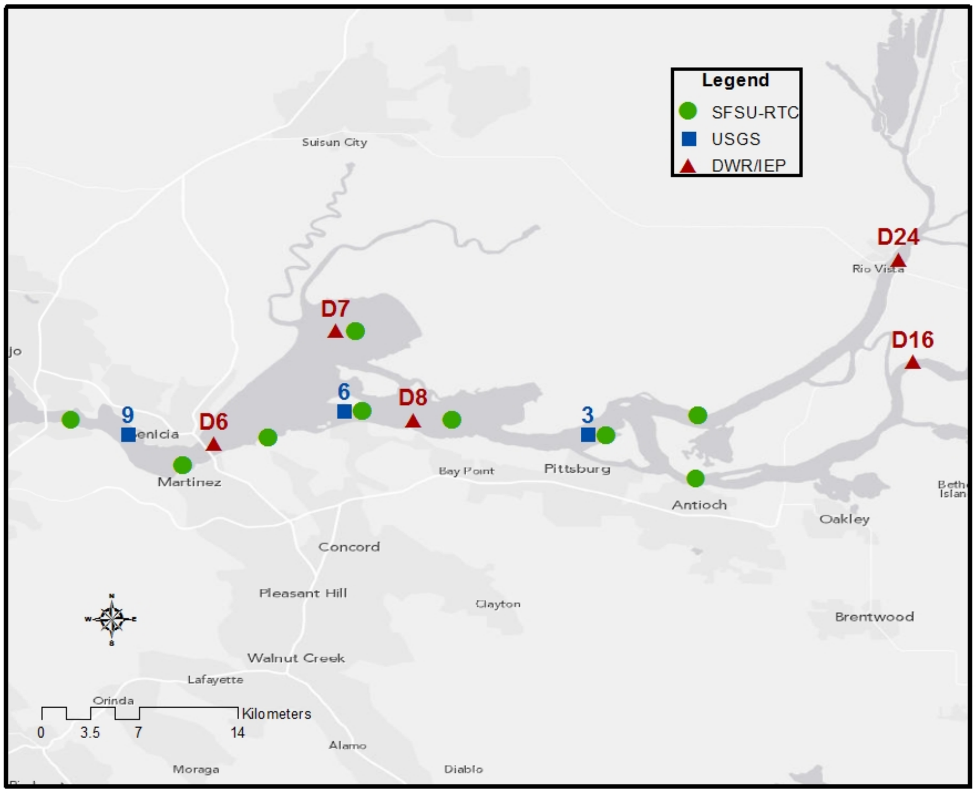

Figure 6.3 Location of DWR/IEP (red triangles), USGS (blue square) and SFSU-RTC (green circle) monitoring stations with nutrient data available.

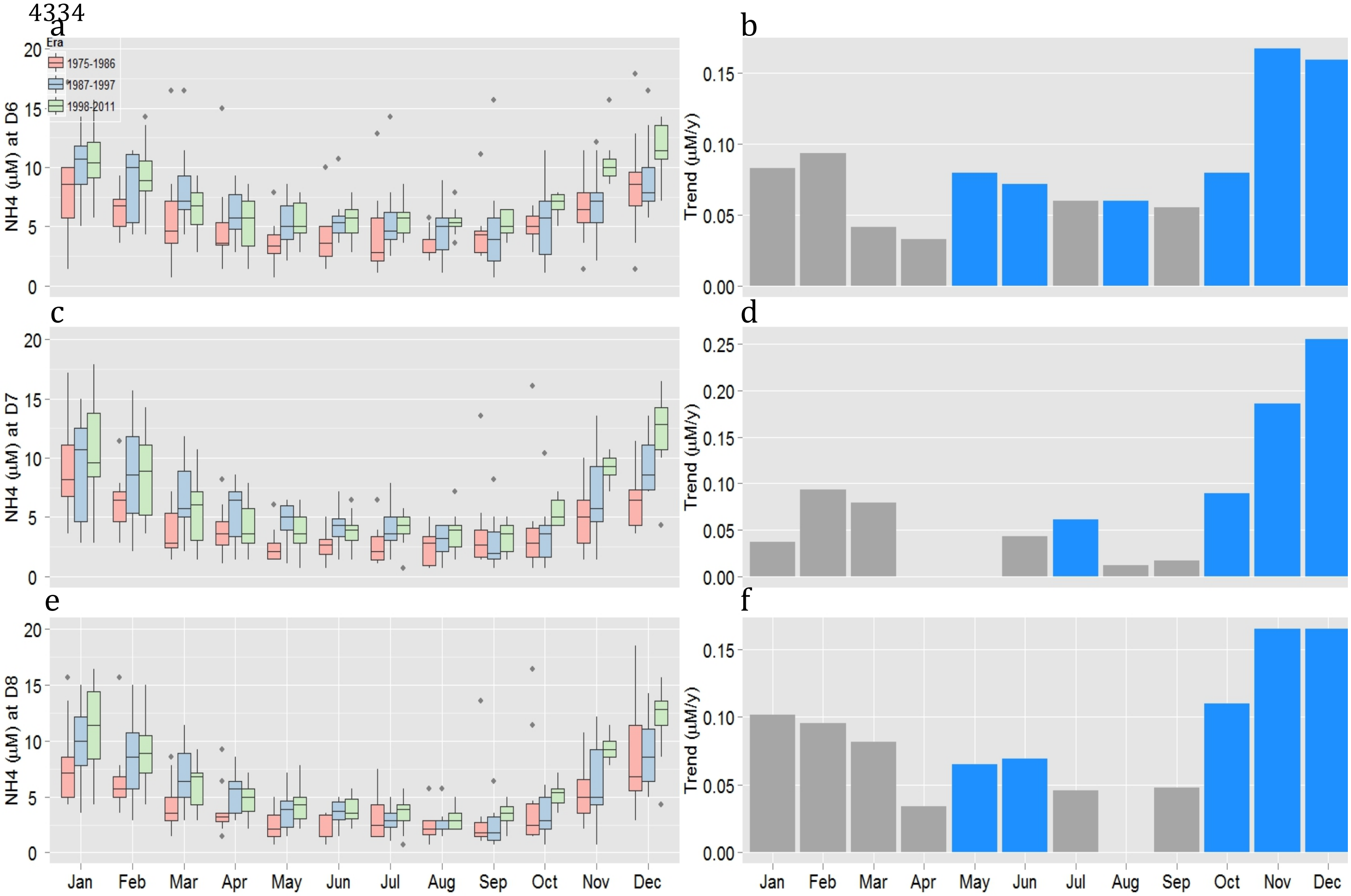

Figure 6.4 Seasonal and temporal variations in NH4 concentrations at DWR/IEP stations D6, D7 and D8. Data were first aggregated into three eras (1975-1986, 1987-1997 and 1998-2011), and then averaged by month within each era (panels a, c and e). Long-term trends were characterized by the Theil slope (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

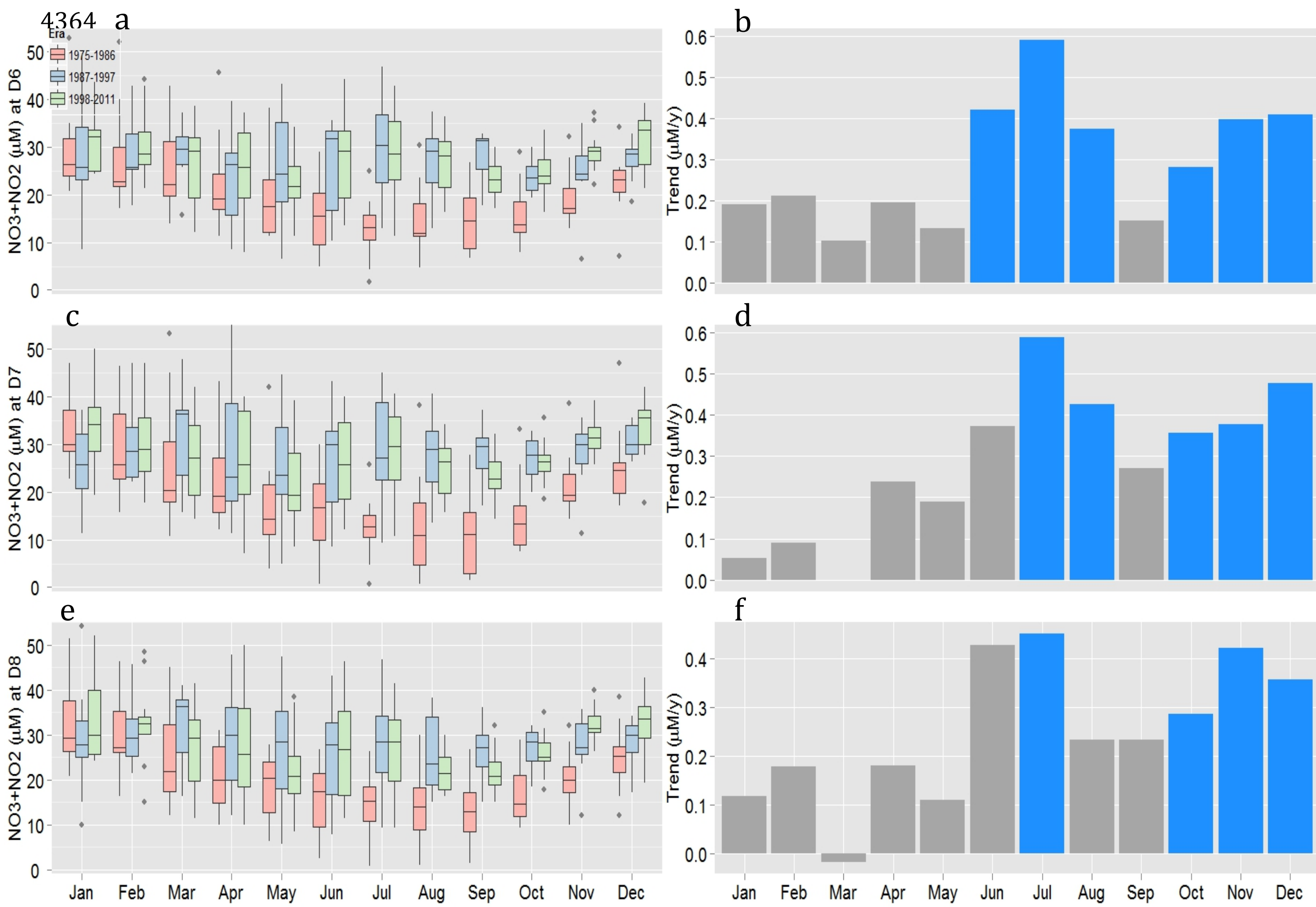

Figure 6.5 NO3+NO2 concentrations at DWR/IEP stations D6, D7 and D8. Calculations are identical to those described in Fig. 6.4. Concentrations are presented in panels (a, c and e) and trends are reported in panels (b, d and e).

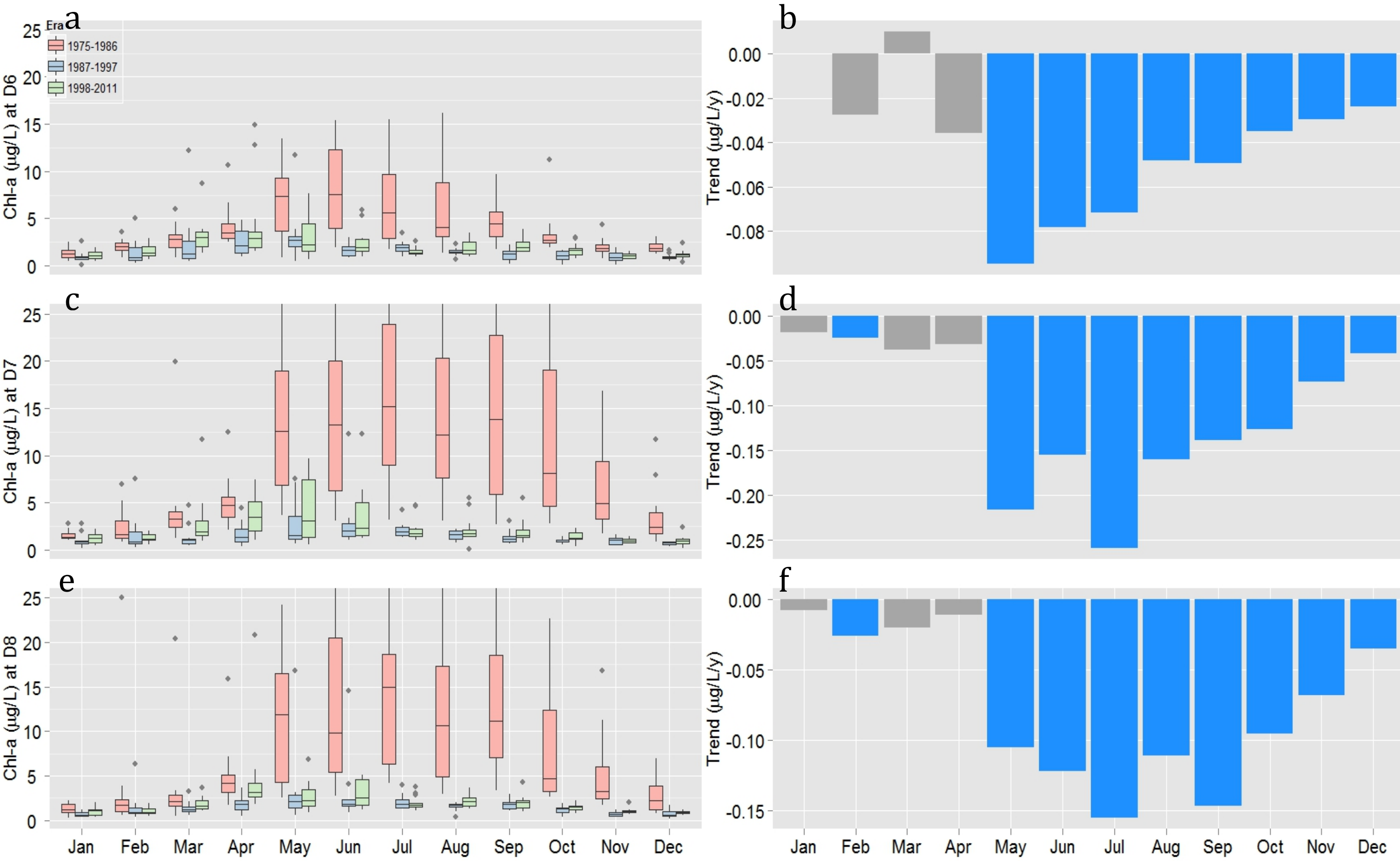

Figure 6.6 Chlorophyll-*a* concentrations at DWR/IEP stations D6, D7 and D8. Calculations are identical to those described in Figure 6.4. Concentrations are presented in panels (a, c and e) and trends are reported in panels (b, d and e).

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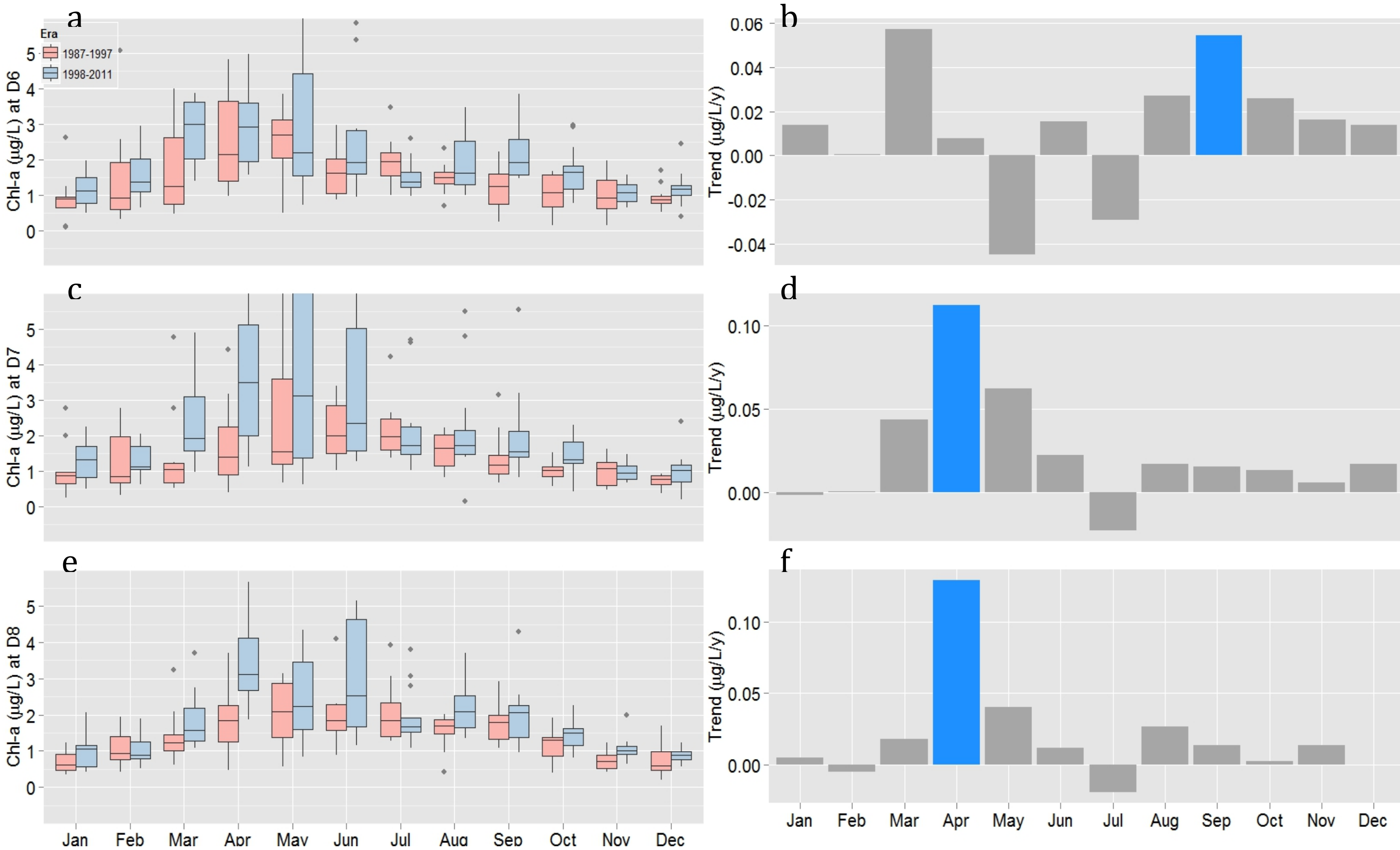

Figure 6.7 Chlorophyll-*a* concentrations at DWR/IEP stations D6, D7 and D8 for the era 1987-2011. The entire time series (1975-2011) was truncated to remove the abrupt effect of the invasion of the clam *Corbula amurensis* (Figure A.6.1.4). Calculations are identical to those described in Figure 6.4. Concentrations are presented in panels (a, c and e) and trends are reported in panels (b, d and e).

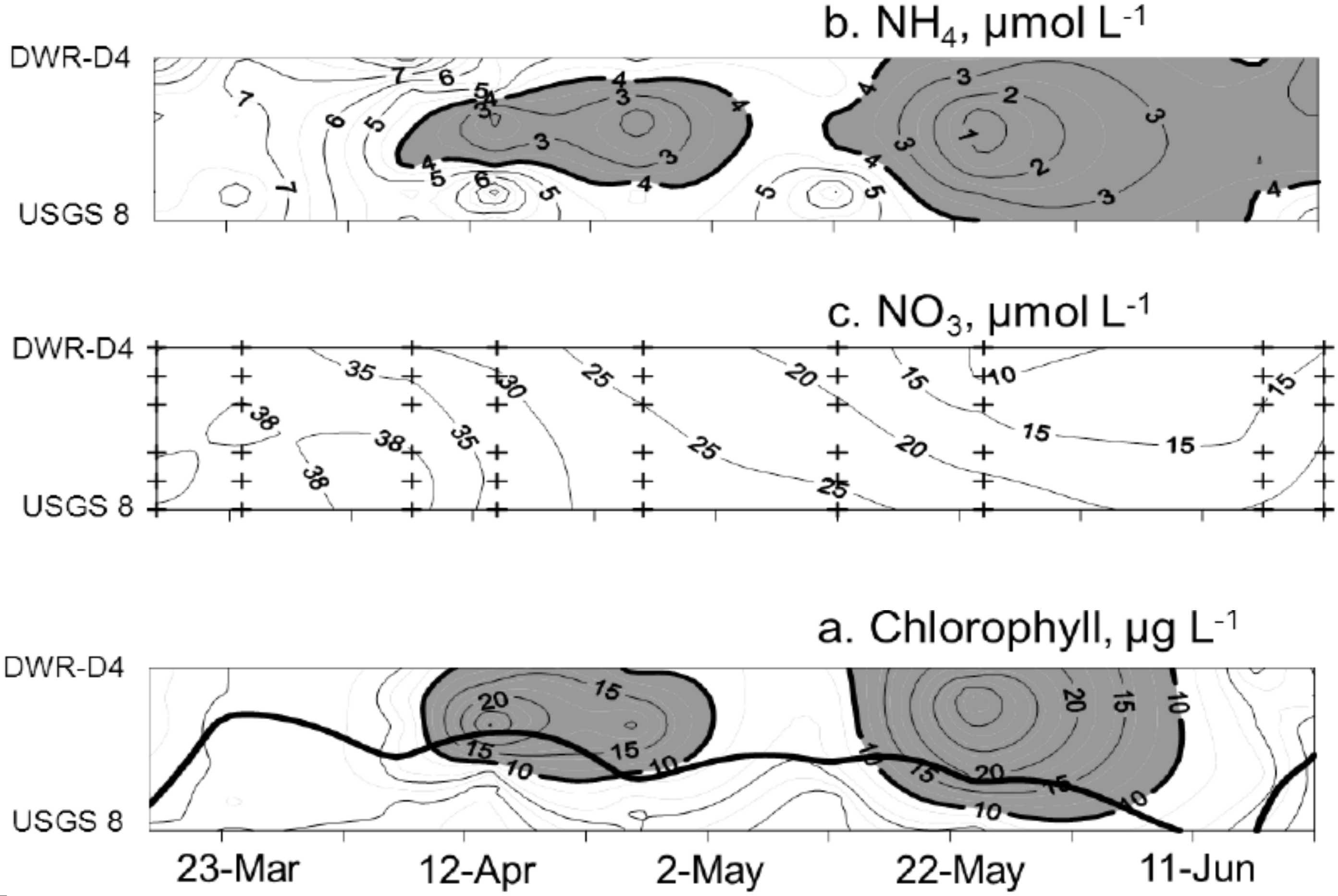

Figure 6.8 Contour plots of NH4, NO3 and chlorophyll-a data collected by SFSU-RTC during Spring 2010 in Suisun Bay. Data were collected on 9 days at 7 stations along a roughly linear transect through Suisun Bay between DWR/IEP-D4 and USGS-8. (DWR/IEP D7 not included here – see Figure 6.9) Figures borrowed with permission from Dugdale et al (2012).

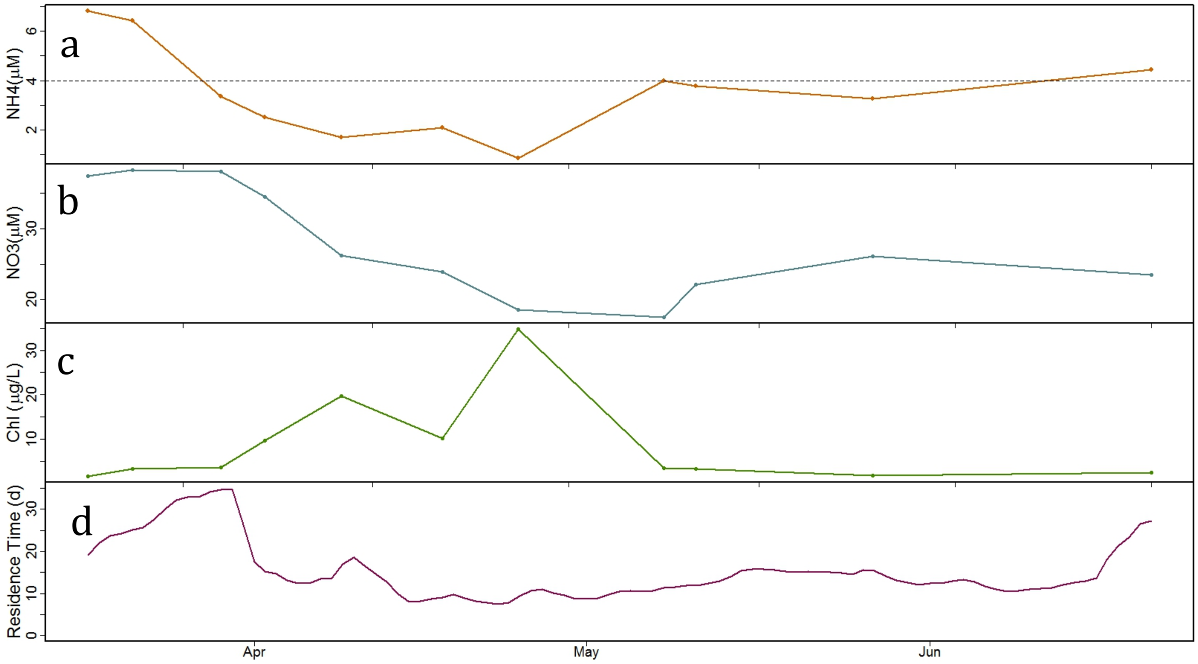

Figure 6.9 Time series of NH4, NO3 and chlorophyll-a data collected by SFSU-RTC near DWR/IEP Station D7 on 9 dates during Spring 2010 in Suisun Bay. Data presented here were not included in Figure 6.8. The dashed line in panel a is at 4µM, the concentration believed to inhibit NO3 uptake and limit primary production (Dugdale et. al, 2007). Residence time was calculated by dividing the volume of Suisun Bay (6.54e11 L) by daily advective flows

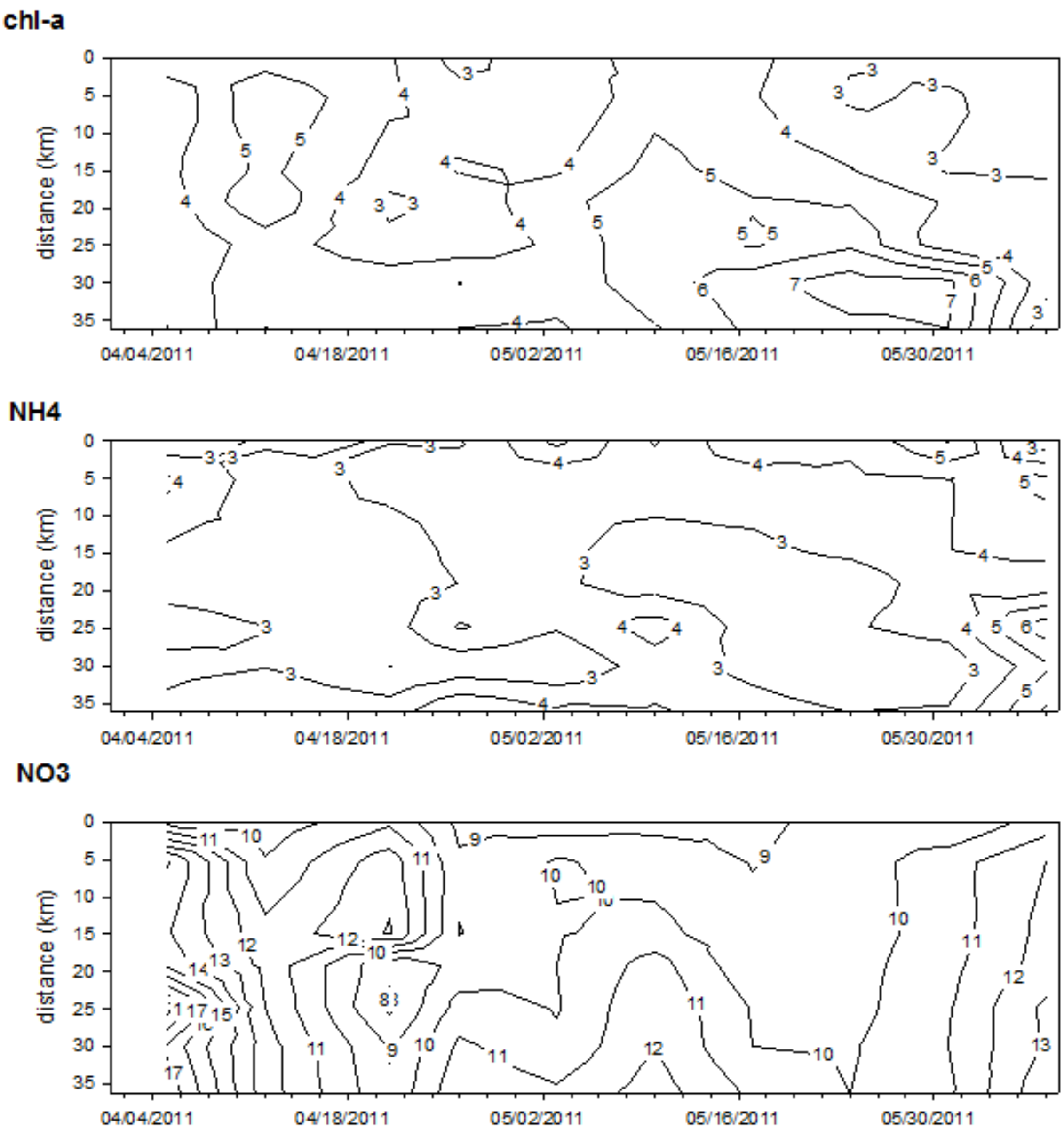

Figure 6.10 Contour plots of of NH4, NO3 and chlorophyll-a data collected by SFSU-RTC on 9 dates during Spring 2010 in Suisun Bay. Data were collected on at 7 stations along a roughly linear transect through Suisun Bay between DWR/IEP-D4 and USGS-8. (DWR/IEP D7 not included here – see Figure 6..11)

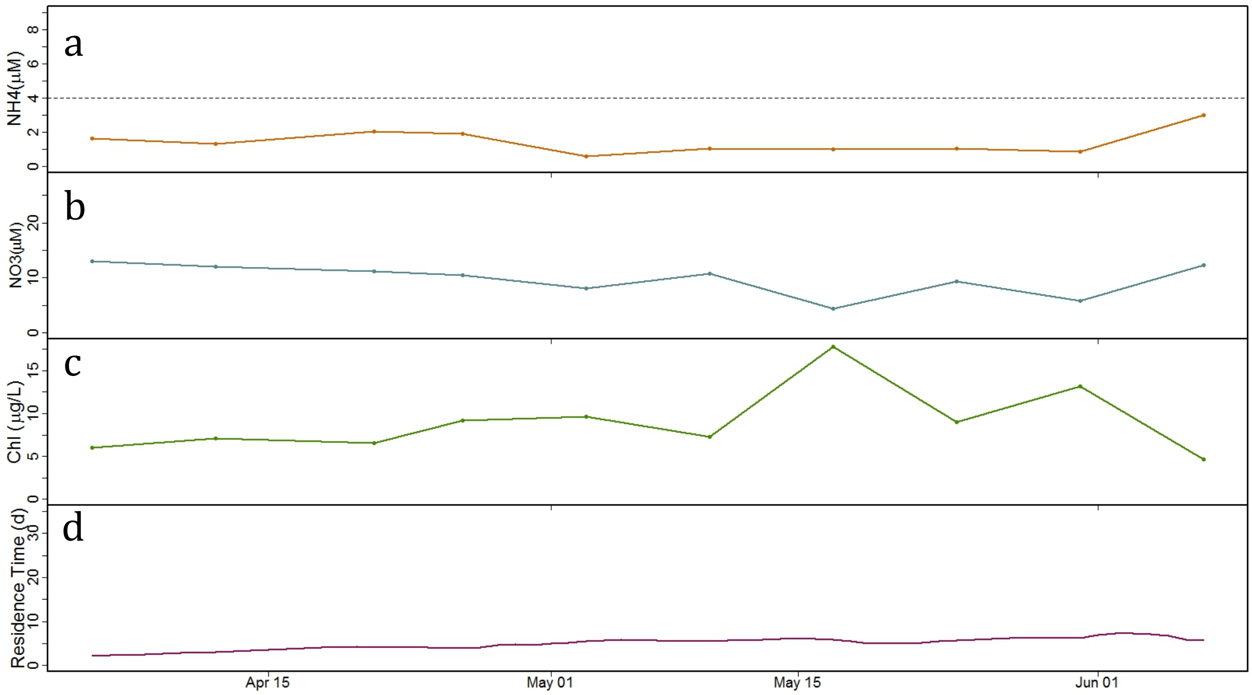

Figure 6.11 Time series of NH4, NO3 and chlorophyll-a data collected by SFSU-RTC near DWR/IEP Station D7 on 10 dates during Spring 2011 in Suisun Bay. Data presented here were not included in Figure 6.10. The dashed line in panel a is at 4µM, the concentration believed to inhibit NO3 uptake and limit primary production (Dugdale et. al, 2007). Residence time was calculated by dividing the volume of Suisun Bay (6.54e11 L) by daily advective flows

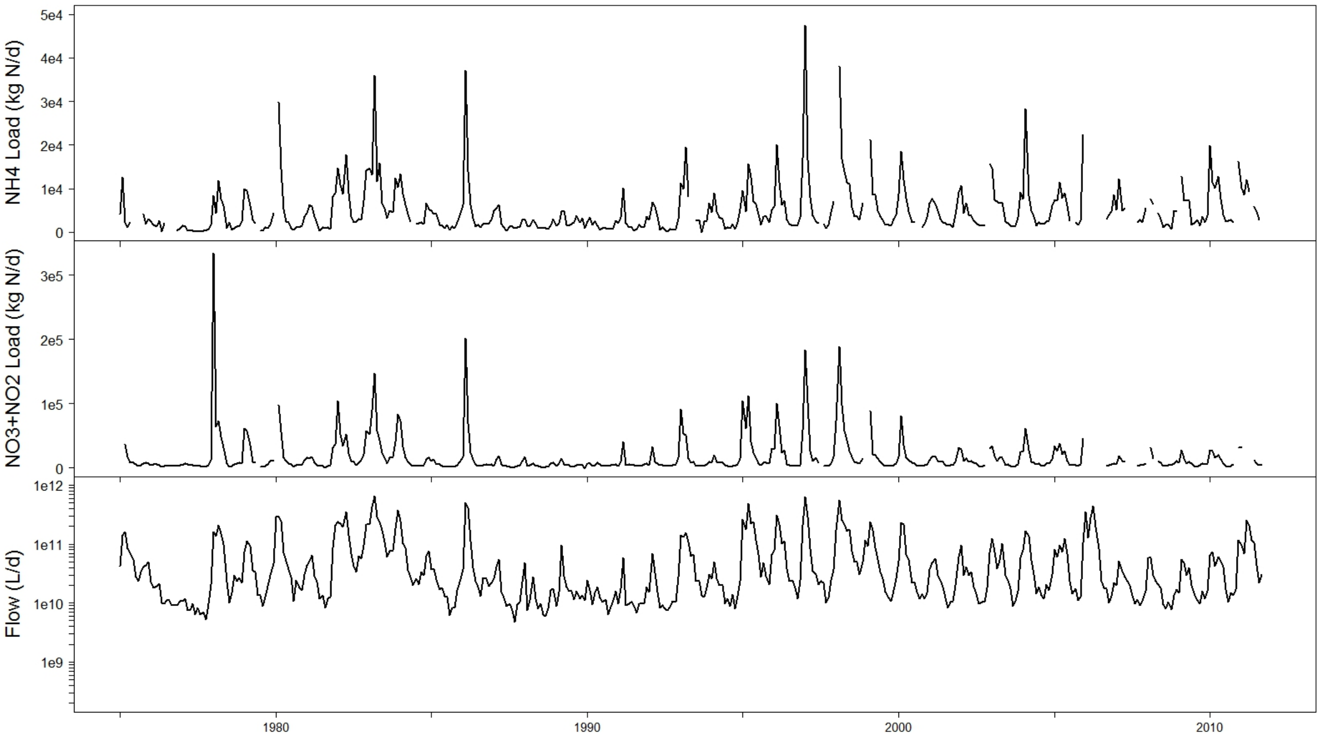

Figure 6.12 Time series of estimated nutrient loads into Suisun Bay from the Delta (panels a and b). Loads were estimated using flow data from DWR DAYFLOW[1] (presented in panel c) and concentration data from DWR/IEP stations similar to the method used by Jassby and Cloern (2000)

[1] http://www.water.ca.gov/dayflow/

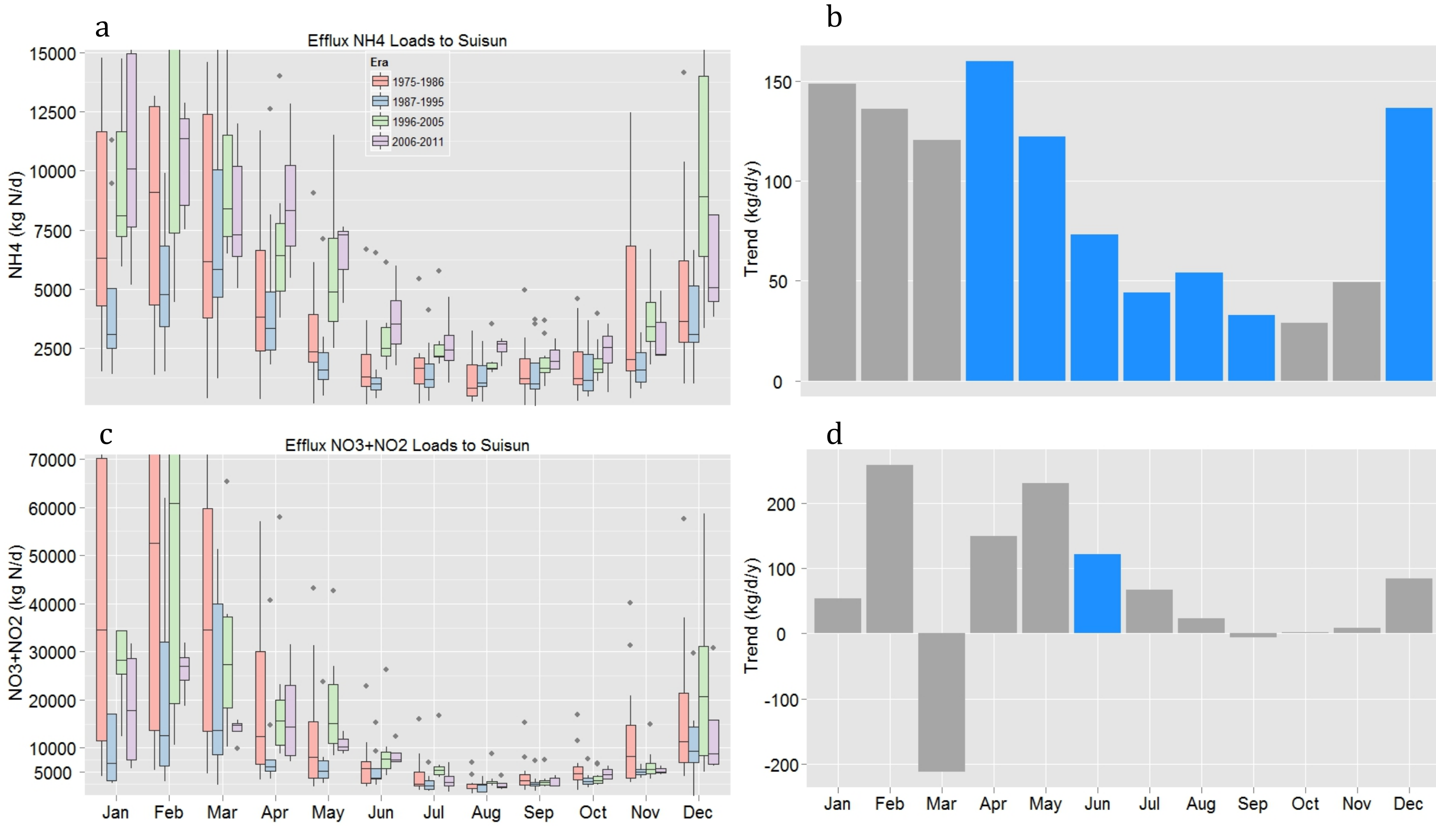

Figure 6.13 Seasonal and temporal variations in Delta efflux loads to Suisun Bay. Data were first aggregated into three eras (1975-1986, 1987-1997 and 1998-2011), and then averaged by month within each era (panels a and c). Long-term trends were characterized by the Theil slope (see description in Section 6.2.3) (panels b and d). Blue bars indicate statistically significant trends with $p<0.05$ as determined by the Kendall Tau test.

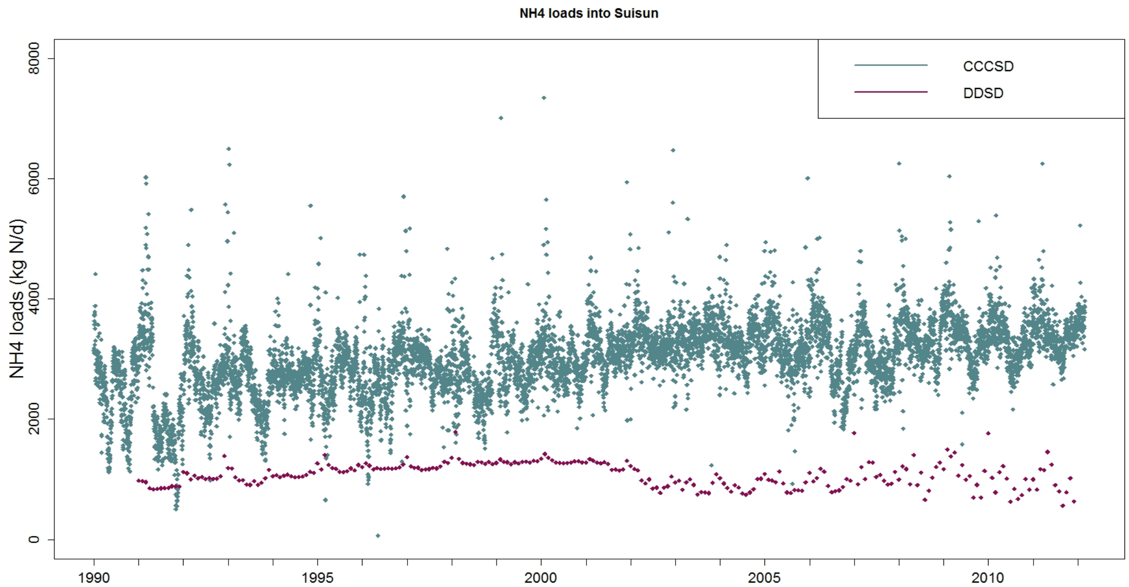

Figure 6.14 Time series of NH4 effluent loads from the two major NH4 dischargers to Suisun Bay: CCCSD and DDSD. Data for trial periods of nitrification at CCCSD (1977-1982, 1987-1988) are presented in Figure A.6.1.5. Nitrification processes at FSSD reduce NH4 loads to approximately 1% of the other two dischargers and are therefore not included here.

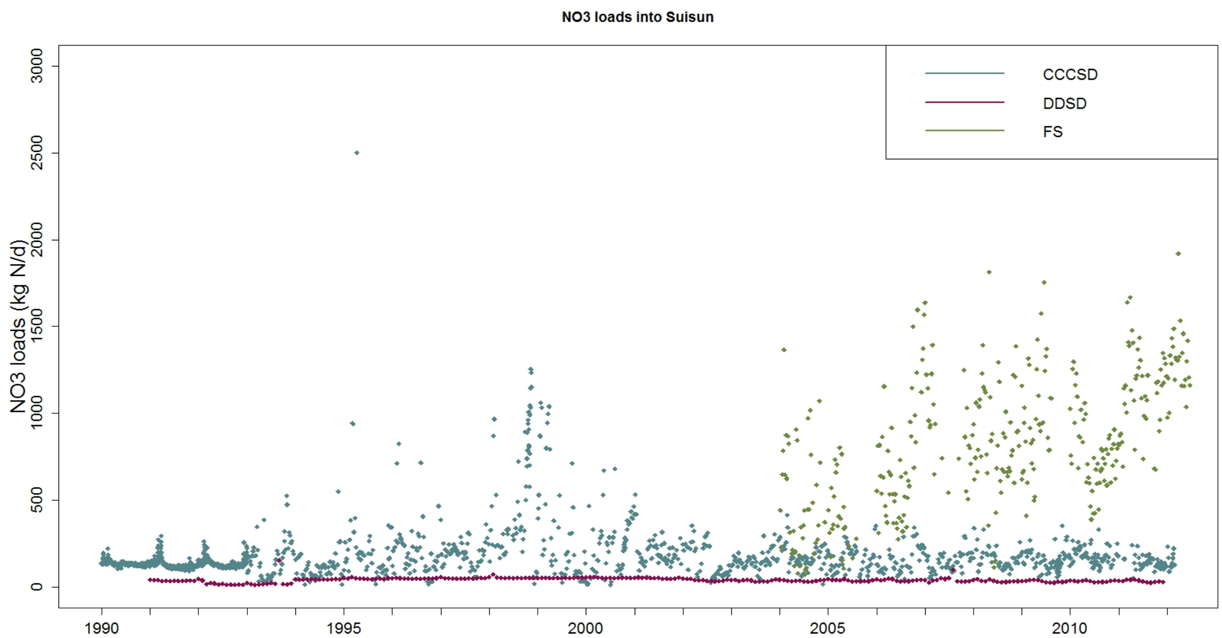

Figure 6.15 Time series of NO3 effluent loads form the three major NH4 dischargers to Suisun Bay: FSSD, CCCSD, and DDSD. Data for trial periods of nitrification at CCCSD (1977-1982, 1987-1988) are presented in Figure A.6.1.6. Nitrification processes at FSSD increase NO3 loads to well above those at either CCCSD or DDSD.

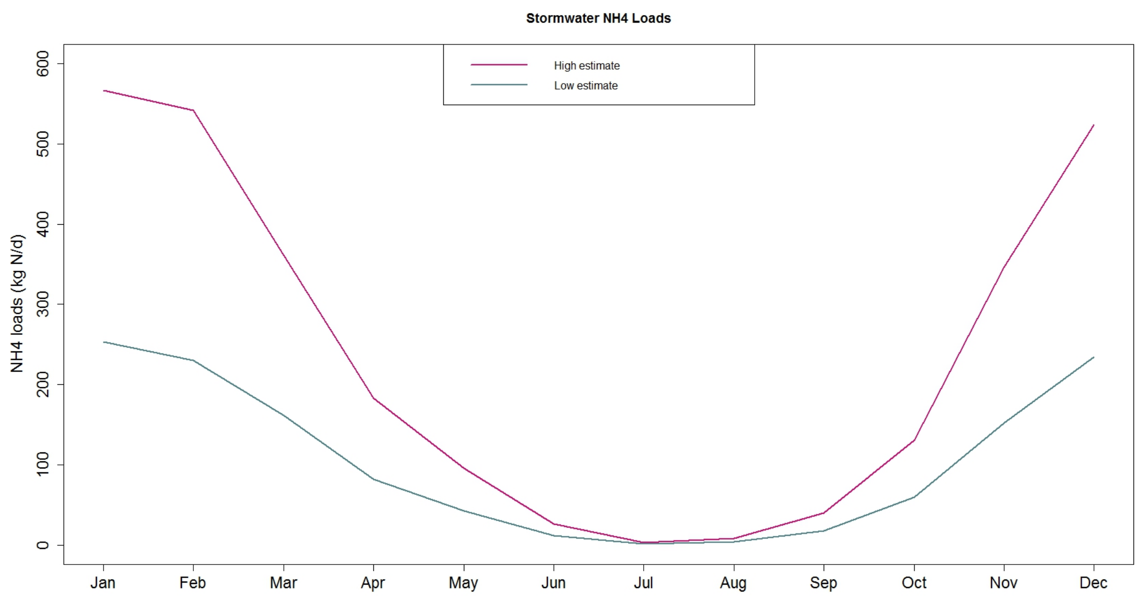

Figure 6.16 Estimated stormwater NH4 loads from two watersheds that drain directly into Suisun Bay. Loads were estimated using monthly average precipitation values, average runoff coefficient for each watershed (weighted by land-use), watershed area and stormwater NH4 concentrations from the literature. See Appendix 6.3 for further information.

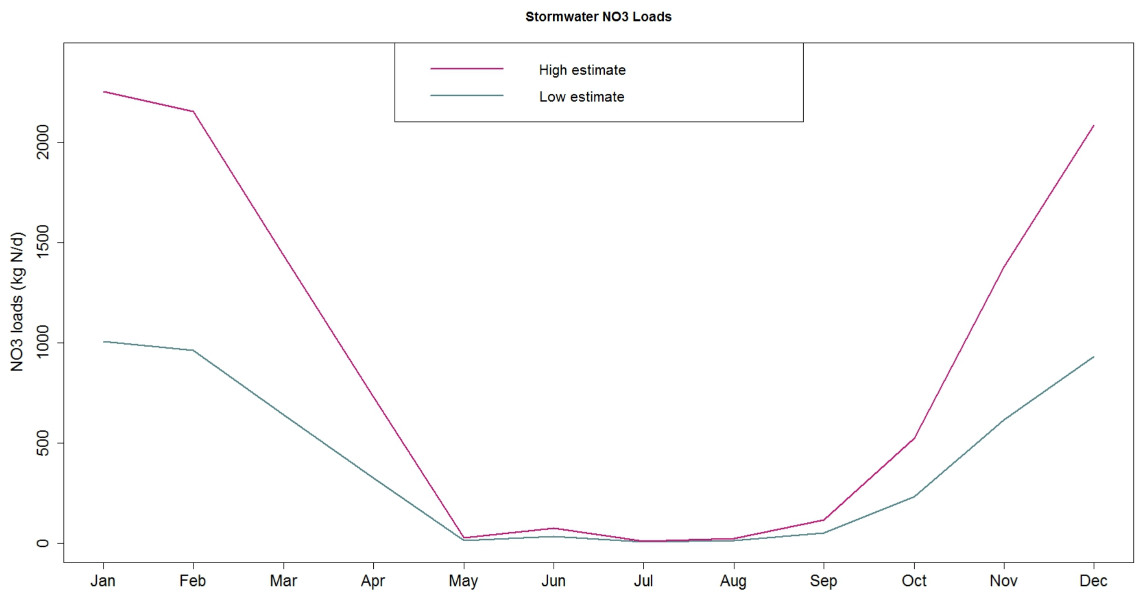

Figure 6.17 Estimated stormwater NO3 loads from two watersheds that drain directly into Suisun Bay. Loads were estimated using monthly average precipitation values, average runoff coefficient for each watershed (weighted by land-use), watershed area and stormwater NO3 concentrations from the literature. See Appendix 6.3 for further information.

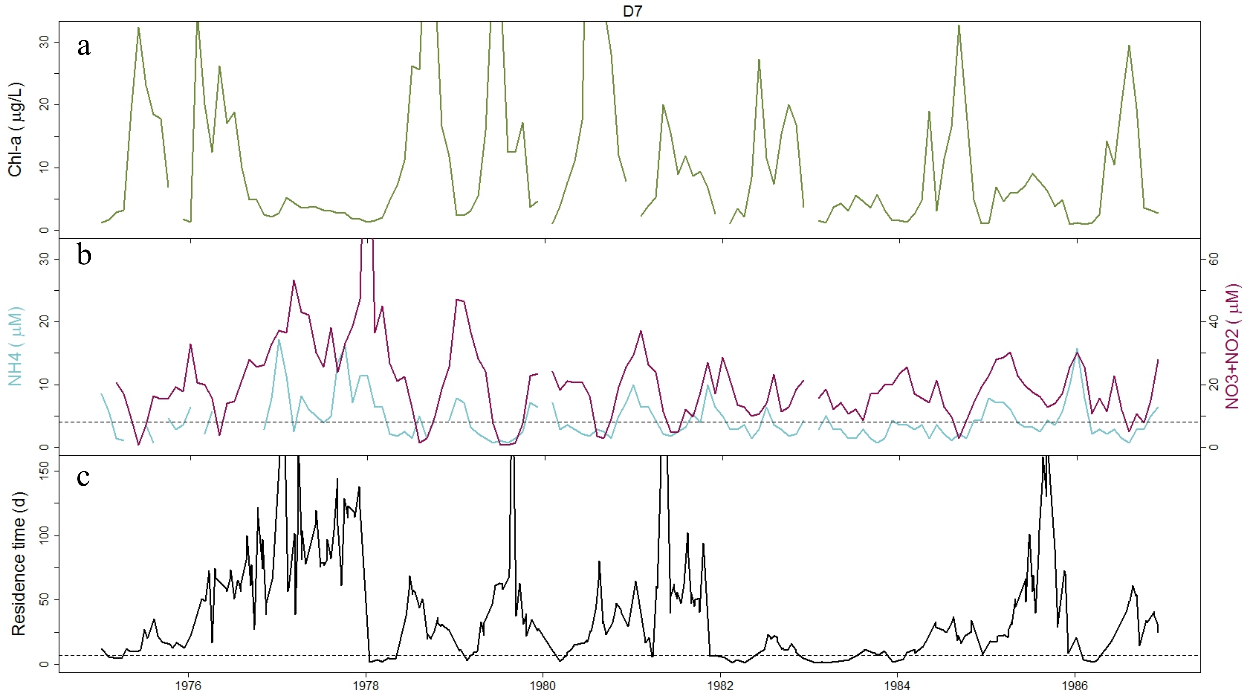

Figure 6.18 Chl-*a* concentrations (panel a), NH4 and NO3 concentrations (panel b) and residence time (panel c) in Suisun Bay for the period 1975-1986. This figure can evaluate the potential for NH4 inhibition of primary production (Dugdale et al, 2007) prior to the influence of the *Corbula* clam invasion (1987) or significant increases in NH4 loading from CCCSD and Sacramento Regional Water Treatment Plant (SRWTP) during the 1990's (Fig. 6.14, Jassby 2008). Residence time was calculated by dividing the volume of Suisun Bay (6.54e11 L) by daily advective flows.

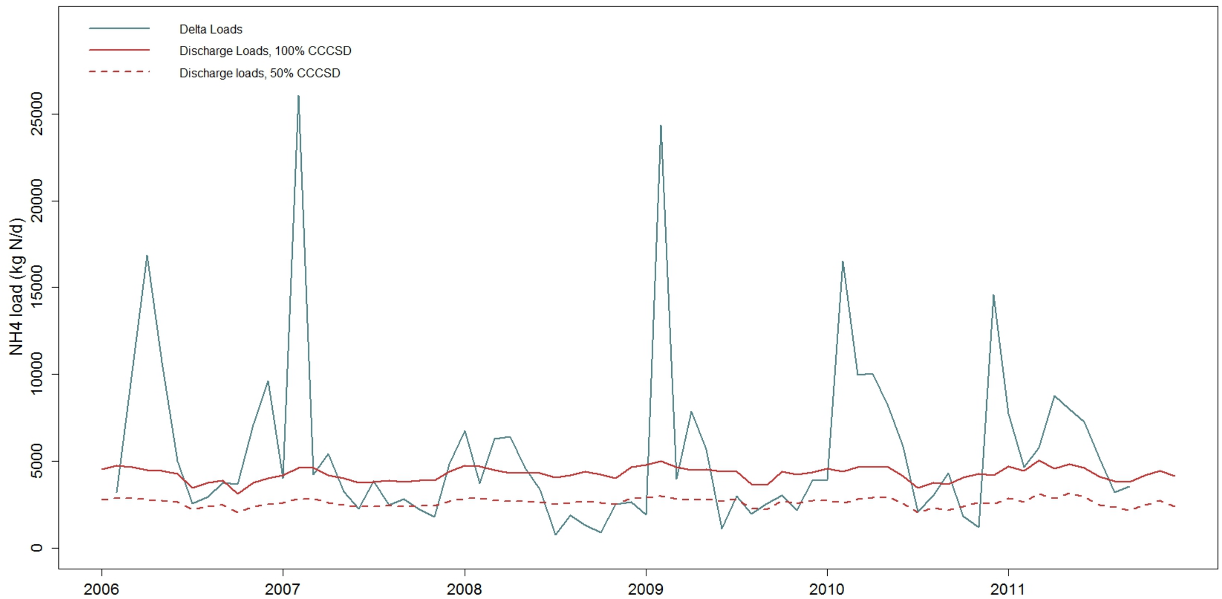

Figure 6.19 Comparison of Delta efflux loads to Suisun Bay (blue line) and direct POTW discharge loads assuming 100% mixing of CCCSD effluent (red solid line) and 50% mixing of CCCSD effluent (red dashed line) for the period 2006-2011. During high-flow periods, loads are dominated by the Delta, however 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

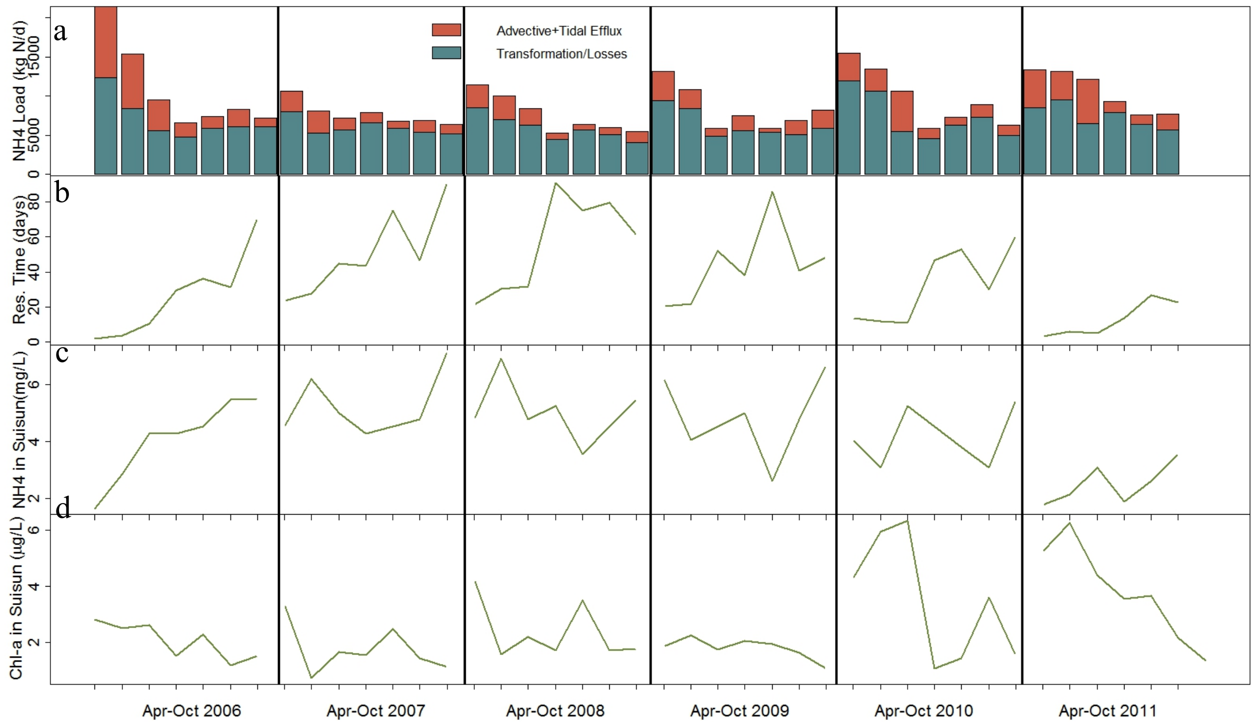

Figure 6.20 a) Comparison of the fate of NH4 entering Suisun Bay during April-October 2006-2011. Transformations/losses are always greater than advective/tidal efflux, particularly in the late summer months. Tranformations/losses are largest in April and May, which may be indicative of phytoplankton updake. **b)** Residence time in Suisun Bay. Residence time was calculated by dividing the volume of Suisun Bay (6.54e11 L) by daily advective flows. As residence time increases, the contribution of transformations/losses to the fate of NH4 increases. **c)** NH4 concentrations in Suisun Bay (average of DWR/IEP stations D6, D7 and D8). With the exception of 2006, concentrations were approximately steady through April-October. **d)** Chl-*a* concentrations in Suisun Bay (average of DWR/IEP stations D6, D7 and D8). Even at times when chl-*a* is not accumulating in the system (e.g. April and May 2006), the magnitude of transformations/losses during these months suggests phytoplankton uptake may be high, especially considereing low observed NH4 during these times as well. Chl-*a* concentrations may be kept low by clam grazing.

7. Recommended next steps

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1. General:

A coordinated nutrient science program needs to 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, a Delta-Suisun nutrient research program could be readily coordinated with the Bay-wide nutrient strategy and with IEP. Developing such a coordinated nutrient science program is consistent with recent recommendations in the Delta Plan V6.0.

***2. NH4 inhibition hypothesis**:*

2.a To develop the scientific questions and the specific studies (and study designs) that are needed to address these questions, a scientific panel should be convened. This panel should consist of regional scientists working on phytoplankton ecology and nutrient issues in the Bay, as well as outside experts. The panel should be challenged to explore the detailed evidence from studies in San Francisco Bay and literature from other systems and identify: scientific 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 consensus document summarizing their observations and recommendations, and that document can serve as the final chapter to a revised version of this report.

2.b. Whether or not NH4 inhibition is a viable mechanism, its potential importance at the ecosystem scale, relative to other factors known to play important roles in limiting primary production rates (e.g., light limitiation) or biomass accumulation (clam grazing, residence time) in Suisun Bay, has not been adequately investigated. Such an analysis could be carried with relatively basic biogeochemical models and existing data, and using parameterizations of the proposed mechanisms. These modeling efforts have benefits far beyond testing the NH4 hypothesis, in that they will provide simultaneously provide a tool for quantitatively synthesizing existing nutrient and phytoplankton data in Suisun Bay and other embayments (e.g., Lower South Bay), identifying data and monitoring needs, and informing the broader modeling strategy for the Bay.

3. NH4 toxicity to copepods:

The chronic toxicity test of Teh et al. (2011) should be replicated. Recognizing that this study has drawn criticism in the past, 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). While other more nuanced questions and complex study designs may eventually be warranted (e.g., effect of food limitation and NH4), replicating the chronic toxicity experiment first, and determining if similar or different thresholds are observed, is a logical next step. The revised study design should include lower NH4 concentrations to establish a no observed effect level (NOEL). The need for carrying out the experiment at different salinities relevant to Suisun Bay also deserves consideration.