Section 2

THE "AMMONIUM PARADOX": A SUMMARY OF MORE THAN A DECADE OF RESEARCH INTO PHYTOPLANKTON PROCESSES AND NITROGEN RELATIONSHIPS IN THE NORTHERN SAN FRANCISCO ESTUARY.

Richard Dugdale, Frances Wilkerson and Alexander E Parker

This review chapter focuses on the role that ammonium (NH_4; reduced form of nitrogen) plays in determining the likelihood of phytoplankton bloom formation and reduced primary productivity in the northern San Francisco Estuary (nSFE). Our goal is to summarize the research that we have carried out since 1997 leading to the concept of an "Ammonium Paradox", in which NH_4 prevents phytoplankton from being able to access the larger pool of nitrogen, which in the nSFE is nitrate (NO_3), resulting in persistent low chlorophyll, lack of blooms and the export of unused NO_3 to the coastal ocean. The Ammonium Paradox is a consequence of three elements: 1) NH_4 inhibition, 2) NO_3 shift-up and 3) a predictable sequence of nitrogen drawdown to initiate blooms. The four sections that follow, describe how different forms of nitrogen (reduced and oxidized) have been recognized as contributing to phytoplankton productivity in different ecosystems (Section 5.1). Then we describe how the research carried out by our group applies these paradigms to the nSFE where typically NO_3 is high, chlorophyll low and NH_4 high enough to interfere with NO_3 uptake, but insufficiently high to fuel phytoplankton blooms alone (Section 5.2). In the third section, we develop a conceptual model based upon the Ammonium Paradox, in which NH_4 acts as a gatekeeper, determining whether the phytoplankton can access the larger pool of dissolved inorganic nitrogen (i.e. NO_3) for growth (Section 5.3). Numerous environmental variables (abiotic and biotic) can impact the NH_4 gatekeeper. Our approach has been to try to understand one of the multiple, synergistic drivers of the low productivity condition in a very complicated estuarine ecosystem. Ultimately a holistic aspect of how they all interact is needed in order to understand phytoplankton growth in the estuary and rivers. The last section (Section 5.4) provides a summary of our assessment of key uncertainties and research priorities looking to the future. There is work yet to be done (and some work that is happening) to investigate these interactions.

5.1. Introduction: Nutrient Paradigms Concerning Reduced Versus Oxidized Form of Nitrogen

5.1.1 High-nutrient, low-chlorophyll estuarine ecosystems

Nutrient pollution is long recognized as a stressor for urbanized estuaries and coasts. With increased population growth, estuaries such as the San Francisco Estuary (SFE) are particularly susceptible to nutrient enrichment (e.g., Painting et al. 2007; Cloern 2001). The traditional paradigm is that nutrient enrichment leads to eutrophication (the production of organic matter) or cultural eutrophication (increased organic matter production resulting specifically from anthropogenic nutrients such as fertilizer runoff or sewage discharge; e.g., Fisher et al. 2006) and results in noxious or harmful algal blooms or poor trophic transfer and hypoxic (low oxygen) conditions, fish kills, and reduced beneficial uses of coastal waters (e.g., recreation). The reality is that estuaries exhibit a broad spectrum of responses to nutrient enrichment, making a single national management strategy elusive (Glibert et al. 2010).

One observed alternative response of high nutrient estuaries is *lower growth* than expected, based upon nutrient input. For example, since the late 1980's there has been persistently low phytoplankton biomass in the nSFE (e.g., Cloern 1996) and measurements of annual primary production in the Suisun Bay (Wilkerson et al. 2015) and the Low Salinity Zone of SFE (Kimmerer et al. 2012; Parker et al. 2012c; Lidstrom 2008) place the nSFE in the lower portion of the oligotrophic category of coasts and estuaries by Nixon (1995) i.e., <100 g C $m^{-2}yr^{-1}$. These rates are among the lowest primary production rates of estuarine-coastal ecosystems of the world that include the Gulf of Finland, Dumbell Bay and Colne (Cloern et al. 2014), and should be considered high-nutrient, low-chlorophyll (HNLC; Cloern 2001) or high-nutrient, low-growth (HNLG; Sharp 2001) and experiencing oligotrophication (Nixon 1990).

Conventional wisdom regarding the SFE is that the availability of photosynthetically active radiation limits primary productivity (Cole and Cloern 1984; Alpine and Cloern 1992), and growth in the nSFE is assumed to be regulated by high suspended sediment concentrations and lack of water column stratification that decreases phytoplankton light availability (Cloern 1987, 1991). Chlorophyll accumulation is proposed to be controlled largely by grazing (e.g., Kimmerer 2004). It has long been assumed that nutrients do not play a role in phytoplankton processes in the SFE as nitrogen (N) and phosphorus (P) are found in excess of phytoplankton nutrient demand and so the estuary is said to be resilient to anthropogenic nutrients and cultural eutrophication (Cloern and Jassby 2012). However, such an assumption is not based on process-based measurements of phytoplankton demand. In fact, we are unaware of published measures of N or P uptake in the SFE prior to 1999 (Wilkerson et al. 2006). Recent studies of the nSFE show either a breakdown in some of this resilience (i.e., potential for cultural eutrophication - Jassby 2008) or other signs of nutrient impacted ecosystem function (Dugdale et al. 2007;

Glibert 2010; Glibert et al. 2011; Parker et al. 2012a). The estuary has generally not followed the estuary eutrophication paradigm but has seen significant changes in the natural phytoplankton bloom cycles and a long term decrease in total chlorophyll-*a*, a proxy of phytoplankton biomass and primary production (Cole and Cloern 1984, Jassby et al. 2002). A growing body of work points to nutrient composition and availability as contributors to the low growth condition (Dugdale et al. 2007; Parker et al. 2012a, b, Glibert 2010, Glibert et al. 2011, 2014a, b).

Similar situations have been described for other estuarine and coastal ecosystems. For example, in the Scheldt Estuary the low algal biomass was noted by Cox et al. (2009) to be "contrary to expectations from the classical eutrophication response ". Nixon (1990) observed a low productivity response to elevated nutrients in Narragansett Bay and termed it oligotrophication. Elevated N as NH_4 from wastewater effluent was implicated in depressed primary production in the Saronikos Gulf of Greece (Dugdale and Hopkins 1978), Wascana Creek, Canada (Waiser et al. 2010), Hong Kong waters (Xu et al. 2012) and along the California coast (MacIsaac et al. 1979) where there was also lower nitrate (NO_3) uptake.

Sharp (2001) suggested that nutrient-enriched estuaries may not respond classically but rather show characteristics of high-nutrient, low-growth (HNLG). Working with nearly 40 years of estuarine data for the Delaware Estuary, Yoshiyama and Sharp (2006) observed primary productivity per unit chlorophyll *a* declined exponentially with increasing NH_4 concentration (<10 µM NH_4) and attributed this effect to a shift in the form of N being used, leading to lower growth. Parker (2004, 2005) also working the Delaware Estuary observed shifts in the estuarine food web, with differences in phytoplankton biomass production but also increased production of dissolved organic matter and microbial loop processes with changing N form.

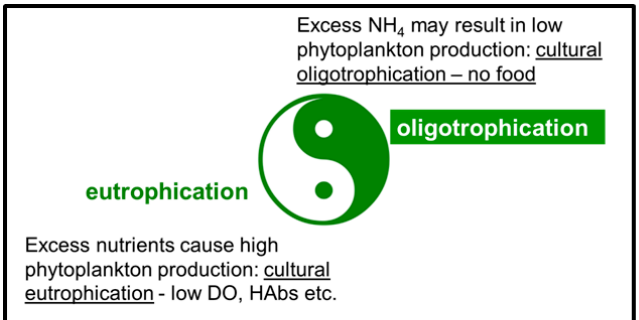

Figure 1. The dual effects of anthropogenic nutrients that may result in high production (cultural eutrophication) or low production (cultural oligotrophication).

Our SFE research over the last 18 years has shown the importance of considering the form of N when evaluating the impact of nutrients in an estuarine system. In the nSFE and Sacramento River, the association of wastewater NH_4 and declining primary production appears related to decreased NO_3 uptake by phytoplankton; thus higher NH_4 leads to lower uptake of NO_3 and carbon (oligotrophication) (Dugdale et al. 2007, 2012; Parker et al. 2012a, b; Wilkerson et al.

2015). Given the relatively limited number of management options available to restore phytoplankton growth, the historical viewpoint and traditional paradigm that " nutrient concentrations don't matter" needs to be re-evaluated in light of insights gained with ten years of nutrient-related peer-reviewed research in the SFE (**Section 5.2: Key Findings**).

5.1.2. New and regenerated production in the ocean

The consequences of phytoplankton use of NO_3 or NH_4 are not a new concept but was developed for the ocean, and summarized as the classic oceanographic paradigm of new and regenerated production (Dugdale and Goering 1967). The oceanographic paradigm recognizes the distinction between phytoplankton production resulting from reduced forms of N, primarily NH_4 and urea, that are regenerated *in situ* (from zooplankton excretion or bacterial remineralization) versus production resulting from the use of oxidized N forms, primarily NO_3, resulting from allochthonous inputs to a system. The concept of new production was developed to understand how to predict the yield (e.g., as a fishery) or potential export (as sinking particles) of carbon in an ecosystem

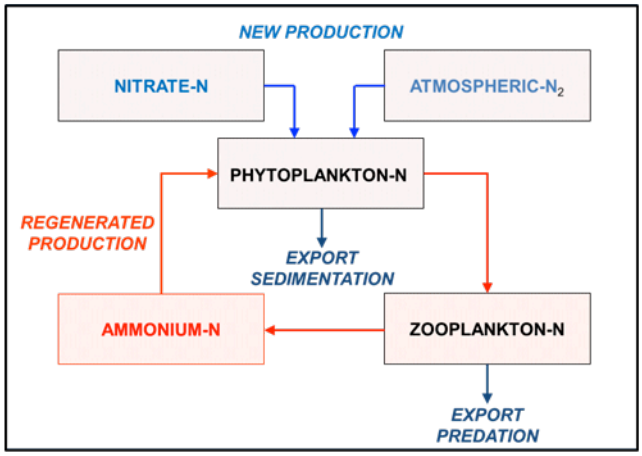

Figure 2. Schematic flow of NO_3 and N_2 (in blue) resulting in new production and export compared to that of NH_4 in regenerated production (red) in the ocean

The insights into new and regenerated production were possible because Dugdale and Goering (1967) used the stable isotope ^{15}N as a tracer in order to separate out the pathways of inorganic N species in transformations, including the uptake and assimilation of NH_4 and NO_3 by phytoplankton (Dugdale and Wilkerson, 1996). In the ocean, and in most natural aquatic and freshwater environments, NH_4 is a transition form of dissolved inorganic nitrogen (DIN), present only as an intermediate product in the bacterial oxidation of NH_4 to NO_3. This process, nitrification, results in NH_4 concentrations being held to low (<1 µmol L^{-1}) values. Consequently,

in the ocean, NH_4 generally occurs primarily in near surface waters as a product of grazing and NO_3 occurs in high concentrations in deep water. High productivity regions in the ocean occur where nutrient- (especially NO_3) rich sub-surface water is brought to the surface by upwelling. High rates of nutrient uptake and high rates of primary production occur due to the large amounts of NO_3 made available to phytoplankton. Little primary production can be achieved based on NH_4 uptake, since very little NH_4 is present and NH_4 supply is controlled by rates of bacterial regeneration and grazing rates.

Recent evolution of phytoplankton, including diatoms, has taken place in an oxidized world, with elevated NO_3 and low NH_4 conditions and algal physiology reflects this (Glibert et al. in review and references therein). The diatoms that were successful in cooler NO_3-rich waters, e.g. in coastal upwelling evolved in order to acquire the abundant NO_3 and assimilate it rapidly in comparison to the low concentrations of NH_4. In the modern oceans, high productivity areas such as upwelling systems (e.g., Peru, Baja California, and Northwest Africa) with high periodic NO_3 at the surface, exhibit both biomass specific and absolute NO_3 uptake rates by phytoplankton (Table 1) that always exceed NH_4 uptake rates (e.g. Wilkerson and Dugdale 2008 and refs therein). The ratio of NO_3 uptake to the sum of NO_3 plus NH_4 uptake is called the "f-ratio" (e.g., Eppley and Peterson 1979) and high primary productivity in the ocean is correlated with high f-ratios. Our research has shown that the same is true in the SFE.

Table 1 High nitrate uptake and f-ratios in coastal upwelling areas

Upwelling Area	ρNO_3, $\mu g\text{-at } l^{-1} h^{-1}$	VNO_3, h^{-1}	mean *f*-ratio	Reference
Oregon	1.29	0.08	0.86	Dickson and Wheeler, 1995,
Monterey Bay, CA	0.55	0.08	0.86	Kudela, 1997
Bodega Bay, CA	0.46	0.08	0.80	Dugdale et al. 2006
15°S, Peru	0.57	0.122	0.82	Wilkerson et al., 1987
Benguela	0.55		0.71	Probyn, 1985; 1992
Cap Blanc	0.36	0.04	0.7	Wilkerson et al. 1987

Most coastal pelagic ecosystems supplied with NO_3 and displaying high new production - e.g., coastal upwelling systems are dominated by diatoms (Estrada and Blasco 1985; Chavez et al. 1991; Lassiter et al. 2006) and typically have short efficient food webs at the base of major natural fisheries (e.g. coastal Peru; Ryther 1969) and high rates organic matter export from the photic zone (e.g., Pilskaln et al. 1996). This is primarily because, amongst the phytoplankton, diatoms are generally NO_3 opportunists. In cool, nutrient-rich environments, the >20 μm biomass (mainly diatoms) uses a disproportionate fraction of total N as NO_3 even when NH_4 is otherwise available (Dauchez et al. 1996; Koike et al. 1986; Lomas and Glibert 1999 a, b; Maestrini et al. 1982, 1986; Nalewajko and Garside 1983; Parker et al. 2010; Probyn 1985; Probyn and Painting 1985; Probyn et al. 1990). Additionally the occurrence of many rapidly growing diatom species

has been highly correlated with the large and/or frequent additions of NO_3 (e.g., Goldman 1993; Lomas and Glibert 1999a; Rothenberger et al. 2009). In SFE, enclosures enriched with NO_3 and held at low light showed increased fucoxanthin concentrations (reflective of diatoms) accompanying NO_3 drawdown (Glibert et al. 2014b.).

In contrast, a proportionately greater flow of organic material through the microbial loop has generally been shown to occur when systems are more enriched with chemically reduced N forms, NH_4 and urea, and the resulting community composition is often dominated by mixotrophic dinoflagellates or (pico) cyanobacteria (e.g., Legendre and Rassoulzadegan 1995; Berg et al. 1997, 2003; LaRoche et al. 1997; Lomas et al. 2001; Glibert 1998, Glibert et al. 2001, 2006, 2010). Many harmful algal bloom (HAB) events, especially those caused by dinoflagellates, raphidophytes or cyanobacteria have also been associated with increased dominance of N in reduced rather than oxidized form (e.g. Kudela et al. 2005).

Changes in the food web resulting from a shift in the dominant form of nitrogen (NH_4 versus NO_3) making up the nutrient source have been described. For example, the long-term increases in NH_4 loadings to the nSFE and Sacramento River may have contributed to changes in zooplankton (larger to smaller, e.g. Winder and Jassby 2011) and fishes as the phytoplankton communities changed from diatoms to "NH_4-tolerant" phytoplankton taxa such as cryptomonads and flagellates (Glibert et al. 2011). This may have also helped to promote an enhanced microbial food web (Bouley and Kimmerer 2006; Gifford et al. 2007; Rollwagen Bollens et al. 2011; York et al. 2013), although there are no long-term data on microzooplankton for the SFE.

5.1.3. Physiological response of phytoplankton to multiple and different nitrogen forms

Interactions

The interactions between the various chemical forms of inorganic N are important considerations in the promotion of phytoplankton blooms. Simultaneous uptake of multiple N sources has been reported in phytoplankton (e.g., Conover 1975; Kuenzler et al. 1979; Harrison et al. 1982; Price et al. 1985; Collos et al. 1989), although some N forms may inhibit the uptake of other forms. For example NO_3 may inhibit NH_4 uptake (e.g. Dortch 1990; Caperon and Ziemann 1976; Yin 1988), NH_4 and urea have been described as inhibiting uptake of each other (Healy 1977; Molloy and Syrett 1988), but these interactions are rarely reported compared to the more common interaction well recognized in the oceanographic and algal culture literature, that of NH_4 inhibition of NO_3 uptake (e.g., Conway 1977; Cresswell and Syrett 1979, Eppley and Rogers 1970 and many later publications).

NH_4 inhibition of NO_3 uptake

More correctly referred to in algal physiology as repression, but commonly termed inhibition, (as we will use here) this phenomenon has been described in both cultures and the field (freshwater and marine) and many of the earlier studies were reviewed in Dortch (1990). The threshold and severity of NH_4 inhibition may vary with species, physiological status and environmental conditions (e.g., Dortch, 1990), and cell size (e.g., L'Helguen et al. 2008). Concentrations as low as 0.1 to 0.3 µmol N L^{-1} NH_4 have been shown to completely inhibit NO_3 assimilation in the subarctic Pacific (Wheeler and Kokkinakis 1990) (Figure 3). Field studies in upwelling areas using the tracer ^{15}N showed the inhibition of NO_3 uptake by NH_4 at low ambient concentrations in the range of 1 - 2.5 µmol L^{-1} (Dugdale and MacIsaac 1971, for Peru; Dugdale et al. 2006 for Bodega Bay, CA) and the same relationship was shown in sewage plumes, e.g., in the Saronikos Gulf, Greece (Dugdale and Hopkins 1978) and the California coast (MacIsaac et al. 1979).

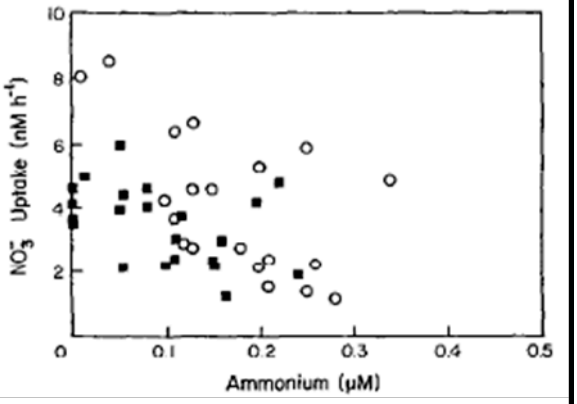

Figure 3 Repression of NO_3 uptake in subarctic Pacific with NH_4 concentrations < 0.5 µmol L^{-1} (from Wheeler and Kokkinakis, 1990). Data from 1987 (squares) and 1988 (circles)

There is a great deal of confusion as to whether NO_3 uptake inhibition is actually a manifestation of NH_4 as a preferred N source, although Dortch (1990) is careful to separate the two. With the exception of N-deficient algae, when both N sources are co-provided, NH_4 is typically drawn down first as it is energetically the least "expensive" N form to metabolize (i.e., preferentially used), due to the of number of electrons required to reduce NO_3 to NH_4 (Losada and Guerrero 1979; Syrett 1981). However, the presence of NH_4 (or its assimilation product(s)), in the cell also interferes with the transport of NO_3 across the cell membrane (Figure 4) and the enzymatic reduction of NO_3 (Syrett 1981; Dortch, 1990; Glibert et al. in review) (Figure 4). This results in down-regulation of NO_3 transporters (NRTs) on the cell membrane. Down regulation of NRTs, i.e., repression of gene transcripts for NRTs has been described in freshwater green algae (e.g.,

(Navarro et al. 1996; Koltermann et al. 2003; He et al. 2004) and in diatoms (Hildebrand and Dahlin 2000; Song and Ward 2007).

As is the case for regulation of transporters by NH_4, so too for NO_3 assimilation, NH_4 suppresses both abundance and activity of the NO_3 assimilatory enzyme, nitrate reductase (NR), For example, Eppley et al. (1969) first described the effect of NH_4 on NR activity. Vergera et al. (1998) showed it to decrease both the amount of enzyme protein synthesized and activity in the marine diatom *Thalassiosira weissflogii.* The molecular basis for this has been shown in diatoms. When NH_4 was present, the gene for NR was expressed, but not translated (Flores et al. 2005, Parker and Armbrust 2005, Poulsen and Kröger 2005, Poulsen et al. 2006). In most algae, the regulation is via the size of the glutamine pool (the product of NH_4 assimilation). When glutamine levels are high (as a result of NH_4 assimilation), NR activity levels are reduced (Campbell 1999). A detailed review of how NH_4 interacts with NO_3 cellular metabolism in different phytoplankton groups is given by Glibert et al. (in review; and this volume).

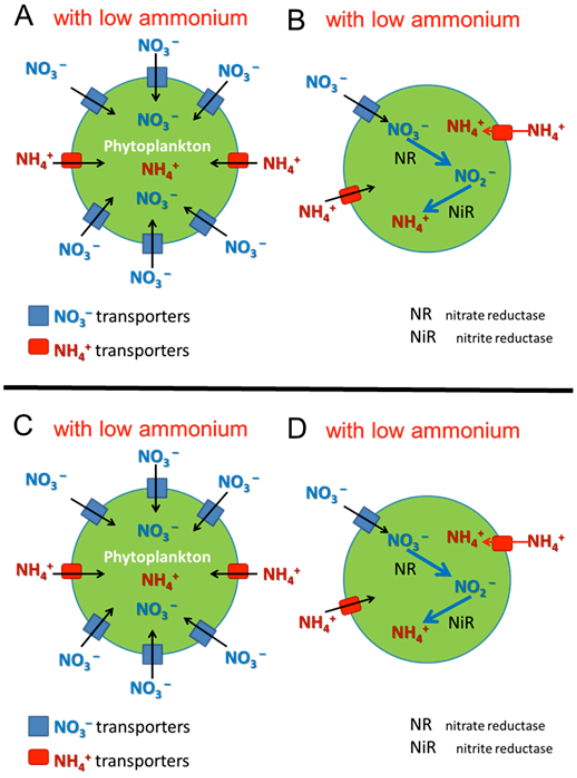

Figure 4 Simplified schematic of the interaction of NH_4 on NO_3 transport and assimilation with low NH_4 impact (A<B) and high repressive concentrations of NH_4 (C, D). High NH_4 blocks NO_3 transporters (crosses) and inhibits nitrate reductase catalysis of NO_3 to NO_2 (red cross)

In field conditions (or in laboratory conditions in which mixed substrates are used), it is the interaction of substrates and uptake kinetics that leads to repression of metabolism, inhibition of NO_3 uptake, and ultimately of growth. These interaction kinetics are regulated by relative substrate concentrations, nutritional and growth state of the cells, and species specific differences. The result is both preferential use and inhibition. This is the underlying physiological basis of the "Ammonium Paradox" (Dugdale et al. 2012).

NH_4 toxicity versus inhibition

Unialgal cultures can grow on elevated NH_4 without NH_4 toxicity (Collos and Harrison 2014). However there is no question that under excessive NH_4 conditions (i.e. 100 µmol L^{-1} or mM levels) NH_4 can be toxic (Keller et al. 1987; Natarajan 1970). High NH_4 concentrations can be

toxic to cells via destabilization of membranes and other metabolic effects (e.g., Britto and Kronzucker 2002).

Parker et al. (2012a, b) reported a process separate from the physiological inhibition of NO_3 uptake by NH_4 affecting phytoplankton growth in the Sacramento River, i.e., NH_4 toxicity and speculated that NH_4 or some unidentified toxicant associated with NH_4 input (i.e., through WWTP effluent) was inhibiting phytoplankton NH_4 uptake and associated C uptake., This has led to some confusion or perception that our research suggests that phytoplankton in the SFE *cannot* grow on NH_4. In fact, each of the studies that we have conducted on phytoplankton N uptake in the SFE has demonstrated that NH_4 is typically assimilated by phytoplankton over other N substrates, but rarely results in a bloom as the ambient NH_4 is insufficient to fuel high amounts of chlorophyll, and the larger N pool, NO_3 needs to be accessed.

Shift-up of NO_3 uptake

The cell physiology concept of "shift up" needs to be considered when studying how phytoplankton may respond differently to NO_3 and NH_4. Shift-up was first described by Schaechter (1968) who found he could increase the growth rate (shift-up) of a cultured bacterium by introducing a more energy-rich substrate. It was then used by Button and colleagues (e.g. Robertson and Button 1989 and references therein) to describe induction or acceleration of uptake of a substrate (toluene) when the substrate availability was increased. This acceleration or shift-up was described for NO_3 uptake (and NO_3 reductase activity) by phytoplankton (Dugdale et al. 1981 and refs therein), first by Ishizaka et al (1983), then MacIsaac et al. (1985) and Wilkerson and Dugdale (1987) (Figure 5) and modeled by Zimmerman et al. (1987) and Dugdale et al. (1990). This acceleration in NO_3 uptake represents the up-regulation of the cellular machinery to process NO_3 in response to its availability (Smith et al. 1992; Berges et al. 2004; Lomas 2004; Allen et al. 2006).

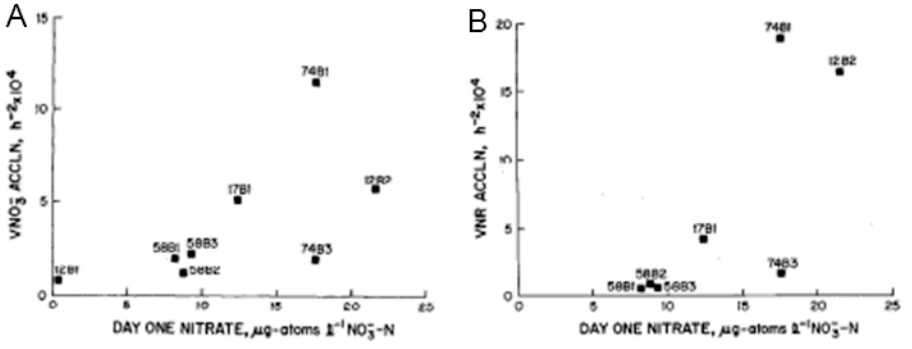

Figure 5 Acceleration (shift-up) of A) NO_3 uptake and B) nitrate reductase activity vs.NO_3 concentration in enclosures filled with upwelled water containing different ambient NO_3 concentrations, from Wilkerson and Dugdale (1987).

Shift-up of NH_4 uptake has not been described to our knowledge. Also, in enclosures using SFE water where this was tested, no acceleration in NH_4 uptake was observed whereas the same water showed acceleration (shift-up) of NO_3 uptake. This is in part a consequence of the difference in the way the NO_3 and NH_4 transporters and assimilation enzymes are regulated. In general, both NO_3 transporters (NRTs) and NR (and transcription of the gene for NR) are induced by the presence of their substrate (NO_3), whereas NH_4 transporters are induced by the absence or deficiency of their substrates, or repressed by increased availability of their substrate (NH_4) (Clarkson and Luttge 1991; Navarro et al. 1996; Crawford and Glass 1998, Daniel-Vedele et al. 1998; Glibert et al. in review). Thus, increasing concentrations of NO_3 yield more NRTs and drives shift-up response, whereas increasing concentrations of NH_4 yield fewer AMTs, and so no acceleration in uptake would be expected. NH_4 addition tends to act at the cellular level as a repressor of NH_4 transport and its assimilation, down-regulating these processes when availability of NH_4 increases in the cell (Flynn and Fasham 1997; Flynn et al. 1997; Lindell and Post 2001; Coruzzi and Bush 2001; Tanigawa et al. 2002; Muro-Pastor et al. 2001, 2005; Ohashi et al. 2011; Post et al. 2012)

5.1.4. The role of NH_4 in suppressing phytoplankton blooms in Suisun Bay

An observable consequence of the interaction of NH_4 and NO_3 on phytoplankton production in the field was described for SFE (Wilkerson et al. 2006, 2015; Dugdale et al. 2007) and the Sacramento River (Parker et al. 2012b).

The nSFE traditionally had phytoplankton blooms of > 30 µg L^{-1} chlorophyll in the 1970's that were the result of full utilization of available DIN - both NH_4 and NO_3 (Di-Toro et al. 1977; Ball and Arthur 1979). These blooms have been uncommon or rare since the late 1980's, (except spring blooms observed in Dugdale et al. 2012 and Glibert et al. 2014a), a change attributed to the appearance of a voracious invasive clam *Potamocorbula amurensis* (Nichols and Thompson 1985; Alpine and Cloern, 1992).

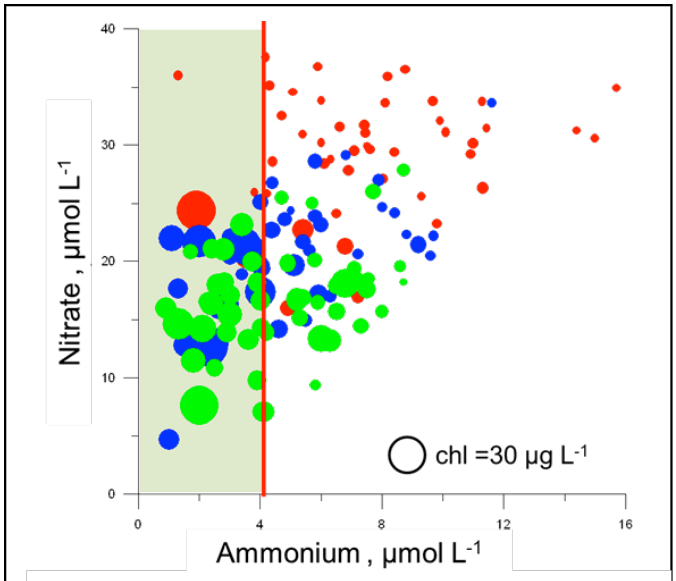

Figure 6 Chlorophyll concentration (bubbles, µg L^{-1}) measured in Suisun (red), San Pablo (blue) and Central (green) Bays showing less chlorophyll at higher NH_4 concentrations

However, the clams are not present at all locations and at all times of the year and so cannot be considered as the sole cause of low phytoplankton. Spring blooms occur in Suisun Bay at a time when populations of bivalves are typically low. Blooms have also occurred when high *P. amurensis* abundance was observed (Dugdale et al. 2012; Wilkerson et al. 2015). NH_4 inputs to the Sacramento River by the Sacramento Regional Sanitation District Wastewater Treatment Plant (Regional San, referred to in previous publications as Sacramento Regional Wastewater Treatment Plant) were increasing at the same time as a result of population increase (Jassby 2008) and these increasing NH_4 concentrations that occurred as the newly upgraded plant came on line in 1982 have been cited as the cause of the reduced phytoplankton concentrations in Suisun Bay (Glibert 2010; Glibert et al. 2011).

Time series data collected monthly (and weekly during the spring months) from 1999 to 2003 in Central, San Pablo and Suisun Bay (the latter in nSFE) indicated that elevated chlorophyll was often associated with low NH_4 and high NO_3 concentrations (Wilkerson et al. 2006; Dugdale et al. 2007) and Figure 6.

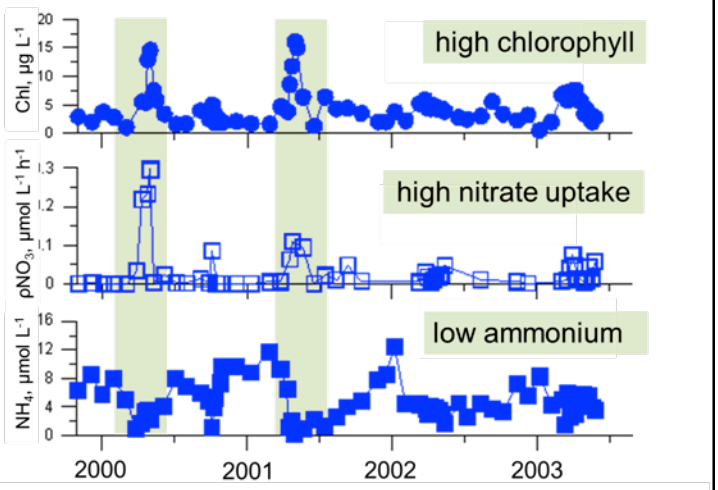

Figure 7 Monthly (and weekly in spring) data from San Pablo Bay showing high chlorophyll accompanied by high NO_3 uptake and low NH_4 concentrations (grey panels) (from Wilkerson et al. 2006).

Nitrogen-15 tracer uptake measurements made starting in 1999, show that phytoplankton in the nSFE were using NH_4 for growth and not reaching high biomass unless NO_3 uptake occurred (Wilkerson et al. 2006; Dugdale et al. 2007) (Figure 7). Use of NO_3 was a common feature of the few spring blooms (>30 µg L^{-1} chlorophyll) observed in the nSFE since 1999 (i.e., in 2000, see Wilkerson et al. 2006; Kimmerer et al. 2012; Parker et al. 2012a, c; in 2010, see Dugdale et al. 2012, in 2014, see Glibert et al. 2014a) and was accompanied by a decline in the ambient

concentration of NH_4 to ~1 µmol N L^{-1} (Wilkerson et al. 2006; Dugdale et al. 2012). This indicated an inhibitory role of NH_4 in modulating blooms as a result of a physiological interaction minimizing access to NO_3 (the larger DIN pool in nSFE) by the phytoplankton.

If NH_4 inhibition were to be relieved by a decrease in NH_4 concentration to below some physiological threshold (e.g., by phytoplankton uptake, nitrification or dilution) then the algae can access the elevated NO_3 pool, accelerate their NO_3 uptake (shift-up) in response, and build biomass (i.e. chlorophyll) at a rate that outpaces grazing losses. This was shown in a set of SFE enclosures (Figure 8; also in Dugdale et al. 2007) that started with either low or high ambient concentrations of NH_4. NO_3 drawdown and uptake (as measured with ^{15}N tracers) did not commence until NH_4 concentrations were below a threshold of ~ 4 µmol L^{-1}. NO_3 uptake accelerated and chlorophyll increased.

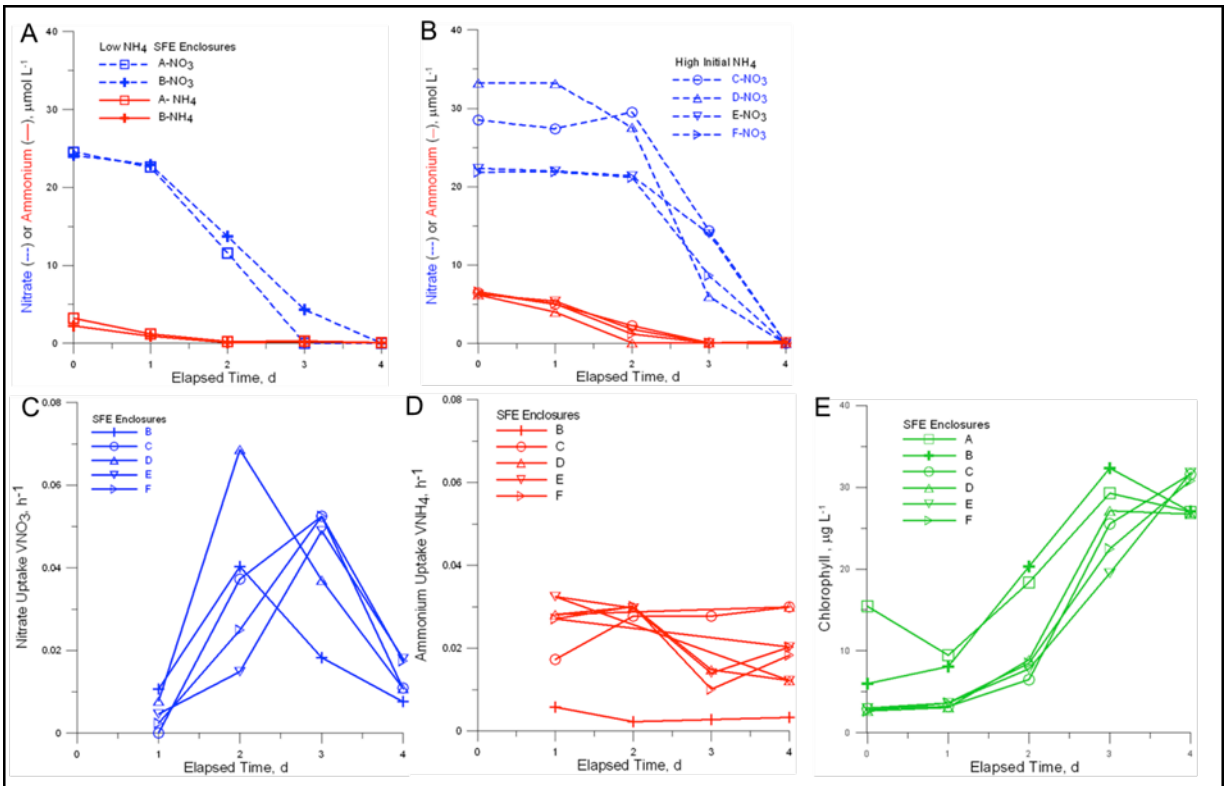

Figure 8 Enclosures filled with water from Central Bay containing low (A) or high (B) ambient NH_4 and tracked for 4 days. NO_3 drawdown (blue) does not start until NH_4 (red) has been drawn down to ~ 4 µmol L^{-1}. In the high NH_4 enclosures this results in a delay in the start of NO_3 drawdown (B). ^{15}N uptake rates show acceleration (shift-up) of NO_3 uptake (C) that results in all NO_3 being drawn down (A, B) whereas NH_4 uptake rate stay constant (D). Chlorophyll accumulated in all enclosures (E). (From Dugdale et al. 2007)

Ammonium uptake did not exhibit shift-up and remained constant throughout the experiment (Figure 6D). As a consequence of shift-up of NO_3 uptake all ambient NO_3 was drawn down in the enclosures by the fourth day of the experiment (Figures 6A, B), regardless of the starting NO_3 or NH_4 concentration.

The conclusions from these early experiments were further tested in additional experimental enclosures (Dugdale et al. 2007; Parker et al. 2010, 2012a; Dugdale et al. 2013), and later in the field in nSFE (Wilkerson et al. 2015), and led to the conclusion that lack of access to the larger pool of NO_3 limits primary production and consequently the accumulation of chlorophyll. The 96 hour enclosure experiments (and verified by field observations) showed there to be a predictable sequence of events enabling a phytoplankton bloom using the available NO_3 (Dugdale et al. 2007), accompanied by high f-ratio and elevated carbon uptake, once NH_4 is at non inhibitory levels (Parker et al. 2012a; Figure 9).

First, phytoplankton N demand is satisfied by NH_4 but with low growth; NO_3 uptake is low or near zero due to NH_4 inhibition. Once the NH_4 has been drawn down to below the inhibitory threshold (< 4 µmol N L^{-1}) NO_3 uptake is enabled, followed by continued growth and NH_4 is further reduced to ≤ 1 µmol N L^{-1}. This allows a rapid shift-up of NO_3 uptake coupled with high C uptake and a bloom rapidly develops. (e.g., Parker et al. 2012a). Of note is the fact that in experiments conducted on samples collected from the Sacramento River and Suisun Bay, and enriched equally in N but with different forms, twice the chlorophyll a was produced with NO_3 compared to enrichment with NH_4 (Glibert et al, 2014b) Similarly, elevated C:N uptake ratios were found by Parker (2004) in enclosures tested in the Delaware Estuary.

In some situations where NH_4 is the major source of DIN this sequence will not play out

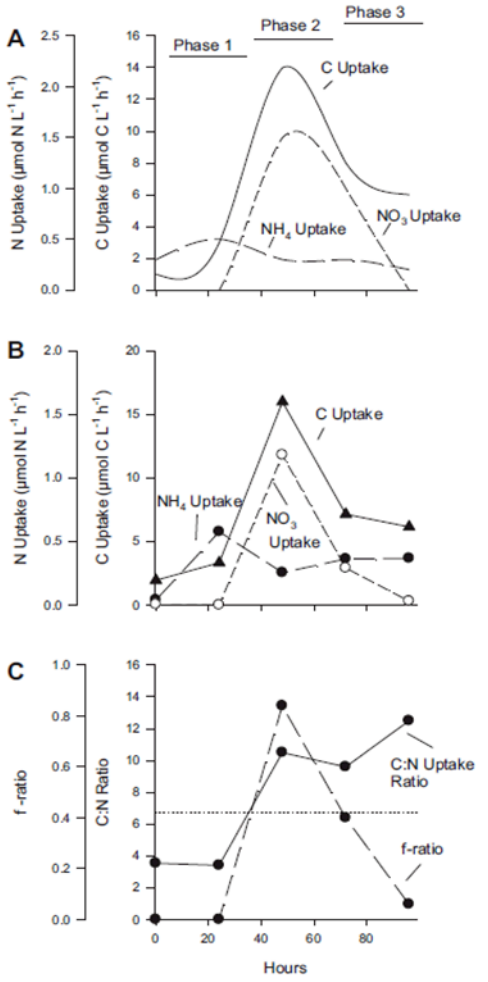

Figure 9 Idealized sequenced observed in enclosures (A) along with data (B.C) measured in enclosures in Parker et al. (2012a)

as the phytoplankton can grow on the NH_4, (although the Ammonium Paradox would predict this would not occur as rapidly as during shifted-up growth on NO_3). This was presumably the situation described by the small basin study of Esperanza et al. (2014) where, with very high residence time, supplied with 1900 µmol L^{-1} NH_4, almost a batch culture of benthic diatoms (i.e. *Cocconeis, Navicula*) and chlorophytes were able to grow to a concentration of 400 µg L^{-1} using the NH_4 and not needing to access the NO_3, since the highest DIN source was NH_4.

In the field, the sequence to access the available NO_3 observed in the enclosures is modulated by river flow conditions and residence time as well as continual inputs of NH_4, e.g., in nSFE, from the WWTPs including Sacramento Regional Sanitation District WWTP (Dugdale et al. 2012; 2013). Wilkerson et al. (2015) described how the sequence could be observed in the nSFE, especially in shallow well lit shoal conditions.

If the sequence for bloom formation is interrupted such that NH_4 is not drawn down to below an inhibitory threshold to enable NO_3 uptake, then the ecosystem is stalled in a low productivity mode. This is the "Ammonium Paradox" (Dugdale et al. 2012). The phytoplankton can grow on the available NH_4 but since NH_4 is not the major DIN pool (typically 3-8 µmol L^{-1}, Wilkerson et al. 2006), they are limited in the amount of biomass they can make using NH_4 alone and that access to the larger DIN pool, NO_3 (typically > 14 - 35 µmol L^{-1}; Wilkerson et al. 2006) is needed to reach chlorophyll concentrations of > 20 µg L^{-1}. To make 1 µg L^{-1} of chlorophyll requires 1 µmol L^{-1} N (see discussion in Dugdale et al. 2012 and also Dugdale et al. 2007) although the ratio can vary from 1 to 4 (as discussed in Glibert et al. 2014 b), so using the ambient NH_4 only in most of the SFE will only yield chlorophyll concentrations of < 10 µg L^{-1} (Figure 10).

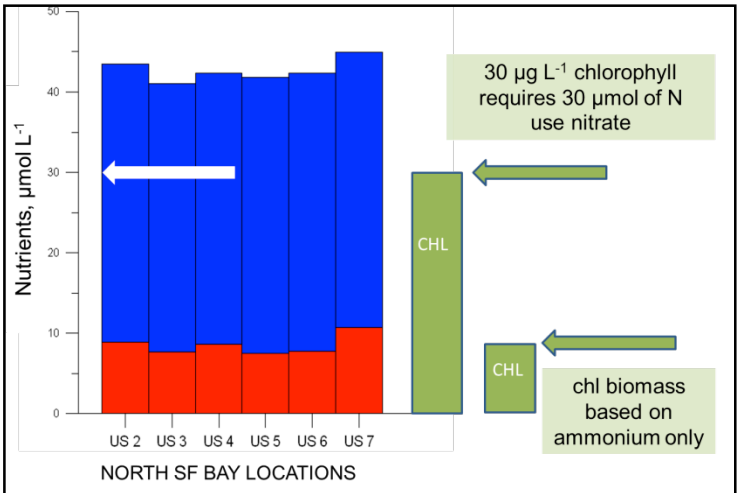

Figure 10. Relative concentrations of NO_3 (blue) and NH_4 (red) making up the DIN pool in Suisun Bay, shown to demonstrate how much chlorophyll can made from each form.

However, the ambient NH_4 is sufficient to inhibit phytoplankton NO_3 uptake and this limits access to the greater pool of available DIN (i.e., NO_3) and so NO_3 does not contribute to phytoplankton chlorophyll production. The un-used NO_3 is instead exported from the estuary.

Whether the "Ammonium Paradox" is realized in a particular set of circumstances depends on the interaction of several elements: 1) physiology of the phytoplankton and 2) environmental factors such as flow, irradiance or toxic contaminants).

5.1.4. Modeling the role of NH_4 in suppressing phytoplankton in nSFE;

A simple flow numerical model developed for this situation by Dugdale et al. (2012; 2013) developed criteria for bloom initiation and offered an optimal window where balanced river (Sacramento River) flow and NH_4 conditions could support rapid phytoplankton growth on NO_3 that result in a phytoplankton bloom in the nSFE. The window requires favorable irradiance conditions, river flow low enough to prevent washout yet high enough to allow NH_4 dilution of effluent to reach threshold levels to enable the NO_3 to be accessed (Figure 11). If water residence time was too low (when there is high river flow, i.e., in excess of 1100 m^3 s^{-1}) to allow phytoplankton to assimilate the inflowing NH_4, or if there was elevated NH_4 loading, phytoplankton growth would be based solely on NH_4 (with lower C production and low biomass accumulation) and NO_3 would go unused and be exported to the Pacific Ocean.

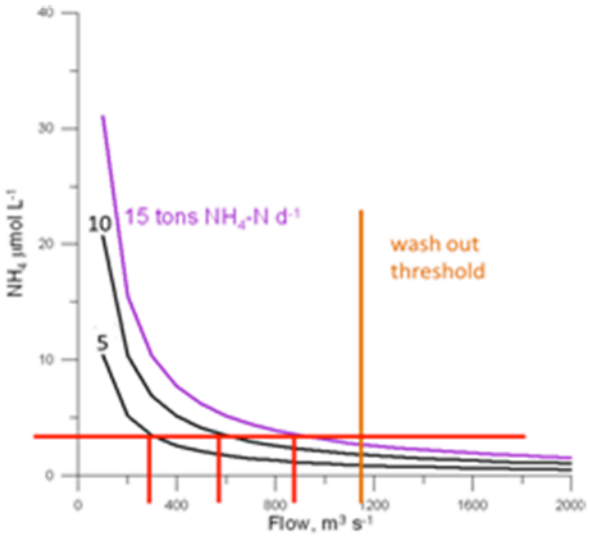

Figure 11. NH_4 concentration inflowing to Suisun Bay as a function of Sacramento River flow at three effluent level (5, 10, and current-day 15 tons NH_4 d^{-1}). The red vertical lines show the river flow that results at Suisun Bay in the concentration criterion of 4 µmol L^{-1}. Washout criterion is shown with vertical brown line. At current discharge levels, flow of 800 to 1100 m^3s^{-1} will be favorable for blooms (from Dugdale et al. 2012)

The subsequent improved numerical model that was developed (Dugdale et al. 2013) and now named NAMFLOW (NutrientAMmoniumFLOW) predicts two stable states: low biomass resulting from high flow and elevated NH_4 or high biomass with low flow and variable NH_4 input. The low flow drought conditions of 2014 resulted in chlorophyll accumulation as

predicted by the model (Figure 12). The model also predicted the blooms described by Ball and Arthur (1979) prior to the clam invasion (Dugdale et al. 2013). The model also allows for bloom formation using only NH_4 when it makes up the bulk of the DIN pool and is in high concentrations, along with long residence time. With flow set to zero, NAMFLOW simulates enclosure experiments (Dugdale et al. 2013).

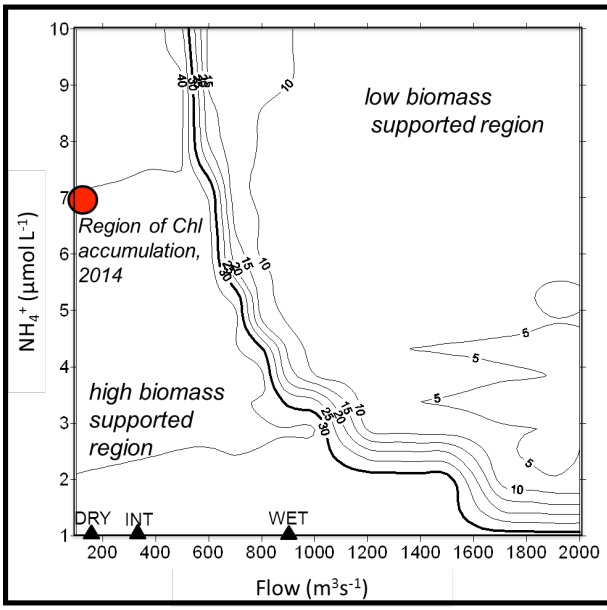

Figure 12 Modeled effect of flow and NH_4 on biomass accumulation in Suisun Bay that yields two steady states- high biomass with low flow and intermediate NH_4 and low biomass at high flows and high NH_4. 2014 .Suisun bloom (red circle) observed in drought conditions (from Glibert et al. 2014a)

5.1.5. Key findings summary for nSFE

NH_4 inhibits NO_3 uptake

We find NH_4 concentrations of >4 µmol L^{-1} inhibit phytoplankton NO_3 uptake to virtually zero and as a consequence we observe high chlorophyll concentrations only when NH_4 concentrations are low (< 4 µmol L^{-1}). As in the ocean, the f ratio is high when productivity is high.

Shift-up (acceleration) of NO_3 uptake occurs

Enclosure experiments confirm that the shift-up phenomenon occurs in SFE waters with the consequence that the higher the ambient NO_3 concentrations, the more rapidly NO_3 uptake occurs. The result is that once NH_4 concentrations are decreased and no longer inhibit NO_3 uptake, explosive NO_3 uptake and chlorophyll synthesis occurs over the course of 3 to 5 days. Note that monthly sampling is likely to miss many of these ephemeral phytoplankton blooms.

A predictable nutrient drawdown sequence results in bloom initiation

In both enclosure experiments and the field, phytoplankton take up NH_4 and reduce the ambient concentrations, such that NO_3 uptake shifts-up and rapid drawdown of NO_3 accompanied by elevated C uptake leads to a chlorophyll increase.

The Ammonium Paradox is a consequence of these elements (NH_4 inhibition, NO_3 shift-up and nutrient drawdown sequence)

NH_4 prevents phytoplankton from being able to access the larger pool of nitrogen which in the nSFE is nitrate (NO_3), resulting in persistent low chlorophyll, lack of blooms and the export of unused NO_3 to the coastal ocean.

Blooms (chl >10 µg L^{-1}) do occur in Suisun Bay in spring

Spring chlorophyll concentrations, comparable to those observed before 1987 by Ball and Arthur, have been reported (e.g. blooms in 2000 and 2010; Wilkerson et al. 2006; Dugdale et al., 2012), and are associated with the same conditions as before 1987; i.e., low NH_4 concentrations and NO_3 uptake by phytoplankton. All NH_4 and NO_3 were drawn down in the historical blooms, implying that turbidity cannot always be invoked for low phytoplankton production in the Suisun Bay. The supply of nutrients to Suisun Bay is primarily via the Sacramento River (Jassby 2008), which behaves mostly as a conduit, delivering nutrients from WWTP effluent with little phytoplankton processing within the river itself (Parker et al. 2012b). Clam abundance in Suisun Bay does not appear to explain spring bloom dynamics in Suisun Bay: clam abundance and occurrence of spring blooms show no correlation.

Flow and concentration criteria exist for bloom formation.

NH_4 and NO_3 interactions are incorporated into models with river flow and N loading creating three criteria necessary for the initiation of spring blooms in Suisun Bay; if any of these criteria are not met, no bloom would be expected:

1. The *Loading Criteria* – NH_4 loading within a range that allows phytoplankton to assimilate all NH_4 entering the embayment
2. The *Concentration Criteria* – NH_4 concentration in Suisun Bay is ~4 µmol L^{-1}.
3. The *River Flow Criteria* – river flow entering Suisun Bay needs to be within a range allowing dilution of NH_4 effluent yet not so high as to washout the phytoplankton.

High and low biomass steady state conditions can be predicted from NH_4 concentrations and river flow

A *more* developed numerical model relating NH_4 inputs and flow, to the use of NO_3 by phytoplankton predicts two stable states (low and high biomass) that fit well with contemporary and historical (i.e. those prior to the clam invasion; Ball and Arthur, 1979) observations.

Multiple and interconnected stressors occur

As summarized above, this chapter focuses on the interaction of NH_4 and NO_3, just one of the multiple, synergistic drivers of the low productivity condition There are important roles to be played by other drivers such as freshwater flow and residence time, clams and other grazers, light and turbidity, nutrient stoichiometry and the seed stock of phytoplankton amongst others.

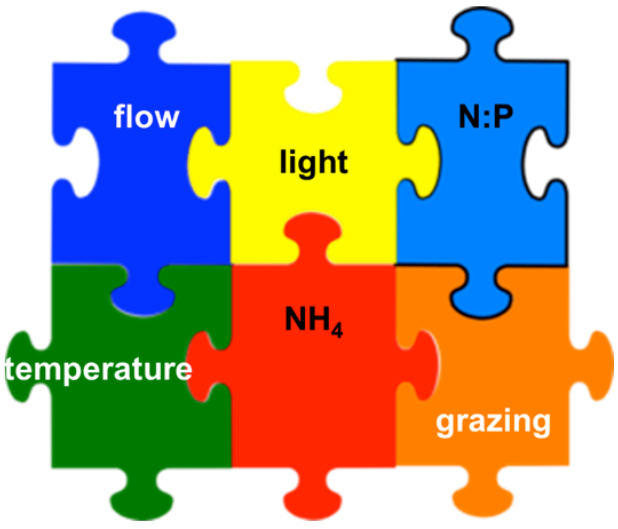

Figure 13. A few environmental parameters involved in phytoplankton bloom occurrence in SFE to be considered with a holistic approach.

Additionally many of these drivers will not just impact phytoplankton directly but will affect the nutrient regime and stoichiometry. For example, grazers will excrete NH_4 in addition to serving as top-down controls on phytoplankton biomass accumulation.

A holistic understanding of how each factor interacts with one another is needed in order to understand the factors that govern phytoplankton growth in estuaries and rivers There is work to be done (including some work that we are currently doing) to investigate the interactions of these to develop a complete understanding phytoplankton bloom dynamics (Figure 13). The emerging pattern is that the strength of the inhibition/repression effect of NH_4 is greater in the spring when

temperatures are low, and other effects, including the stoichiometry of N:P may play a greater role in the late summer and fall (Glibert, Section 6).

Next we review to date our publications supporting our key findings about NH_4 and NO_3 interactions and phytoplankton production in the nSFE, focusing on physiology and rate processes. The consequences on phytoplankton community structure are discussed in the Glibert chapter, as is the role of N:P stoichiometry in nutrient metabolism, primary productivity and trophodynamics.

5.2. Key Findings: A Chronology of our Publications

5.2.1. Chlorophyll blooms occur when NH_4 is low and phytoplankton use NO_3

(Wilkerson et al. 2006 Estuaries and Coasts 29: 401-416)

This is the first of a series of papers from our research group reporting nutrient uptake processes by phytoplankton in Central, San Pablo and Suisun Bays. It describes a three year monthly time series that sampled intensively (weekly during spring months) from 1999 to 2003. Ephemeral higher chlorophyll episodes in spring accompanied by elevated NO_3 uptake by the larger cell-sized phytoplankton and low NH_4 concentrations were recorded in spring. Most of the year NO_3

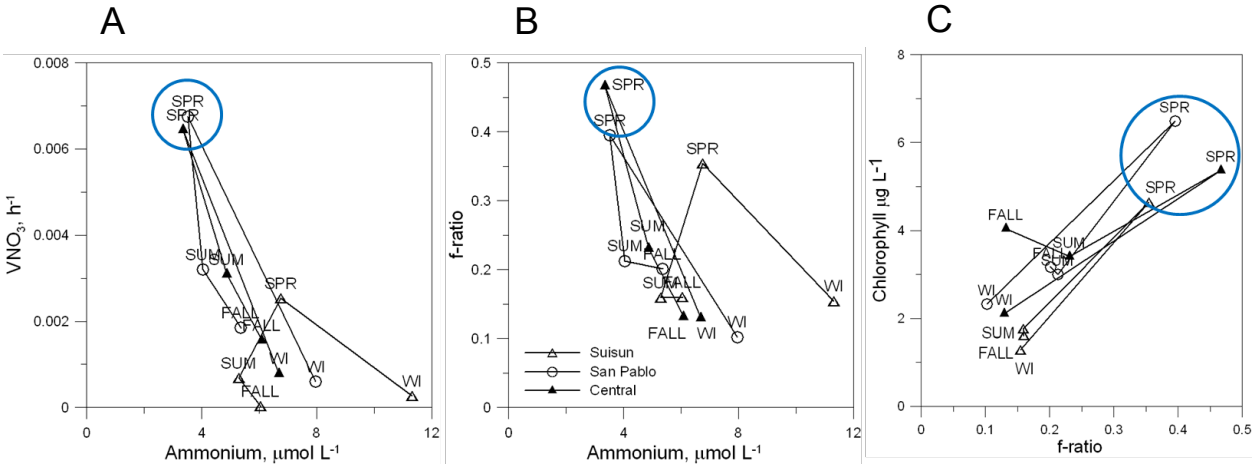

Figure 14. Mean seasonal (Spring-SPR, Winter-WI, Summer-SU and Fall-FALL) NO_3 uptake (A) and f-ratio (B) versus NH_4 measured from 1999-2003 in Suisun, San Pablo and Central Bays. NO_3 uptake and f-ratios are highest in spring when the highest chlorophylls were also measured, at high f-ratios (C).

and NH_4 uptake rates were in the range observed in oligotrophic oceans, with growth supported by NH_4.

Increases in phytoplankton biomass occurred only with sudden bursts in growth rate outpacing temporarily the losses. The bursts resulted from NO_3 uptake that occurred in the presence of low non-inhibitory NH_4 when presumably the stratification improved the irradiance conditions as in the wet spring of 2000. The HNLC condition observed most of the time was attributed primarily to poor light availability modulated by the interaction between NH_4 and NO_3 and the relative contribution of the two different N forms to the total DIN pool available to the phytoplankton.

NO_3 uptake and f-ratios were greater at low NH_4 concentrations, especially in the spring (Figure 14) when elevated chlorophyll was observed. Regressions of N uptake by larger phytoplankton cells versus all cells showed that NO_3 uptake was dominated (88% of total) by the larger phytoplankton (> 5 µm in diameter) in contrast to NH_4 uptake that was carried out by both the smaller and larger cells.

5.2.2. NH_4 inhibits NO_3 uptake by phytoplankton

Shift-up of NO_3 uptake occurs

(Dugdale et al. 2007 Estuarine Coastal and Shelf Science 73: 17-29)

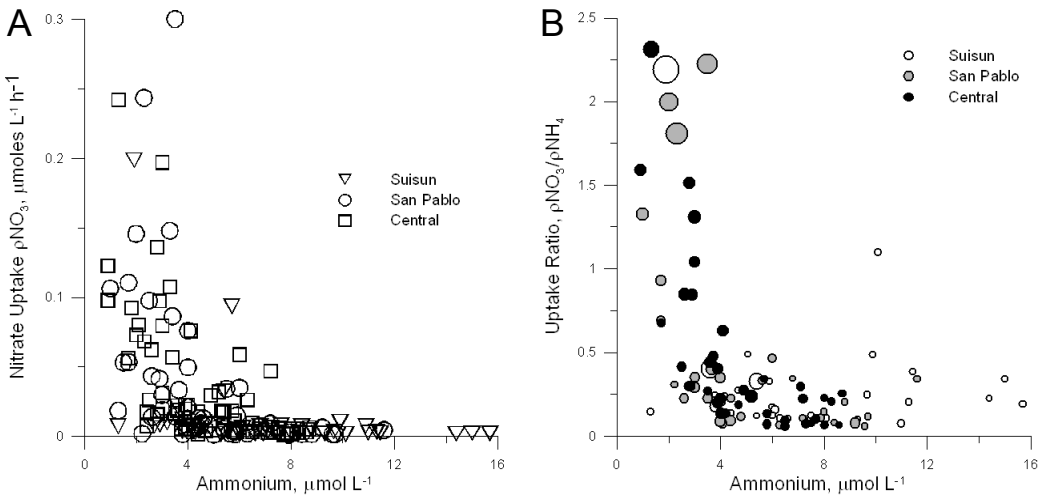

Figure 15. NO_3 uptake (A) and NO_3:NH_4 uptake ratio (B) versus NH_4 concentration measured from 1999-2003 for Suisun, San Pablo and Central Bays showing hyperbolic relationships and maximum values of uptake or ratio with $NH_4 < 4$ µmol L^{-1}. The circle size in B represents chlorophyll concentration that is greater at lower NH_4 values when the NO_3:NH_4 uptake ratio is highest.

In this paper the time series data of NH_4 and NO_3 uptake rate measurements made between 1999 and 2003 were re-examined and an inverse hyperbola of NO_3 uptake versus NH_4 concentration was presented confirming NH_4 inhibition of NO_3 uptake and quantifying the threshold value of NH_4 greater than ca 4 µmol L^{-1} for the SFE. Above 4 µmol L^{-1} the ratio of NO_3:NH_4 uptake was

0.5 or less whereas <4 µmol L^{-1} the ratio was up to 2.5, and was accompanied by elevated chlorophyll.

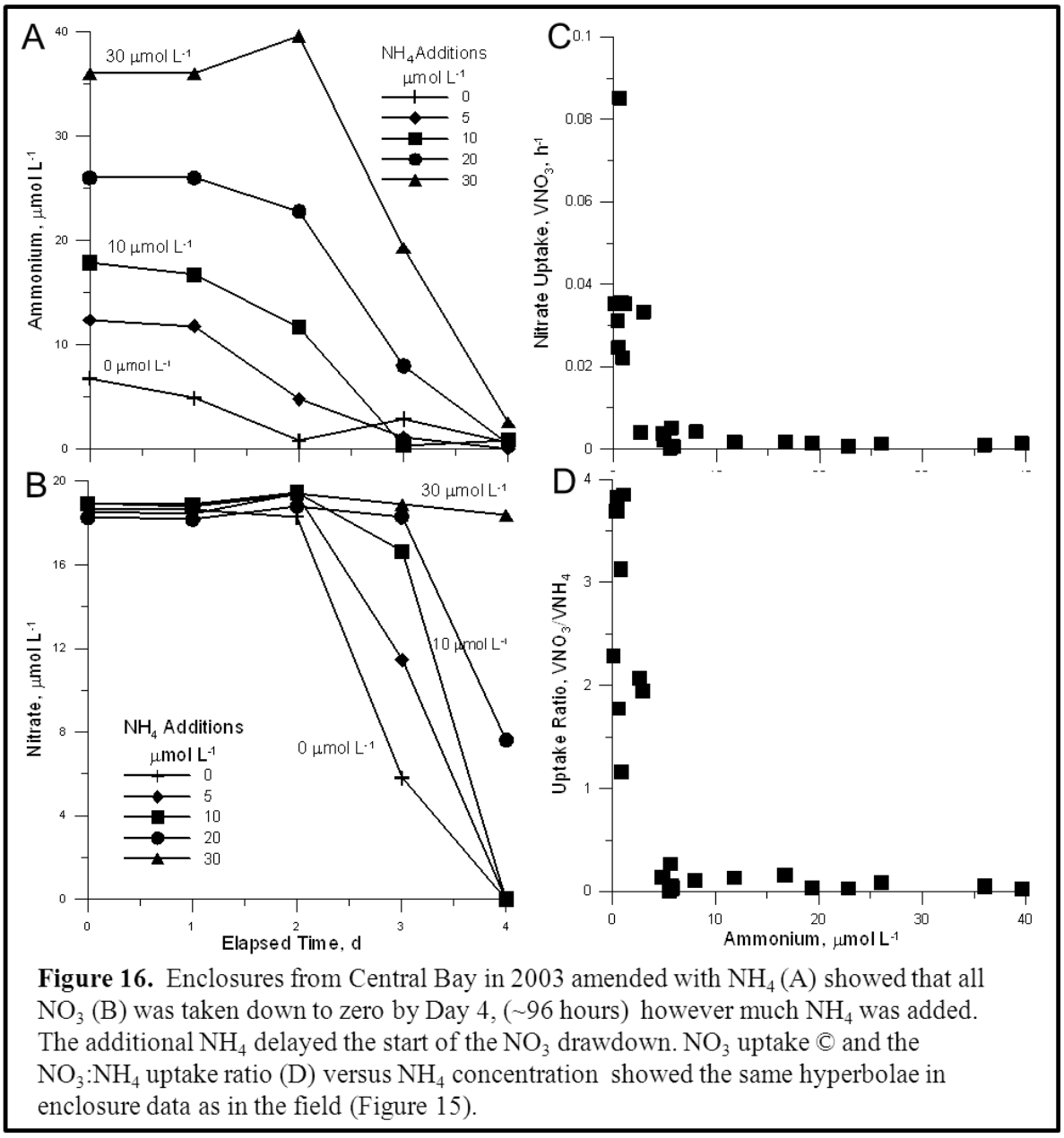

Figure 16. Enclosures from Central Bay in 2003 amended with NH_4 (A) showed that all NO_3 (B) was taken down to zero by Day 4, (~96 hours) however much NH_4 was added. The additional NH_4 delayed the start of the NO_3 drawdown. NO_3 uptake © and the NO_3:NH_4 uptake ratio (D) versus NH_4 concentration showed the same hyperbolae in enclosure data as in the field (Figure 15).

In addition, a consistent sequence of events leading to rapid chlorophyll accumulation was described that included reducing ambient NH_4 to a critical range of 1-4 µmol L^{-1} to allow NO_3 uptake. Calculations were made to predict the length of time that favorable irradiance conditions were required in order that the ambient NH_4 will be drawn down below this threshold, and showed this to be too long for blooms to occur in Suisun Bay with current nutrient conditions.

The paper also showed results from enclosure experiments with Central Bay water that confirmed that no NO_3 was taken up by phytoplankton until NH_4 concentrations were reduced to

low concentrations (a result of phytoplankton uptake; Figure 8, Figure 16) and also that after NH_4 was reduced NO_3 uptake rates were higher than NH_4 uptake rates and chlorophyll accumulated (Figure 8). These experiments found that NH_4 uptake rates did not change with time, whereas NO_3 uptake rate accelerated (shifted up) as the NO_3 became available and the time elapsed for complete exhaustion of NO_3 (after reduction of NH_4 to non-inhibiting concentrations) was independent of initial NO_3 concentration. The greater the initial NO_3, the faster it was taken up, confirming the shift-up phenomenon seen in the ocean. Enclosures with added NH_4 and showed that the time at which NO_3 drawdown starts could be manipulated. The higher the NH_4, the longer the time before NO_3 started to decline (Figure 16).

The possible role of increasing anthropogenic NH_4 inputs to the nSFE from wastewater treatment plants on the low primary productivity of SFE was considered. High NH_4 concentrations were probably related to the lack of spring blooms some years in Suisun Bay. Clams, often thought to be the cause of a productivity crash about 1987 are not present in significant numbers in spring and so not a factor at that time of year. However, a decline in average chlorophyll was noted in the decade prior to 1987 and correlated with increasing NH_4 discharge.

5.2.3. There is a consistent sequence for bloom formation that requires NH_4 drawdown Carbon uptake tracks NO_3 uptake

(Parker et al. 2012a Estuarine and Coastal Shelf Science 104-5:91-101

Using enclosure experiment data collected during 2005, the sequence of nutrient drawdown and biomass increase described by Dugdale et al. (2007) was shown to be repeatable using water from throughout the nSFE in different seasons (spring, summer and fall). Water from Central and San Pablo Bays behaved remarkably similar to each other with the time to drawdown of the ambient NH_4 pool (typically ~5 μmol L^{-1}) between 24 and 48 hours after the experiment was initiated. Following NH_4 exhaustion, the much larger NO_3 pool (20 to 30 μM) was rapidly drawn down over 48 to 96 hours. The entire DIN pool that was initially enclosed was converted to phytoplankton biomass within the 96 hour time frame. As reported by Dugdale et al. (2007) specific uptake rates (V, h^{-1}) for NO_3 were consistently higher than for NH_4. An experiment designed to measure maximum rates showed VNO_3 was approximately 20% higher than VNH_4.

Enclosures filled with water from Suisun Bay showed a similar drawdown sequence but the time required to exhaust NH_4 was extended to between 48 and 72 hours; and in some cases NH_4 was not exhausted after 96 hours. As a result, the time to initiate NO_3 drawdown was delayed or never observed. This difference in time required to exhaust NH_4 was in part ascribed to elevated initial NH_4 concentrations in Suisun Bay that are typically 3 to 5 μmol L^{-1} higher than NH_4 in Central Bay, a consequence of being in closer proximity to sources of NH_4 loading. Phytoplankton required additional time to assimilate the extra NH_4 concentration. Additionally, NH_4 uptake, reported both as VNH_4 uptake, h^{-1} as well as NH_4 transport or ρ, μmol N L^{-1} h^{-1},

738 (Dugdale and Wilkerson 1986) was lower for phytoplankton growing in Suisun Bay compared to
739 those in Central or San Pablo Bay.
740
741 The relatively low NH_4 uptake and subsequent lag in NH_4 drawdown observed in Suisun Bay
742 was investigated in more detail. Because of the potential for bias in V due to detrital particulate
743 N (Garside, 1991), specific C and N uptake were also normalized to chlorophyll and cells L^{-1}
744 (Kudela et al. 1996) and showed the same trends as the traditional measure of V, normalized to
745 PON; i.e., the lag in Suisun NH_4 uptake was not explained by detrital N. As a result, the lower
746 initial NH_4 uptake observed in Suisun Bay could not be explained but it was speculated that
747 because the station where water was collected was adjacent to a WWTP and the US National
748 Defense Reserve Fleet ("Mothball Fleet"), the potential that an unknown contaminant was also
749 introduced to the Suisun enclosures. This is consistent with a "Bad Suisun" hypothesis.
750
751 Parker et al. (2012a) also measured rates of carbon (C) uptake and dissolved inorganic carbon
752 (DIC) drawdown in the enclosures and found that C followed the patterns observed for
753 phytoplankton N uptake and drawdown (Figure 9). Carbon uptake to N uptake ratios were
754 generally <6:1 when phytoplankton were growing on NH_4 (the result of NH_4 inhibition of NO_3
755 uptake) early in the enclosure experiment (Phase I). Once phytoplankton had exhausted NH_4 and
756 were able to access the NO_3 pool, C:N uptake ratios were ≈ 6:1 (Phase II). Finally, late in the
757 experiment (72 to 96 hours) C:N uptake ratios >6 : 1, reflecting NO_3 exhaustion and continued
758 phytoplankton C uptake (Phase III). The fate of this C was not determined; it may have gone into
759 further increases in phytoplankton C biomass or into the dissolved organic C pool, as observed in
760 the Delaware Estuary by Parker (2004).
761
762 The C:N uptake ratio was consistent with a well-established concept in phytoplankton studies,
763 the Redfield Ratio that provides an estimate of C : N requirements of "typical" phytoplankton.
764 The Redfield Ratio includes the phytoplankton stoichiometry for phosphorus. The Redfield Ratio
765 has been extended specifically for diatoms that require silicon for production of frustules
766 (Brzezinski 1985) yielding a ratio of 106 C:16 N:1 P:16 Si. Elemental drawdown ratios,
767 estimated from the drawdown of DIC and nutrients in the 2005 enclosures, yielded ratios
768 generally consistent with the Redfield ratio, reflecting the observed production of mostly diatoms
769 (*Skeletonema costatum, Leptocylindrus minimus* and small centric diatoms). Drawdown ratios
770 from enclosures collected in Suisun Bay consistently yielded ratios that were relatively low in C
771 drawdown (and presumably production) despite having a similar final phytoplankton community
772 composition to Central and San Pablo Bay enclosures. Carbon drawdown was 40 to 60% of
773 expectation based on N drawdown and assuming the Redfield Ratio.
774
775
776
777

5.2.4. NH_4 in Sacramento River downstream of WWTP decreases production
Possible toxic substance carried with NH_4 in effluent
First estimates of pelagic nitrification and increase in NO_3 downstream

(Parker et al. 2012b Marine Pollution Bulletin 64: 574-586)

This study followed from a series of seasonal transects in the Sacramento River during 2008 and 2009 (Parker et al.2010) that included enclosure experiments using water collected immediately upstream and downstream of the Sacramento Regional Sanitation District WWTP that, at the time of the surveys, discharged approximately 180 million gallons of secondary treated municipal waste (equivalent to ~15 tons of NH_4) into the river daily. The subsequent hypothesis tested by Parker et al. (2012b) was that the Sacramento Regional Sanitation District WWTP discharge would significantly change the nutrient biogeochemistry downstream and these changes would be observable in the phytoplankton C and N physiology.

The effluent discharge into the Sacramento River resulted in a variable, but several fold increase in NH_4 concentration downstream of the effluent discharge. (Figure 17) Nutrient concentrations upstream of the WWTP were also variable in NO_3 concentration but were generally modest (1 to 12 μmol L^{-1}), but consistently had low (<1 μmol L^{-1}) NH_4. The variability in NO_3 concentrations at stations upstream of the WWTP in the Sacramento most likely reflect N supply from the large Sacramento River watershed while the variation in NH_4 concentrations in the Sacramento River from the WWTP likely is due to differences in river discharge as well as WWTP discharge activities required within this tidal section of the river (and mandated in the NPDES permit to maintain dilution requirements). The result is that NH_4 concentrations in the WWTP discharge receiving waters may vary between ~20 μM NH_4 to >100 μM (Parker et al. 2010; Parker et al. 2012b; Travis, *in prep*)

As a result of the spatial patterns in NH_4 and NO_3 concentrations, there was measurable phytoplankton NO_3 and NH_4 uptake at stations upstream of the Sacramento Regional Sanitation District WWTP, but NO_3 uptake was reduced to zero and uptake was exclusively on NH_4 immediately downstream of the WWTP outfall. The finding of NH_4 inhibition of NO_3 uptake noted in Wilkerson et al. (2006), Dugdale et al. (2007) and Parker et al. (2012a) appeared to hold for the Sacramento River.

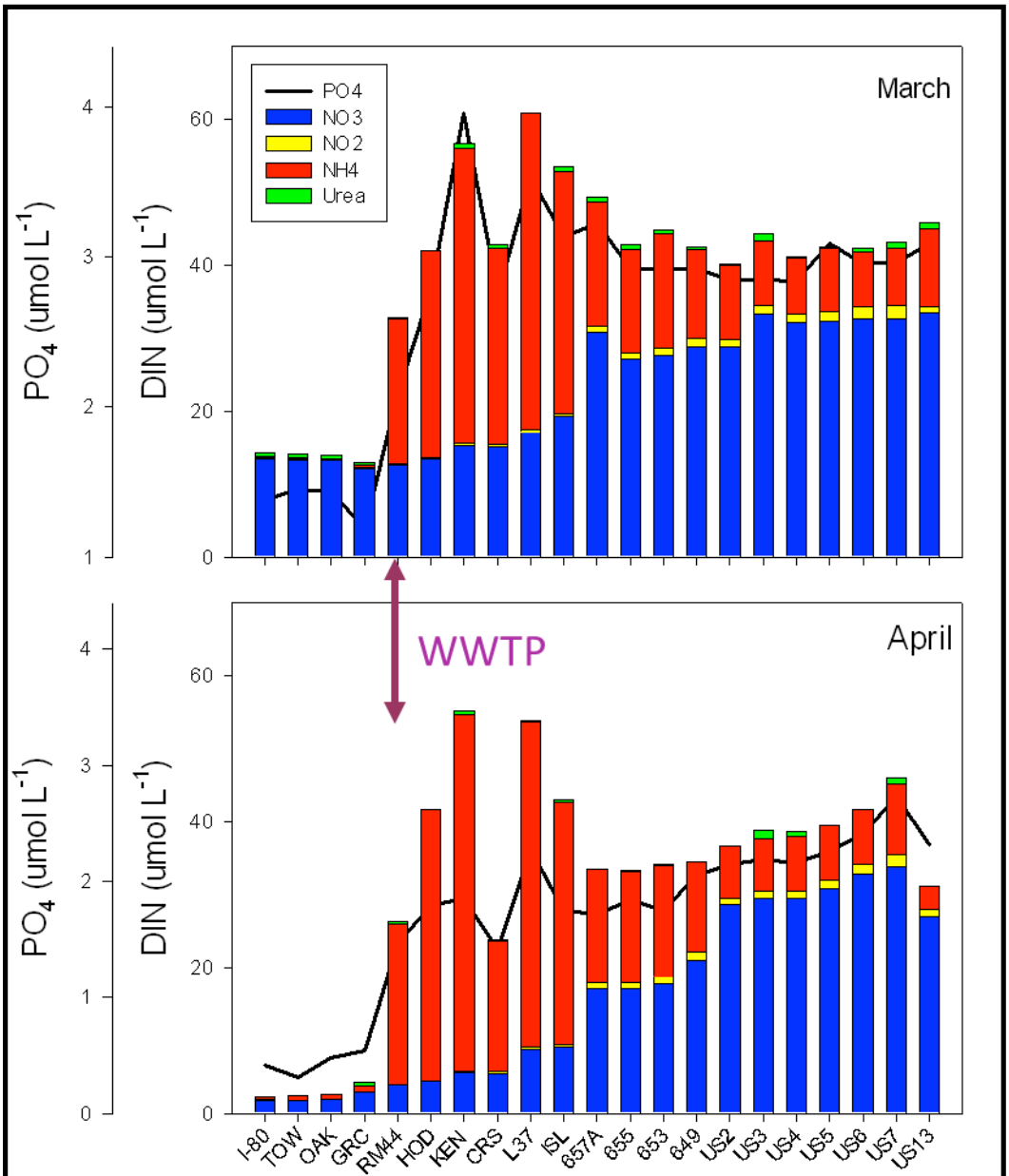

Figure 17. Nutrient concentrations in Sacramento River going downstream from 180 to Suisun Bay shows the input of NH_4 (red) at RM44 (near the WWTP). NO_3 is typically the larger component of the DIN pool. The increase in NO_3 and NO_2 (yellow) going downstream, reflective of nitrification is clear.

At the highest NH_4 concentrations (i.e., >20 µmol L^{-1}) an inverse relationship between NH_4 concentration and NH_4 uptake occurred suggesting NH_4 inhibition of NH_4 uptake (Figure 18), as has been suggested in other systems (e.g., Yoshiyama and Sharp 2006), that either NH_4 or an unknown toxicant associated with NH_4 was inhibiting phytoplankton. Parker et al. (2010) conducted a laboratory experiment in which Sacramento River phytoplankton were exposed to

increasing concentrations of NO_3, effluent-NH_4 or NH_4Cl and found that effluent-NH_4 concentrations inhibited NH_4 uptake at concentrations >8 µmol L^{-1}). We completed additional experiments during summer 2014 (Travis, *in prep*, Parker et al. *in prep*) confirming effluent-NH_4 inhibition of NH_4 uptake, albeit at higher concentrations than reported earlier (>40 µmol L^{-1}).

A decline in chlorophyll concentrations were observed beginning at the most upstream stations in the Sacramento River and continuing through the region of the river that received the WWTP effluent load. Carbon uptake rates (µmol C L^{-1} d^{-1}), reflective of dissolved inorganic carbon drawdown and autotrophic biomass production, as well as carbon assimilation number (mg C (mg chlorophyll-a)$^{-1}$ d^{-1}), reflective of phytoplankton carbon physiology, were lowest in the region of the river with the most elevated NH_4.

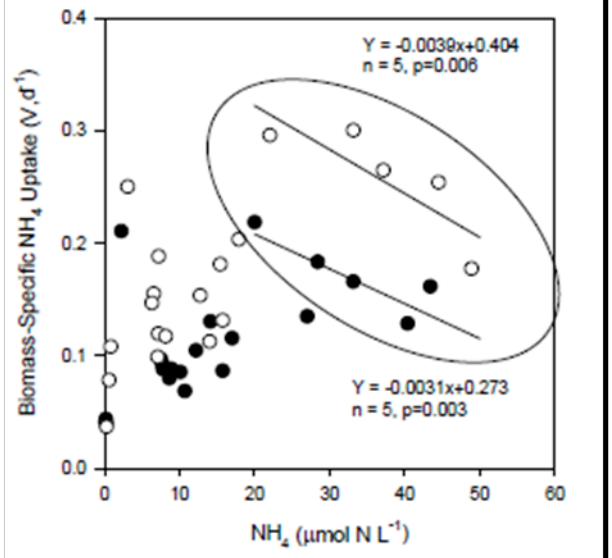

Figure 18 NH_4 uptake versus NH_4 concentration showing decreased uptake at elevated NH_4 (linear slopes), from Parker et al. 2012b

The phytoplankton NH_4 and NO_3 uptake rates measured by Parker et al. (2012b) were insufficient to reduce DIN concentrations downstream of the WWTP effluent discharge yet NH_4 concentrations declined downstream and were mirrored by increases in NO_2 and NO_3 concentrations (Parker et al. 2010; Foe et al. 2010) (Figure 17). This pattern is indicative of nitrification, the two step chemosynthetic oxidation of NH_4 to NO_3 by ammonia oxidizing archaea and bacteria. Estimates of nitrification based on an inventory of DIN at two points along the river transect along with estimates of river flow, suggested that nitrification, and not phytoplankton uptake, to be the dominant microbial *in situ* process influencing the distribution of river inorganic N. Subsequent measurements of pelagic nitrification using ^{15}N labeled NH_4, (Parker et al. in prep) suggest that pelagic nitrification rates are low in the Sacramento River, consistent with other pelagic nitrification rate measurements in the SFE (Damischek, *in prep*). This points to the benthos as a potential hotspot for nitrification activity. The dominant microbial nutrient processes in the Sacramento River result in little reduction the DIN pool via assimilation, but rather a partial shift of N species from NH_4 to NO_3; the Sacramento River acts mostly as a conduit, delivering wastewater effluent N loads to Suisun Bay.

5.2.5. Flow required in addition to NH4 to determine criteria for blooms

Blooms occur in Suisun Bay

Ammonium Paradox defined

(Dugdale et al. 2012. Estuarine and Coastal Shelf Science 115: 187-199)

Weekly cruises made to Suisun Bay in spring 2010 (from mid-March through June) sampled two large blooms, with peak chlorophyll concentrations of 30.9 and 21 µg L^{-1} in April, roughly the same range as observed for Suisun Bay by Ball and Arthur (1979) in the decade of the 70's and thought not to occur after 1987 following introduction of the clam *P. amurensis* in Suisun Bay (Alpine and Cloern 1992). The closely spaced sampling of these blooms in space and time allowed development of a set of criteria necessary for bloom development based on a box model for NH_4 input from the upstream Sacramento wastewater treatment plant but including the effect of flow on the NH_4 environment and phytoplankton assimilation.

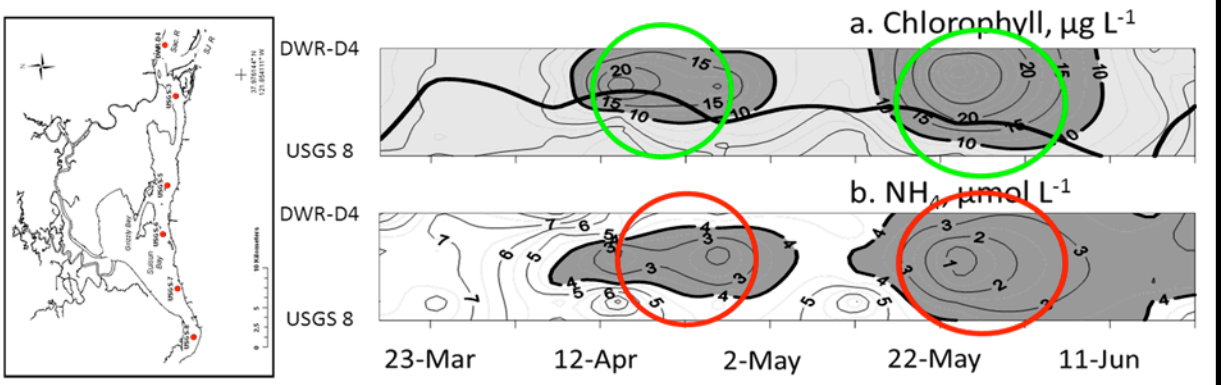

Figure 19 A) Chlorophyll or B) NH_4 plotted as surface contours on a space time plot of 10 cruises from 23 March to 11 June 2010 in Suisun Bay (locations on map) showing occurrence of two blooms (circles), both accompanied by low NH_4 concentrations

The blooms of 2010 both occurred as NH_4 concentrations decreased, in accord with previous findings (see above) and were composed primarily of diatoms, voracious users of NO_3 when NH_4 concentrations are low and non-inhibitory. There were apparent contributions of the 2010 blooms to the food web. Zooplankton (calanoid copepod adults) were nine-fold more abundant in May 2010 and the fall mid-winter trawl data showed increases of 70-194% for delta smelt and longfin smelt compared to 2009. The similarity between the spring 2010 blooms and the 1969-1979 blooms (Ball and Arthur, 1979) suggest that under some conditions, the Bay may revert to the high productivity conditions with positive consequences for the higher trophic levels, including fish. These early bloom conditions shared another characteristic with the 2010 bloom, i.e., low NH_4 concentrations.

Based on earlier measurements of NH_4 uptake by phytoplankton in Suisun Bay, criteria for bloom initiation were developed. The first was a loading criterion stating that loading of NH_4 to the Bay must not exceed the capacity of the phytoplankton to absorb it, otherwise NH_4 concentration will not decline. A value was set based on mean measurements of NH_4 uptake (Wilkerson et al. 2006) and then compared to the loading to Suisun Bay. Calculations were made for a range of loadings at the SRTWP, from 5 tons NH_4-N d^{-1} to the current 15 tons NH_4-N d^{-1}. Estimates were made of the loss of NH_4 from the discharge point to the entrance of Suisun Bay; about 75% due primarily to nitrification. When applied to calculate the loading of NH_4 to Suisun Bay, the conclusion reached was that the current Sacramento Regional Sanitation District WWTP loading exceeds the capacity of the phytoplankton NH_4 uptake, so that NH_4 will not be drawn down to below threshold levels.

The second criterion was that the ambient NH_4 concentration must decrease to 4 µmol L^{-1} or less so that NO_3 (the largest component of the DIN pool) could be accessed by the phytoplankton to build biomass. A minimum flow of 800 m^3s^{-1} was calculated as the minimum flow that would achieve the necessary dilution of discharged NH_4 (Figure 11).

A third criterion was that the loss rate of phytoplankton from outflow could not exceed the specific phytoplankton growth rate, calculated from the specific NH_4 uptake rate of the phytoplankton. A flow calculated from measured NH_4 uptake rates, the washout flow, was 1100 m^3s^{-1} (Figure 11). Flow in spring 2010 was below that level.

The cause/s of the reduction in NH_4 that enabled the 2010 bloom, was found in a series of peaks in flow that occurred in mid-April through the first week in May that resulted in NH_4 concentrations only slightly over the 4 µmol L^{-1} criterion. A 10% decrease in NH_4 loading at Sacramento Regional Sanitation District WWTP from 2009 to 2010 also occurred and this decrease occurred during the bloom period to within the loading criterion.

The low productivity of the nSFE was attributed to the chronic high NH_4 condition and labeled as the "Ammonium Paradox", the situation where the larger component of the DIN pool is NO_3, but uptake of this larger fraction of N is prevented by the smaller component of the pool, NH_4. Biomass of 30 µg L^{-1} of chlorophyll can only be built with the assimilation of 30 µmol L^{-1} of DIN, and cannot be built on the relatively low (but inhibitory) concentrations of NH_4 in SFE, rarely more than 10 µmol L^{-1}. The Ball and Arthur (1979) blooms in the 1970s used all available DIN, including NO_3 and these blooms were considered to be ultimately nutrient limited. The "Ammonium Paradox" does not apply to these conditions.

5.2.6. Flow and NH_4 determine one of two productivity states

(Dugdale et al. 2013 Ecological Modelling 263: 291-307)

This paper integrates the interactions of phytoplankton growth, NH_4 loading and flow in a basin (Suisun Bay) with inflow and outflow by developing a biogeochemical model. The major take-home is that two stable biomass states can occur; high biomass that results from low flow and a wide range of NH_4 concentrations and low biomass with high flow and high NH_4, i.e., low flow is more forgiving of high NH_4 than high flow, in agreement with earlier observations (Ball and Arthur, 1979) of Suisun Bay (and SFE) in low stable state at most times.

The model uses measurements of NH_4 and NO_3 uptake made in SFE and in enclosures of bay water that have shown NO_3 uptake (i.e., use of the larger DIN pool component) to be necessary for bloom formation and that NH_4 concentrations above ~4 µmol L^{-1} to inhibit NO_3 uptake and prevented access to the larger fraction of the DIN. It also incorporates the shift-up phenomenon observed in the SFE enclosures from which acceleration (shift-up) rates were calculated. A box model with N as currency was constructed with inputs and outputs to a model volume, Suisun Bay. The model was first run with zero flow to simulate the enclosures and was then validated with data from a number of different enclosure experiments from a range of locations within SFE.

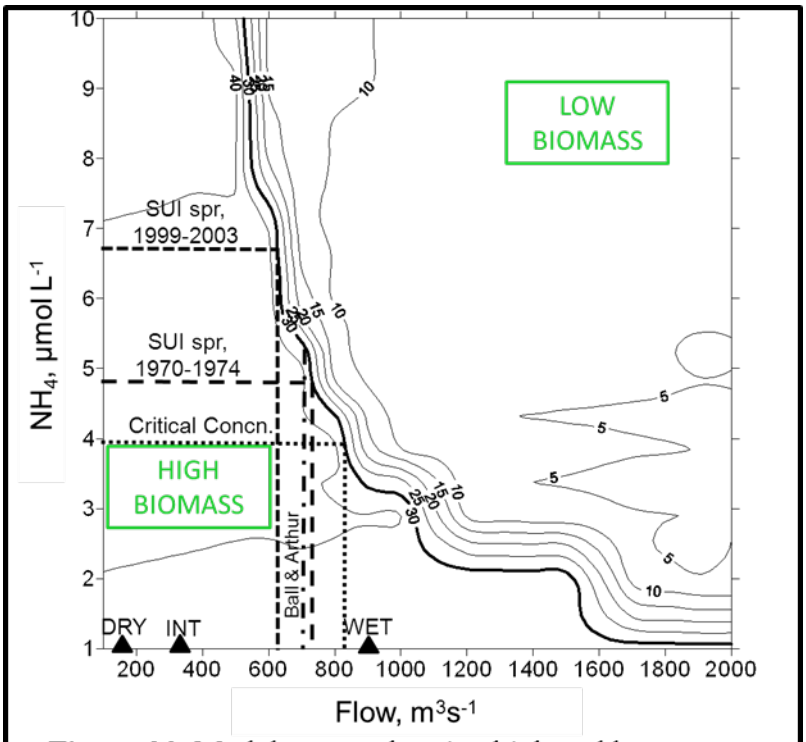

Figure 20. Model output showing high and low phytoplankton biomass states that result from flow and NH_4 interactions overlaid with historical data using mean NH_4 from Suisun Bay in the 1970's and post 1999 and including the maximum flow reported for blooms by Ball and Arthur (1979). Arrows shows flows from Peterson et al. (1985).

The interaction of flow, NH_4 and NO_3 was studied with the aid of the model, varying source NH_4 concentrations and flow. These model runs revealed a system with two stable steady states, low and high phytoplankton biomass (Figure 20). At low flow rates, relatively elevated source NH_4

conditions still allowed bloom development. At high flow rates, the system becomes more sensitive to NH_4 and blooms occur only at low NH_4 concentrations in the inflowing water. The boundaries for flow and NH_4 that designate low or high biomass state agreed remarkably well with conditions in the period before 1977 when Ball and Arthur (1979) made their investigations into the productivity of the low salinity zone. They observed very high chlorophyll concentrations were with NH_4 and NO_3 concentrations nearly zero at the height of blooms, and when relatively low flow conditions prevailed, in the range of 110 to 700 m^3s^{-1}.

Model results were applied to typical flows for different water years, dry, intermediate and wet. Dry and intermediate years should allow blooms with source NH_4 concentrations up to 10 µmol L^{-1}, but NH_4 concentrations in wet years would have to be ≤ 4 µmol L^{-1}.

5.2.7. Ammonium paradox observed during drought conditions and high biomass occurred as modeled with low flow

(Glibert et al. 2014 Journal of Experimental Marine Biology and Ecology 460: 8–18)

In this paper sampling along the Sacramento River and Suisun Bay was made during the second drought year in California (2014). Reduced river flow created conditions conducive to a spatially large and physiologically healthy phytoplankton population (based upon measures of quantum yield i.e., elevated Fv/Fm, using a variable rate fluorometer) in Suisun Bay where a diatom bloom (> 20 µg L^{-1}) dominated by *Entomoneis* was observed. This bloom is conceptualized as a "window of opportunity" response by these diatoms to multiple factors promoted by the drought, including longer residence time for cell growth and biomass accumulation, and longer time for in-river nitrification to occur, reducing sewage-derived NH_4 to a level where diatoms could access the elevated NO_3 (~ 50 µmol L^{-1}) for uptake and growth.

The implication is that management practices that favor higher rates of flow may narrow this "window of opportunity" for phytoplankton growth, potentially leading to low productivity and food limitation for fish. As in Dugdale et al. (2012, 2013) we described how under high flow, a condition of "washout" may develop where both chlorophyll and unassimilated nutrients are transported out of the Bay.

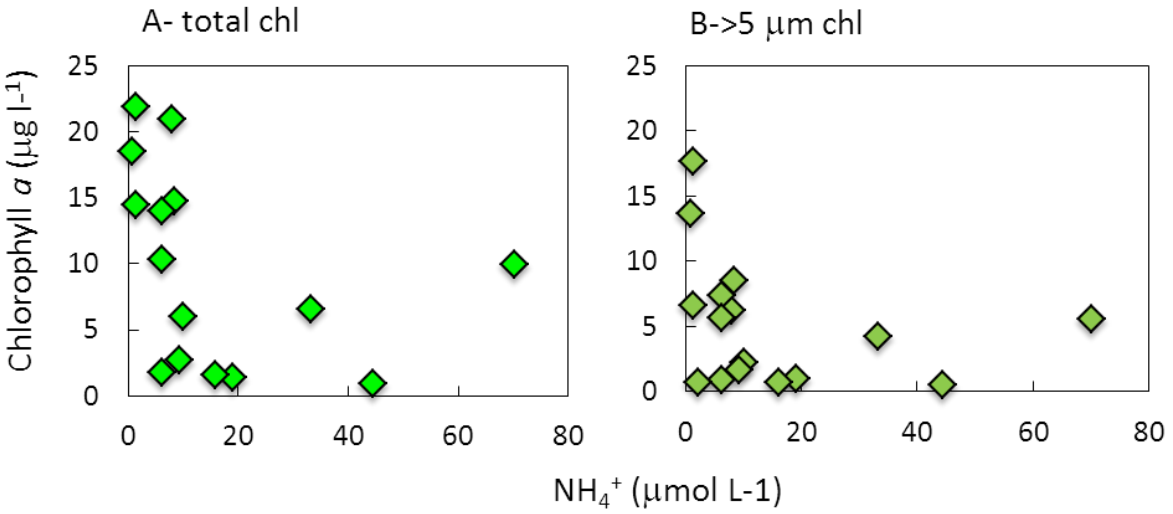

Figure 21. Chlorophyll in A) all phytoplankton cells (as measured with GF/F filter) or in B) cells > 5 µm diameter versus ambient NH_4 concentration show the inverse hyperbola relationship consistent with Dugdale et al. (2007), Parker et al. (2012a) (Dugdale et al. 2012).

As in the previous papers (e.g., Parker et al. 2012b) both biomass and photosynthetic efficiency (based on variable fluorescence, Fv/Fm) precipitously declined down the Sacramento River when cells were exposed to sewage effluent and NH_4 levels of 70 µmol L^{-1}. Further downriver, substantial rates of nitrification occurred, based on increasing levels of NO_3 and NO_2 in proportion to decreasing NH_4 concentrations.

Consistent with the NH_4 inhibition element of the Ammonium Paradox (Dugdale et al. 2012), when chlorophyll data were plotted as a function of NH_4 concentration, virtually all of the high biomass observations were found when NH_4 concentrations were reduced to below 10 µmol L^{-1}, and this was the case also for cells that were >5 µm in size. (Figure 21).

5.2.8.. Bloom sequence occurs in the field

(Wilkerson et al. 2015 Aquatic Ecology 49: 211-233)

This study points to the importance of treating inorganic N separately as NH_4 and NO_3 rather than lumping together as DIN, and the need to measure uptake rates to offer a mechanistic way to understand, predict and avoid cultural eutrophication. The dynamic changes in rate processes (nutrient and C uptake) in response to flow are central to understanding estuarine productivity that is difficult to evaluate using biomass alone.

The predictable nutrient drawdown sequence that results in bloom initiation, previously observed in enclosures was tested in the field using NH_4 and NO_3 uptake data, and nutrient and

chlorophyll concentrations measured weekly in spring 2011 and 2012 and found lack of access to NO_3 (the greater contributor to the DIN pool) limits primary production and consequently the accumulation of chlorophyll which leads to the Ammonium Paradox in nSFE. The study also provided the first depth integrated nutrient uptake rates to better constrain our published criteria (Dugdale et al. 2012; 2013) for bloom formation.

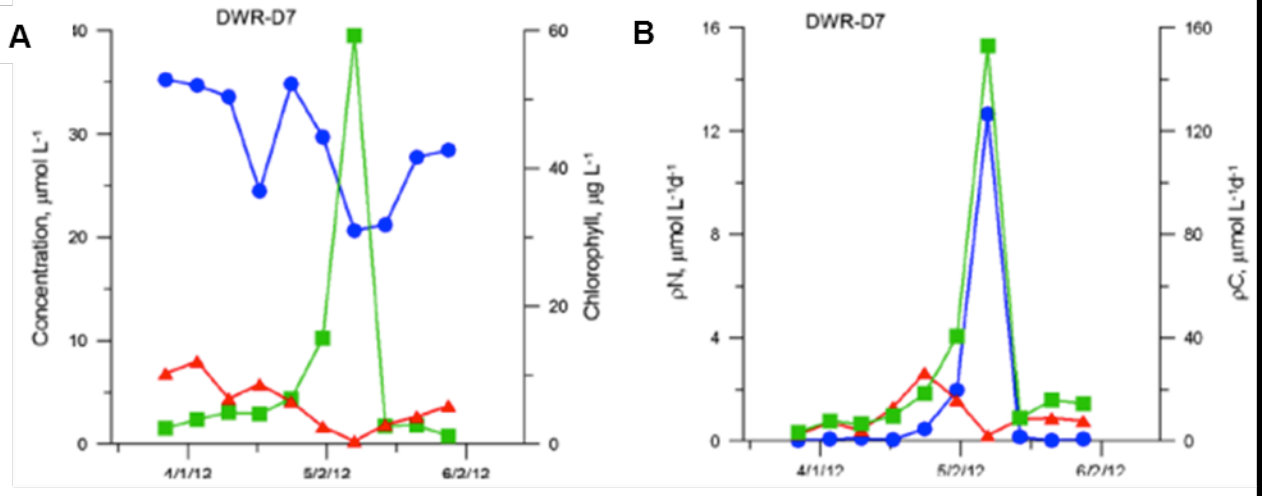

Figure 22. Weekly time series of nutrient concentrations (A) and uptake rates (B) measured during spring 2012 at shoal location in Grizzly Bay showing the sequence of A) NH_4 (red) drawdown (and B, uptake in red), followed by NO_3 drawdown in blue (and uptake, blue) with a bloom of chlorophyll (green) resulting. Consistent with Parker et al. 2012a, high C uptake rates (green) accompanied the high NO_3 uptake rates (B).

In 2011 when flow conditions were high (maximum of 2405 m^3 s^{-1}) there were lower nutrient concentrations than at low/normal flows of 2012, e.g., NO_3 of 10 µmol L^{-1}compared to 30 µmol L^{-1}), with low N uptake and primary production rates. As in the 2014 drought data, in spring 2012 with low flow (maximum of 1304 m^3s^{-1}) there was elevated chlorophyll and blooms occurred, especially in shallow well lit shoals where chlorophyll reached 60 µg L^{-1}.

As expected from the enclosure data described in Dugdale et al. (2007) and Parker et al. (2012a), higher levels of chlorophyll and primary productivity resulted from uptake of ambient NO_3 by phytoplankton, and f-ratios >0.5. This was enabled by phytoplankton uptake of NH_4 to below inhibitory levels as shown (Figure 22) for shoal station DWR-D7 that consistently had elevated chlorophyll in spring

5.3. Big Picture Synthesis and Conceptual Model

5.3.1. Challenging the "nutrients don't matter in the SFE" Paradigm

Many of the management decisions for the San Francisco Estuary are based on statistical analyses of ecological data collected through long-term monitoring programs. Bay scientists have always stressed the multifaceted interacting drivers that shape the ecology of the Bay. Our research efforts over the past decade serve to reinforce these ideas and suggest *not* that nutrients are the only driver (e.g. Figure 13), but rather that nutrients, as a bottom-up control on primary production, are *a key* driver that has been poorly understood, and that serves to shape the ecological character of the system. Importantly, nutrient effects are directly related to effects of other stressors. For example, as shown by Glibert (Section 6), the amount and proportion of nutrient excretion by grazers change as nutrient availability changes for the algae, and, flow interacts both abiotically and biotically with nutrient fluxes from the sediment.

Our work has emphasized both observational and experimental research, with the goal of elucidating underlying phytoplankton processes in order to predict blooms in the nSFE. Our results show that nutrients play a regulatory role in the ecology of the Bay, even though they are often found at concentrations assumed not to limit growth. We have speculated that by relying on broad ecosystem-scale analyses, based largely upon correlation, the scientific community has missed key nutrient processes, triggers, and dynamics associated with phytoplankton blooms. The *"nutrients don't matter in the SFE"* paradigm has been reinforced by correlation analyses over time. Specifically we see the following erroneous assumptions about nutrients in the SFE:

1. Ambient nutrients at elevated concentrations are assumed to be saturating for phytoplankton uptake.
2. It is reasonable to combine NO_3 and NH_4 into a single pool of dissolved inorganic nitrogen (DIN).
3. There are no interactions between NO_3 and NH_4 (i.e., inhibition).
4. Phytoplankton uptake kinetics of NO_3 and NH_4 are similar and do not influence phytoplankton bloom dynamics.

Phytoplankton rate measurements have been largely limited to a small number of investigators, i.e., our group and collaborators, for the past several decades and process-based studies with respect to nutrients have been largely lacking for the SFE; we are unaware of nitrogen uptake rate measurements made prior to 1996 when we established the nutrient and phytoplankton laboratories at the Romberg Tiburon Center of San Francisco State University and began to apply the ^{15}N tracer method to the SFE.

We started collecting data of concentrations of nutrients (including NH_4) and phytoplankton rate processes (i.e. ^{15}N labeled nutrient uptake and ^{13}C or ^{14}C uptake) in Central Bay in 1997 and have an *extensive data set that include* data of *processes and rates.* Such an extensive data set with rates, which include ~ 390 cruises in SFE (and also water collection for experimental enclosures) is better designed to interpret biogeochemical interactions and phytoplankton processes, than the more traditional monitoring (see discussion in Wilkerson et al. 2015). Rather than use statistical correlations between parameters to invoke process, our nutrient rate data offers a direct mechanistic approach to inform compared to traditional water quality monitoring.

There are times and locations in which the benthic grazing (top-down) hypothesis is insufficient to explain observations and these occasions provide an opportunity to explore other competing hypotheses about bottom-up controls. The *"nutrients don't matter in the SFE"* paradigm was first challenged when we began our measurements of NH_4 and NO_3 uptake rates in Central Bay and showed that blooms occurred with low NH_4 concentrations and with NO_3 uptake. The inhibition of NO_3 by NH_4 at low concentrations, well-known in the scientific literature, was quickly identified as a feature of the SFE ecosystem.

Not quite 20 years later, the data that we collected, representing thousands of stations and many thousands of estimates of N and carbon uptake, nutrient and chlorophyll concentrations, has been summarized through publications in the reviewed literature. The *"nutrients don't matter in the SFE"* paradigm is now being replaced with more sophisticated estuarine nutrient conceptual models, including the Ammonium Paradox concept, in which NH_4 is seen to occupy a "gatekeeper" role in SFE phytoplankton productivity, as described in the conceptual model that follows, the statistical efforts directed towards numerical nutrient endpoint criteria classification (e.g. Sutula et al. in prep.) and the advancement of concepts such as ecological stoichiometry (e.g. Glibert, Section 6), the effects of which may operate at all food web levels, whether nutrients are limiting or not.

The Gatekeeper conceptual model is consistent with our finding that blooms do occur quite often in Suisun Bay where the clam, *P. amurensis* occurs and that these blooms occur when NO_3 is taken-up after NH_4 concentrations are reduced to non-inhibiting levels. Criteria for blooms have been established for Suisun Bay, depending largely upon nutrients and Delta outflow. We are also approaching our goal of the ability to predict spring blooms in the nSFE and more generally primary productivity of the SFE with our published model describing the way in which flow and NH_4 interact and lead to either a high phytoplankton biomass or low phytoplankton biomass ecosystem.

5.3.2. Conceptual Model -The Ammonium Gatekeeper

We have developed a conceptual model applying a holistic approach for understanding how NH_4 and NO_3 interact to enable phytoplankton growth and blooms in Suisun Bay (Figure 23). The key concept is that NH_4 acts a gatekeeper, controlled by multifaceted abiotic and biotic factors, that either allows access by the phytoplankton to NO_3 (gate open), that is the dominant DIN form that supports blooms, or the situation in which NO_3 uptake is inhibited by NH_4 (gate closed), limiting

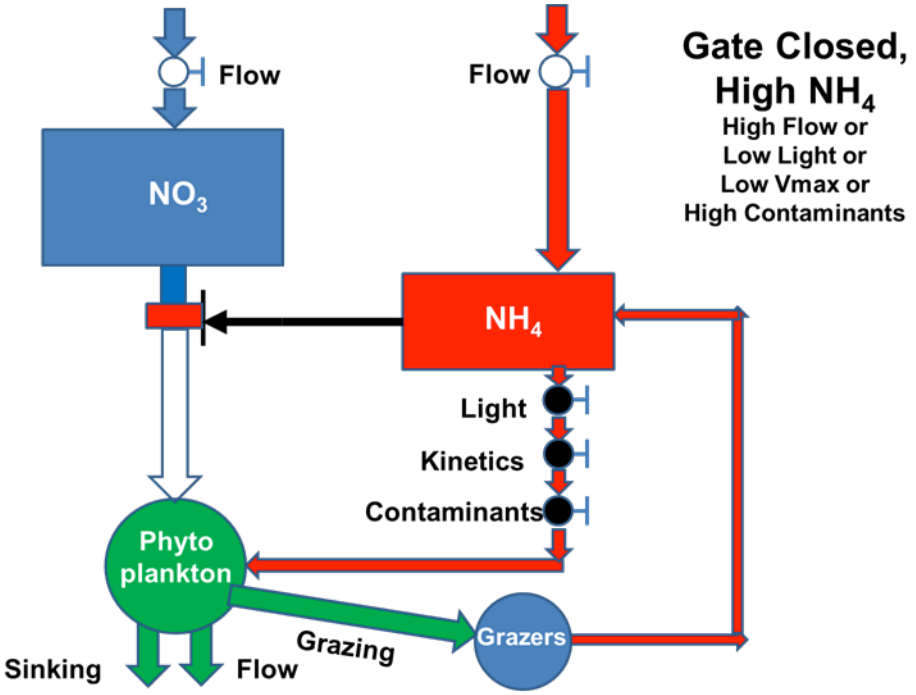

Figure 23 Conceptual model showing the NH_4 gate closed; i.e. flow of NO_3 to the phytoplankton is blocked. High flow ensures elevated nutrients reaching the phytoplankton. Any one of a variety of valves (e.g. low light or high contaminant toxicity) can close shutting down phytoplankton uptake of NH4 thereby increasing the NH_4 pool.

phytoplankton growth to that based on NH_4 (Figure 27), that is the Ammonium Paradox..

The conceptual model depicts the control of a set of variables on the uptake of NH_4 which in turn affect the mass balance of NH_4, that ultimately controls access of the phytoplankton to the larger pool of NO_3 shown here as a valve (but is actually a kinetic effect, inhibition of NO_3 uptake by NH_4).

The gate is closed when NH_4 is above the inhibition threshold, shutting down phytoplankton NO_3 assimilation and NO_3 is therefore unused. The gate can be closed (a result of elevated NH_4 concentrations) by high flow (and NH_4 discharge) or any one of the three factors that result in changes to phytoplankton physiology: 1) low light, 2) unfavorable NH_4 uptake kinetics, 3) additional unidentified contaminants (e.g., herbicides) or another unlisted factor (valve), for example temperature.

These factors (and likely others not represented here) will decrease the NH_4 uptake capability of the phytoplankton so that the high ambient NH_4 will not be drawn down to below threshold values. Other variables may also act to close these valves and enable the NH_4 gate. For example, grazing reduces the standing stock of phytoplankton and limits the ability of the phytoplankton to absorb inflowing NH_4 and therefore allowing NH_4 concentrations to increase. Similarly, high flow makes the system more sensitive to source NH_4 concentrations by reducing residence time and shortening the time available for phytoplankton to reduce ambient NH_4. As flow continues to increase it will ultimately "wash out" the phytoplankton population. The three physiological parameters in Figure 24 will be described in more detail

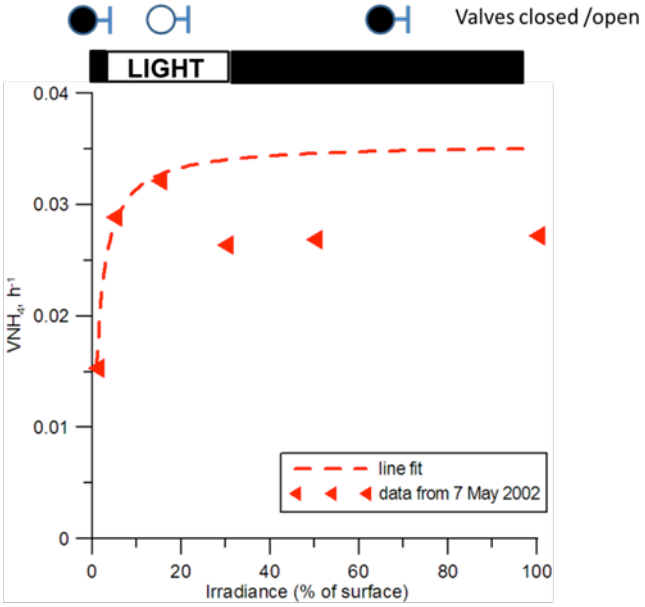

Figure 24. The "light" valve will close (black) when there is insufficient or too much light such that the phytoplankton cannot draw down the NH_4 to below inhibitory levels.

Results from ^{15}N tracer incubations conducted in Central Bay shows that inhibition of uptake occurs at higher light (Fig. 24). A curve fit to the Michaelis Menten hyperbolic equation gives a K_{LT} (half saturation constant for light; MacIsaac and Dugdale, 1972, similar to a K_S for nutrient uptake) of 1% surface irradiance. At irradiances > 15% surface irradiance, NH_4 uptake is reduced, as is often observed in photosynthesis. In this situation at both low and high irradiances light has restricted the uptake of NH_4 tipping the mass balance of NH_4 and forced the gate to close.

NH_4 uptake versus NH_4 concentration, also from Central Bay samples, shows a Michaelis-Menten hyperbolic relationship with the calculated K_S of 1.3 µmol L^{-1} (Figure. 25; Parker et al. 2012a) and indications of decreased uptake > 20 µmol L^{-1}. Unfavorable kinetics, that may be a result of the dominant phytoplankton species, may keep NH_4 uptake low and so close the gate.

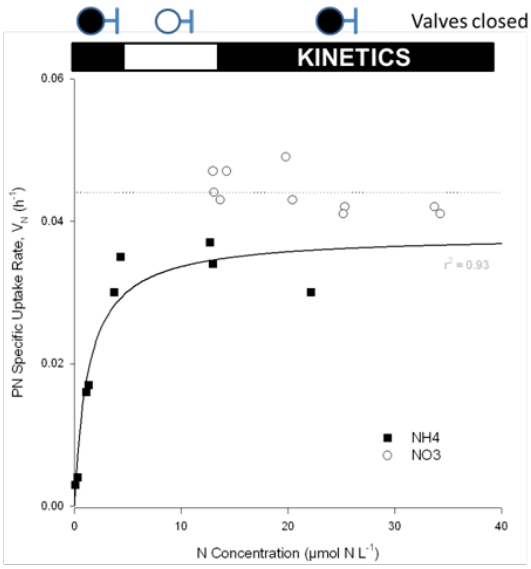

Figure 25. The "kinetics" valve will close (black) when NH_4 kinetics are unfavorable. This NH_4 uptake vs NH4 plot from Parker et al. (2012b) shows lower uptake at low and high NH_4 concentrations. The valve will open when the kinetics are at Vmax and NH_4 drawdown will open the gate.

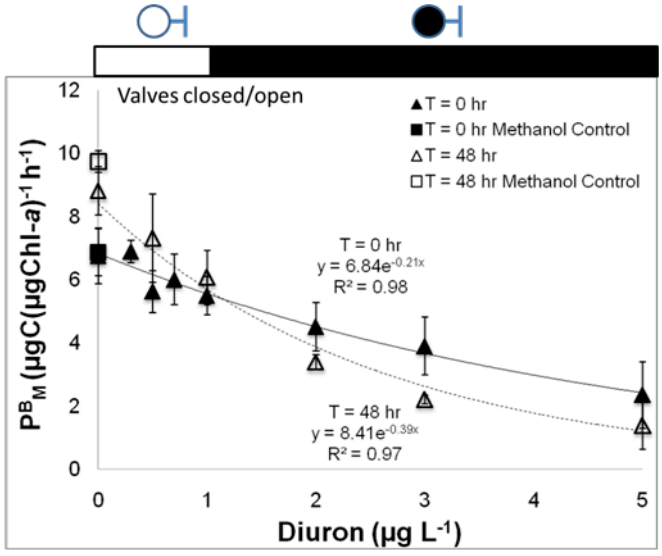

Figure 26. Maximum photosynthetic activity vs diuron concentration (Blaser et al. 2011) shows that high concentrations will decrease phytoplankton C (and likely NH_4) uptake rate and close the gate (black).

Finally, some toxic contaminant or chemical could decrease phytoplankton health and physiology so decreasing NH_4 uptake capacity. For example, Blaser et al. 2011 demonstrated that the herbicide diuron at concentrations > 2 µg L^{-1} reduced C uptake by SFE phytoplankton (Figure 26). The algal physiology would be disrupted and this would close the valve and disable access to NO_3.

To open the NH_4 gate and access NO_3, all parameters (flow, light, NH_4 uptake kinetics, toxic contaminants and any not listed here) need to be favorable with open valves that will ensure NH_4 concentration will be reduced to below threshold levels for NO_3 uptake and assimilation. Low rate of inflow of NH_4 is balanced by favorable light, favorable NH_4 kinetics and lack of toxic contaminants. As a consequence, NH_4 concentration is low, inhibition is released, the gate is open allowing the phytoplankton to access the large NO_3 pool. Using shift-up kinetics for NO_3 uptake, results in increases in biomass that overcome grazing, sinking, and washout losses. Grazers return some NH_4 to the NH_4 pool via excretion and regeneration.

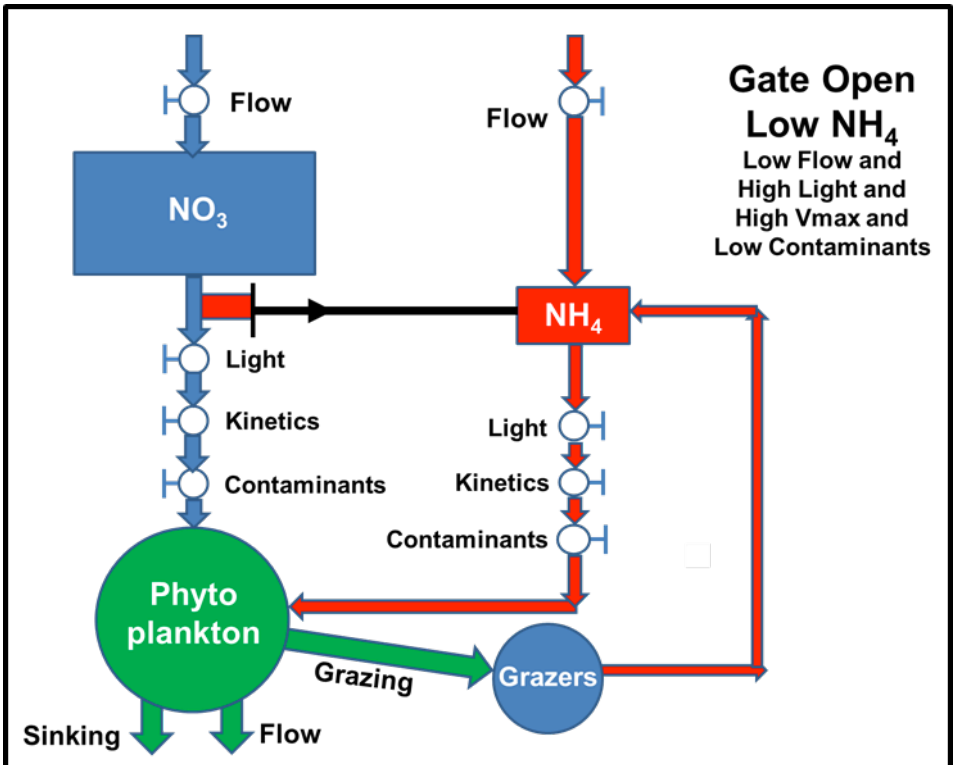

Figure 27 Conceptual model showing the NH_4 gate open; i.e. phytoplankton can access NO_3 as all valves are open (i.e. there are favorable flow, light, kinetics, and contaminant conditions).

The uptake of NO_3 by the phytoplankton will be similarly influenced by the same parameters as for NH_4 uptake (e.g., temperature, light, kinetics, contaminants) but presumably since these were favorable for NH_4 uptake, they should also allow maximal NO_3 uptake.

It is important to distinguish between indirect effects of NH_4 brought about by environmental variables described above, and the direct effects brought about by concentration and discharge which directly influence the ambient NH_4 concentration; i.e. flow plus concentration determine the loading of NH_4 to the system and when it exceeds the ability of the phytoplankton to absorb the loading, NH_4 increases and the gate closes. NH_4 concentration acts as an indicator of ecosystem condition. In this scenario, NH_4 itself is a major problem and potentially could be controlled directly through management, e.g., by controlling effluent concentrations or flow or both. Both changes apparently occurred by serendipity and enabled the spring 2010 bloom (Dugdale et al. 2012) and are planned for the future (2021; California Regional Water Quality Control Board 2015, Central Valley region order R5-2013-0124).

In summary, elevated NH_4 and a closed gate for access to NO_3, indicates the potential for reduced phytoplankton biomass and productivity, but does not necessarily mean that NH_4 inputs are the cause. A successful search for the proximate cause of the elevated NH_4 would then allow an assessment of whether possible mitigation actions might exist.
Recent efforts to produce a nutrient plan for SFE have struggled to find a nutrient related problem and have defaulted to raising concerns about cultural eutrophication in one of the world's least productive estuaries; evidence of a eutrophication threat is limited spatially within the Bay (i.e. South Bay, ref...). The more obvious and serious problem, especially in nSFE, is the oligotrophic condition and low food for higher trophic levels. By understanding the role of NH_4 as gatekeeper to the use of NO_3, and that of the relative proportion of all major nutrients and phytoplankton blooms we may be able to manage the ecosystem for increased productivity- i.e., to reverse the POD.

5.3.3. An Overview of Bloom Occurrence

Using our dataset we have examined when and where blooms may occur in the nSFE and Sacramento River as well as the processes that lead to blooms. In Suisun Bay, phytoplankton blooms (i.e. > 10 $\mu g\ L^{-1}$) were observed often at the shoal station (DWR-D7 in Grizzly Bay) when sampled, i.e. in spring of 2002, 2011, 2012, 2014. Elevated chlorophyll was measured throughout Suisun Bay in 2000 and 2010 (Dugdale et al. 2012). Since 2010 blooms in the confluence/Suisun Bay region appear to have become more regular in spring – likely due to low flow conditions. Our Suisun Bay bloom observations and model predictions (the model in Dugdale et al. 2013, NAMFLOW) are in agreement with the hypothesis of Ball and Arthur (1979) who found that chlorophyll concentrations of > 20 $\mu g\ L^{-1}$ occurred only when Delta Outflow was between 110 - 700 $m^3 s^{-1}$. Bloom occurrences are apparently not related to the variability in abundance of *P. amurensis* as blooms occurred at both below and above average abundances of *P. amurensis* (see Dugdale et al. 2012 and Wilkerson et al. 2015).

Blooms were seen in the Sacramento River (red) during fall whereas spring blooms (blue) were observed in the seaward direction from the confluence through Suisun Bay (Figure 28).

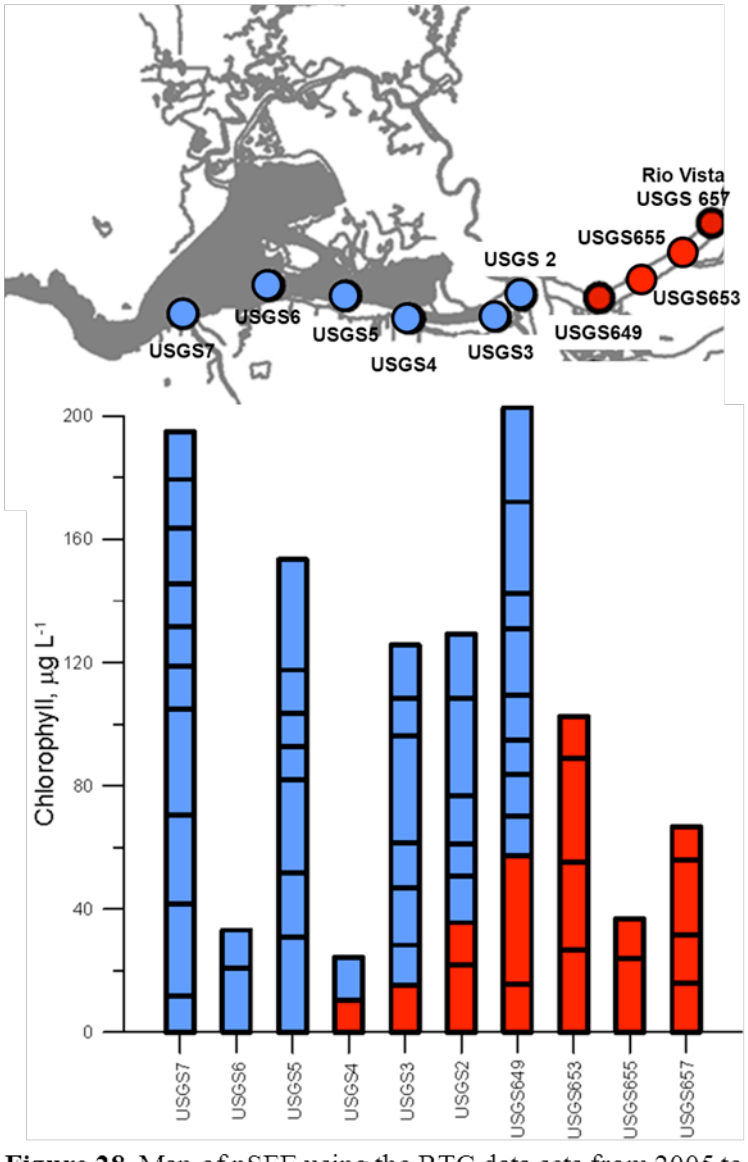

Figure 28. Map of nSFE using the RTC data sets from 2005 to 2015 to show occurrences of phytoplankton blooms , chlorophyll > 10 µg L^{-1}. Blue are spring, and red, fall blooms. Chlorophyll concentrations > 10 µg L^{-1} are stacked, delineated by black horizontal lines. For example at the river location USGS 653 four fall blooms were observed.

For the Suisun Bay blooms, a plot of chlorophyll > 10 µg L^{-1} vs. NH_4 concentrations (Figure 29A) shows the expected relationship from the NH_4 inhibition element of the Ammonium

Paradox and is virtually identical to that we have shown previously (e.g. Glibert et al. 2014a); blooms occur at NH_4 concentrations ≤ 6 µmol L^{-1}, and most at <4 µmol L^{-1}. The highest chlorophyll, 60 µg L^{-1} occurred with NH_4 almost undetectable. Ball and Arthur (1979) reported spring blooms in Suisun Bay from 1966 to 1977 to range from 30 to 40 µg L^{-1} while our data set has a range of 10 to 60 µg L^{-1}.

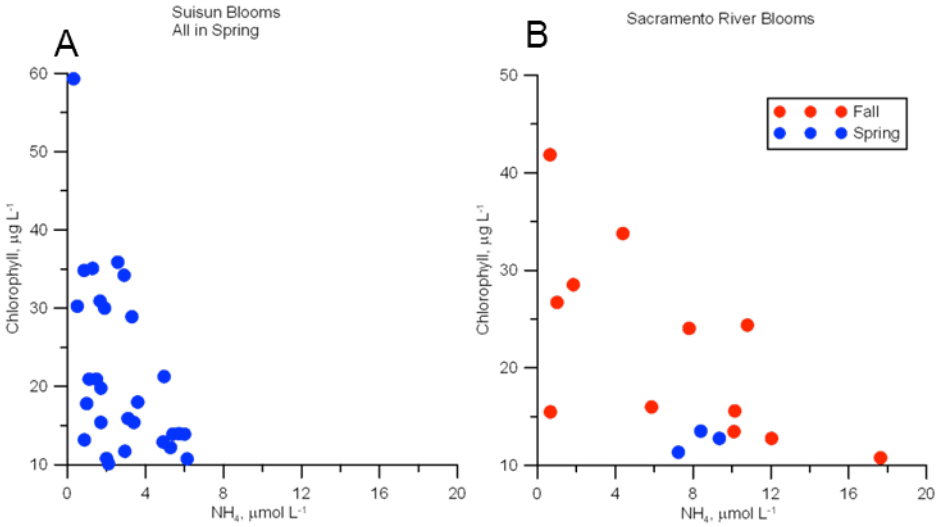

Figure 29. Surface bloom data of chlorophyll concentration versus ambient NH_4 from 2005-2014 showing an inverse hyperbolic relationship in (A) Suisun Bay spring blooms and (B) a less clear relationship for the mostly fall blooms of the Sacramento River.

A similar plot for the Lower Sacramento River blooms (Figure 29B) shows a weaker relationship to NH_4 concentration, although the highest chlorophyll concentrations are at the lowest NH_4 concentrations. Some blooms are associated with elevated NH_4 concentrations. It appears that seasonality (temperature) may be important. It may be that P or N:P ratios play a stronger role in determining the likelihood of *fall* blooms and that NH_4 plays a greater regulatory role in *spring* blooms (Glibert, Section 6). This would be consistent with our emerging understanding of the metabolic interaction of NH_4 with NO_3 under different temperature conditions (e.g., Glibert et al. in review). Ball and Arthur (1979) reported chlorophyll spring blooms concentrations of 25 to 40 µg L^{-1} in the Western Delta which includes the lower Sacramento River.

Criteria for Suisun Bay, how well do they work?

We can make preliminary predictions of spring blooms in Suisun Bay based in a set of criteria developed in Dugdale et al. (2012) and elaborated in Wilkerson et al. (2015). The loading criterion requires that the NH_4 loading to Suisun Bay must not exceed the capacity of the phytoplankton to absorb the inflowing NH_4. This criterion is difficult to evaluate as it depends on

knowledge of the NH_4 uptake rate of the phytoplankton population prior to the bloom initiation period. The loading criterion was first estimated at 1.58 mmol N m^{-2} d^{-1} (Dugdale et al. 2012) and then updated as a range, 0.88 to 2.02 mmol N m^{-2} d^{-1} (Wilkerson et al. 2015). The NH_4 concentration criterion of 4 μmol L^{-1} in water entering Suisun Bay requires flow > 800 m^3s^{-1} to provide sufficient dilution of the present day wastewater discharge from Sacramento Regional Sanitation District Wastewater Treatment Plant of 15 tons NH_4 d^{-1}. However if flow is too great (i.e. above the washout criterion of 1100 m^3s^{-1}) phytoplankton growth cannot keep up with the NH_4 loss rate. This means a flow of between 800 and 1100 m^3s^{-1}will be beneficial for bloom initiation.

Criteria applied to historical data

Ball and Arthur (1979) found that blooms only developed in Suisun Bay in the range of flows from 110 to 700 m^3s^{-1}, but there was less NH_4 discharge from the wastewater treatment plant at the time of their studies (1969-1977). At the discharge rate of NH_4 from the wastewater treatment plant feeding the Sacramento River in 1987, 5 tons N d^{-1} (Jassby, 2008) the Dugdale et al. (2012) model would calculate a flow of 280 m^3 s^{-1} to reduce the discharge value to the NH_4 concentration criterion of 4 μmol L^{-1} in Suisun Bay (Figure 2, Dugdale et al 2012, also Figure 11 this chapter). The Ball and Arthur (1979) flow limits would have met the flow criteria and provided low NH_4 water to Suisun Bay. NH_4 concentrations in April were low at the time of their studies. For example, at Station D9, Honker Bay, the average NH_4 concentration in spring was 2.9 to 3.6 μmol L^{-1} for years 1970 to 1974. The loading criterion cannot be evaluated for their data set as no phytoplankton NH_4 uptake measurements were made. The Ball and Arthur (1979) data were also plotted in Dugdale et al. (2013) (Figure 20, this chapter) and fit the high biomass predictions from our flow and NH_4 model for Suisun Bay phytoplankton productivity.

Criteria applied to the 1987 decline of the chlorophyll in Suisun Bay

The collapse of the phytoplankton populations in Suisun Bay in 1987 has been attributed to the arrival of the invasive clam, *P. amurensis* (Alpine and Cloern, 1992). However, an alternative hypothesis was presented that linked increased NH_4 inputs from the Sacramento Regional Sanitation WWTP and changing N:P ratios to the decline in diatoms and chlorophyll biomass (Glibert, 2010; Glibert et al. 2011). Cloern et al. (2015) found no relationship between NH_4 and chlorophyll concentrations in Suisun Bay during the years just before and after the phytoplankton decline. The Cloern et al. (2015) analysis combined data from a shoal station, DWR-D7 and a channel station, DWR-D8. Shoal and channel stations, however, have quite different seasonal productivity patterns. The shoal station, DWRD-7 showed evidence of bloom initiation in spring each of three sampling years (Wilkerson et al 2015) whereas channel stations did not. Combining data from these two types of locations likely smoothed out the data, obscuring critical details.

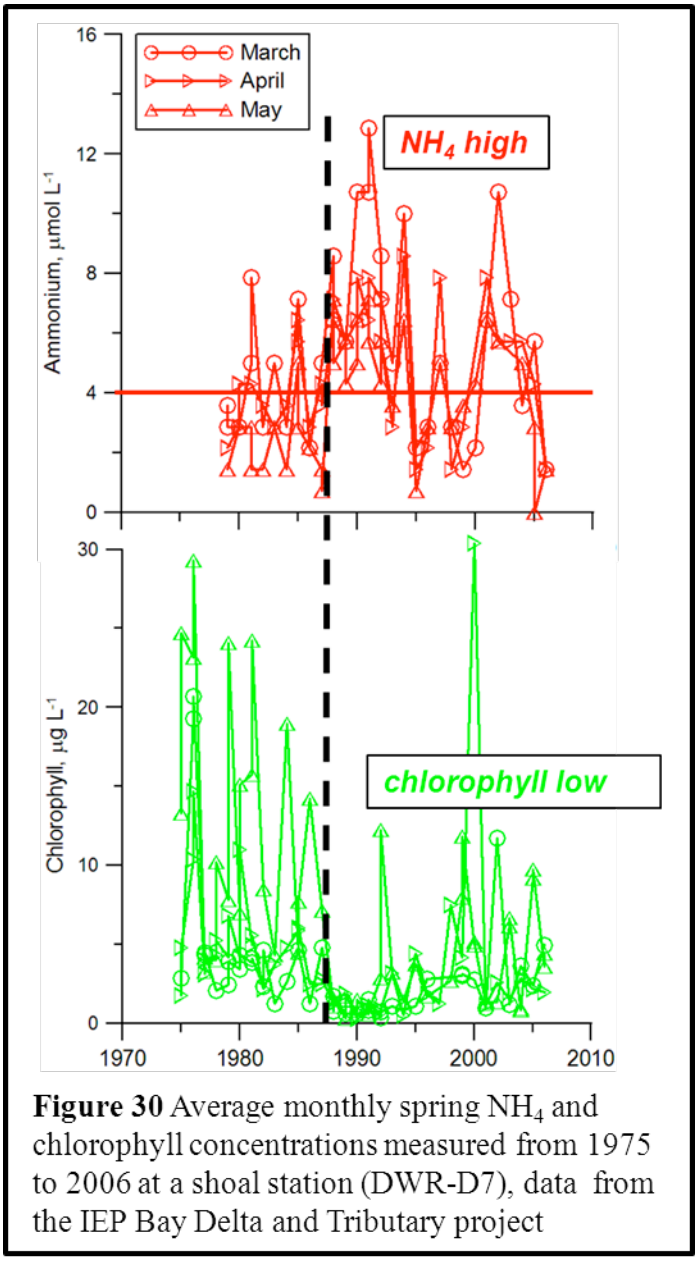

Figure 30 Average monthly spring NH_4 and chlorophyll concentrations measured from 1975 to 2006 at a shoal station (DWR-D7), data from the IEP Bay Delta and Tributary project

The most easily applied criterion for explaining chlorophyll increases, 4 µmol L^{-1} NH_4 as the threshold concentration for bloom initiation, can be applied to the conditions at DWR-D7 (where blooming normally occurs) (Figure 30). NH_4 concentrations were mostly below this criterion prior to 1987 (left of the dotted line in Figure 30) and then rapidly increased. In contrast chlorophyll concentrations were high when the NH_4 was lower and then dropped precipitously after 1987 (dotted line) when NH_4 increased above 4 µmol L^{-1}. According to the NH_4 gatekeeper conceptual model, since only one criterion has to be negated to prevent blooms, the 1987 crash of chlorophyll is predicted by the NH_4 concentration criterion.

5.3.4. Contribution of the Ammonium Paradox to understanding SFE productivity

The information provided by the NH_4 / NO_3 interactions and outcome on phytoplankton production can support the Ocean-Bay-River models that are coming on line (i.e., ROMS-CoSiNE/ SCHISM-CoSiNE, CasCADE, SunTANS etc), that combine hydrology, climate, biogeochemistry and primary productivity. These models can be used to evaluate changes in river discharge, or to predict how changes in the coastal ocean such as the recent phenomenon known as the "warm water blob" (Bond et al. 2014) will influence the pelagic food web of the low salinity zone. Our physiological and flow models (e.g., Parker et al. 2012a; Dugdale et al. 2012, 2013) and uptake kinetics (e.g., Lee et al. 2015) should be incorporated into these large modeling efforts for hypothesis and management scenario testing. Our dataset represents a unique resource for those engaged in the emerging effort to manage nutrients in the SFE by developing numeric nutrient endpoint criteria. We hope that our long term data set is used to inform this understanding rather than relying upon data collected in other systems to test nutrient scenarios.

Finally, measuring rate processes and nutrient transformations that occur in the major rivers that feed the SFE are key to understanding phytoplankton productivity in the northern SFE, including the LSZ, known to be important habitat for the fishes of concern. However, the Sacramento River acts as a pipe delivering nutrients that are rarely taken up and converted to biomass. Instead the impact of nutrients discharged into the rivers will be realized at the river confluence and locations seaward towards the ocean.

5.4. Uncertainties and Next Steps

The integration of the Ammonium Paradox into models of Suisun Bay function will allow for continued testing and refinement of hypotheses about the role of nitrogen in modulating phytoplankton response. Specifically, work should focus on identifying and characterizing the parameters that act as "valves" in the NH_4 gatekeeper conceptual model, including the role of light, variable N uptake kinetics, how it operates under different stoichiometric conditions (N:P), as well as additional contaminants associated with nitrogen loading. Recognition by the Bay's scientific community that seasonal and inter-annual phytoplankton cycles reflect a multitude of controls operating at different spatial and temporal scales, will require resisting the temptation to assume that managing conditions to control for NH_4 *OR* benthic grazers *OR* the position of X2, in isolation, is unlikely to result in desired management outcomes.

5.4.1. Light field

Within the oceanographic literature and in some estuaries, it is well established that phytoplankton N uptake, like C uptake is light-dependent, with differences observed between

NH_4 and NO_3 uptake. Nitrate uptake has been shown to be strongly light-dependent with rates in the dark that are typically very low. In contrast, phytoplankton NH_4 uptake appears to behave differently; NH_4 uptake is less light-dependent than NO_3 uptake with rates in the dark that vary between 30% and 95% of the maximal uptake rate in the light (Pennock, 1987; Boyer et al. 1994). As a consequence the calculation of daily uptake from short term mid-day incubations is typically accomplished by multiplying the measured hourly rate by 18 for NH_4 and 12 for NO_3. This procedure has been verified by studies measuring uptake throughout the diel cycle (e.g. McCarthy et al. 1996).

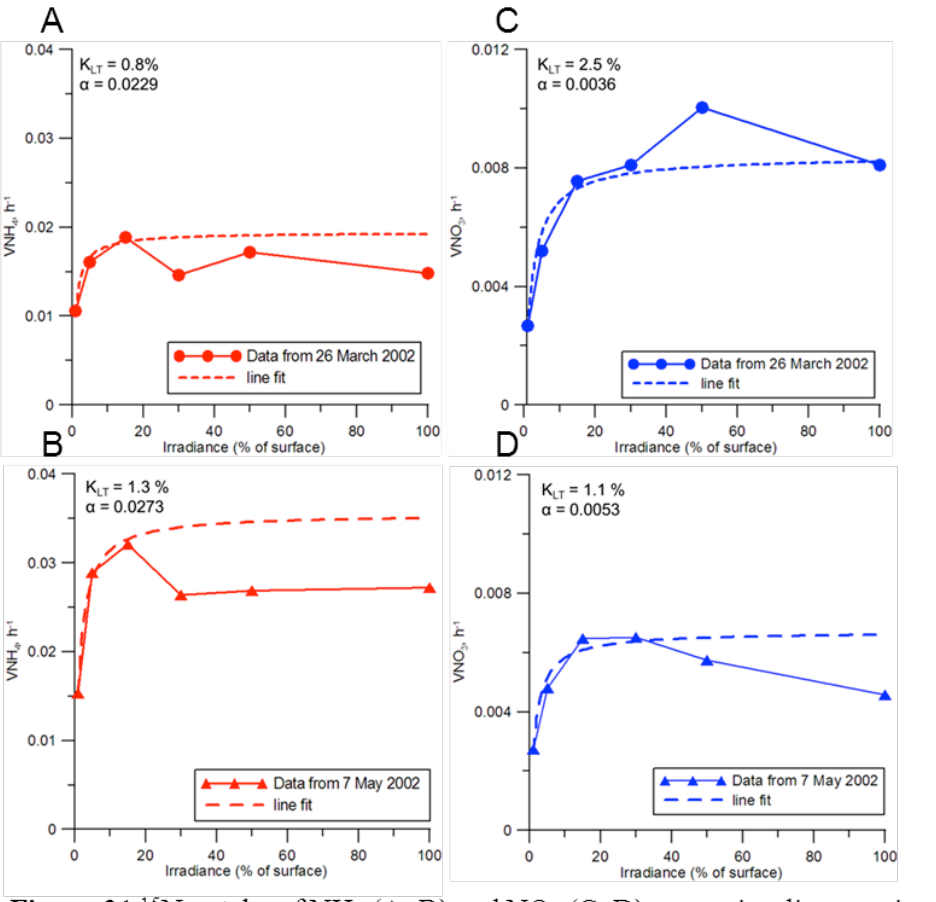

Figure 31 ^{15}N uptake of NH_4 (A, B) and NO_3 (C, D) versus irradiance using water collected from Central Bay

Both NH_4 and NO_3 uptake show Michaels-Menten kinetics in response to light (MacIsaac and Dugdale, 1972) and the Michaelis constant (irradiance for half V_{max}) is termed K_{LT} and typically NO_3 uptake displays a higher K_{LT} compared to NH_4 (MacIsaac and Dugdale, 1972). Published data on NH_4 and NO_3 uptake kinetics for the SFE are lacking.

Examples of uptake vs light relationships in the SFE were made using Central Bay water (Figure 31) and shows the hyperbolic curves were followed up to 15% of surface irradiance and then uptake declined for both nutrients. The same decline in uptake was observed in enclosure studies

in an upwelling area off the coast of Peru (MacIsaac et al 1985, their Figure 7). The implication is that these phytoplankton were shade adapted. K_{LT} values for both NH_4 and NO_3 uptake were ~ 1% of surface irradiance. The specific affinity (α) values (initial slope of the curve for NH_4 were an order of magnitude higher than for NO_3 (value), indicating that less light is required for NH_4 uptake compared with NO_3.

A direct demonstration of the effect of irradiance on NH_4 and NO_3 uptake was also observed in an enclosure experiment using water from Station USGS649 (confluence). The enclosures were held at different irradiances (50%, 25%, 10, 5%, 1% of surface) and nutrient drawdown tracked over five days (Dugdale et al. 2012 IEP presentation) (Figure 32). NH_4 drawdown (Fig 32A) to near zero in four days occurred at 25 and 50% of surface irradiance allowing NO_3 uptake and drawdown to begin and to reduce the NO_3 concentration to about half of the initial value in the following day (i.e. by day 5)(Figure 32B). Chlorophyll concentrations (Figure 32C) increased first with the use of NH_4 and then with NO_3 to a concentration of nearly 20 μg L^{-1}.

The results of this experiment follow clearly the NH_4 gatekeeper paradigm. Improved irradiance allowed NH_4 uptake to reduce NH_4 concentration to below NO_3 inhibition level, opening the NH_4 gate and enabling NO_3 uptake and access to the larger pool of DIN and additional chlorophyll production. In the darker enclosure NH_4 was drawn down but not sufficiently to enable much NO_3 drawdown. Additionally nitrification may be occurring in these darkened enclosures so adding to the NO_3 pool.

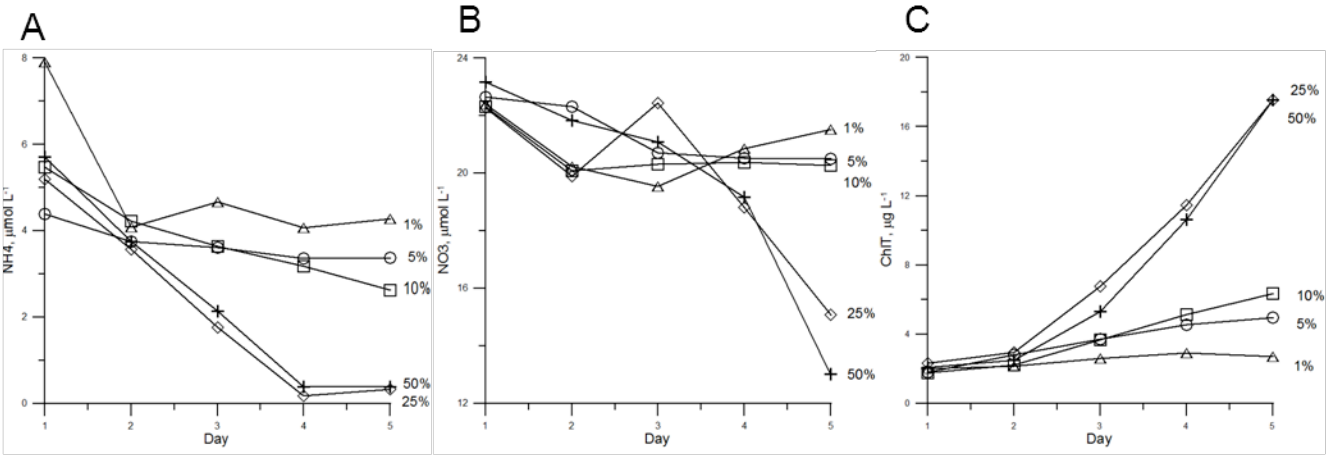

Figure 32. Enclosures filled with Central Bay water and held at different irradiances (shaded with window screening) showed (A) NH_4 drawdown, followed by (B) NO_3 uptake and (C) chlorophyll accumulation in the enclosures given 25% and 50% of surface irradiance

The interaction of light and nitrogen form may also influence the fate of new chlorophyll synthesized. Glibert et al. (2014b) showed that the chlorophyll increase (per μmol of N) in

enclosures amended with NO_3 and incubated at low light was twice that in enclosures amended with NH_4 and low light. A similar relationship occurred for fucoxanthin suggesting this chlorophyll to be largely made up of diatoms. This was not seen with added NO_3 and high light. This is another difference resulting from using NO_3 or NH_4 and the phytoplankton physiology that occurs and also suggests why diatoms do well in the high NO_3 and low light conditions.

Another aspect of light is water clarity and water depth of the phytoplankton ecosystem. As noted in Wilkerson et al. (2015) for Suisun Bay, although water transparency was greatest in the channel locations during high flow conditions such as spring 2011, and showed increasing trend going upstream towards the Sacramento River, blooms were more likely in the shoals (as also recognized by Lucas et al. (1999).. The lowest Secchi depths (i.e. transparency) were measured in Grizzly Bay at the shoal station DWR-D7. However, due to the shallow depth (1.6 m) at DWR-D7, PAR nearly always reached the bottom of the water column and mean Zeu/Zm was 1.03 in 2010, 1.18 in 2011 but 0.66 in 2012, indicating all the water column could be used for photosynthesis in contrast to the deeper channel stations where only the upper water column is available for photosynthesis. Thus the NH_4 gate may open in the turbid shoal and enable a bloom as sufficient NH_4 drawdown may occur (as observed in Figure 23 and in Wilkerson et al. 2015). In all years (2010, 2011 and 2012) of the Wilkerson et al. (2015) study, the shoal station showed elevated average chlorophyll concentrations compared to the channel stations.

A key next step is to more fully evaluate the relationships between irradiance and NH_4 and NO_3 uptake. Further evaluation of how the SFE phytoplankton respond to available irradiance in turbid but shallow shoals compared to those in deep but clearer water channels is needed. We have acquired a considerable data base that could be used for these purposes.

5.4.2. Uptake kinetics

A challenge in characterizing nutrient uptake kinetics from field populations is that nutrient concentrations may be elevated in water in which the phytoplankton are to be tested. For example Parker et al. (2012a) could not evaluate NO_3 uptake kinetics as the ambient NO_3 was likely at saturating concentrations. At the same time, overreliance upon nutrient uptake kinetics obtained under culture conditions, where nutrient concentrations can be tightly controlled, may deviate considerably from N kinetics carried out using natural populations. Recently, we have successfully "aged" water in order to reduce ambient nutrients in order to circumvent this problem in the Delta and characterize N uptake kinetics for *Microcystis spp.* (Lee et al. 2015). This approach shows promise and could be applied in other regions of the estuary.

Key next steps include developing a better understanding of the nutrient uptake kinetics of Suisun Bay (and SFE) phytoplankton. Experiments specifically designed to confirm and characterize the physiological basis for accelerated uptake ("shift-up kinetics") are needed. One

approach is to see if the response of shift-up can be evaluated by measuring changes in the activity of the enzyme NR with changing NO_3 and NH_4 substrate concentrations, following the work of Berges et al. (2004). Moreover, dynamic models of nutrient kinetics, not just fixed constants, need to be developed (e.g., Smith et al. 2009, Glibert et al. 2013, Glibert chapter, this report).

5.4.3. Contaminants

Very little is known about how chemicals supplied both from non point and point sources and introduced into the Sacramento River and Suisun Bay influence phytoplankton nutrient metabolism. Preliminary studies suggest that acute and chronic effects of the herbicides diuron and imazapyr decrease the uptake of both NO_3 and NH_4 in SFE phytoplankton (Blaser 2011 thesis).

Such chemicals may occur within the wastewater effluent stream as a result of stormwater runoff (Brooks et al. 2009). Because NH_4 loading to the SFE is overwhelming through municipal wastewater treatment (e.g., Hager and Schemel 1996; Jassby 2008), at least some of the reported measured NH_4 impacts to phytoplankton physiology may be due to other unidentified contaminants present in effluent, for which NH_4 serves as a "tracer". The large numbers of known and unknown contaminants in municipal wastewater (pharmaceuticals, personal care products, and industrial pollutants) are known to negatively impact aquatic invertebrate and vertebrate species (e.g., Bolong et al. 2009), including for the SFE (Brooks et al. 2009), although making direct links between contaminant mixtures and effects on biological communities is known to be difficult (Thompson et al. 2007).

Our studies showing NH_4 impacts on phytoplankton in the SFE and Delta have acknowledged the possibility that negative impacts on phytoplankton may be through these contaminants:

> "The high NH_4 condition, the result of wastewater loading to the northern SFE (Jassby, 2008), is potentially exacerbated by some additional stress that results in low NH_4 uptake rates. Owing to its proximity to the Sacramento/San Joaquin Delta, which receives nearly half of California's surface water, there are a large number of potential contaminants including herbicides and pesticides (Kuivila and Hladik 2008; Weston and Lydy. 2010; Werner et al. 2010), and metals (Johnson et al. 2010)." (Parker et al. 2012a)
>
> "It is unclear in the present study whether NH_4 or some other component of the sewage effluent (of which NH_4 concentrations act as a ''tracer'') is responsible for the relationship observed here between VNH_4 and NH_4 concentrations although experimental additions of SRWTP effluent into Sacramento River water collected upstream of SRWTP influence showed the same result (Parker et al. 2010)." (Parker et al. 2012b).

Sampling stations occupied during these studies were in close proximity to municipal wastewater effluent NH_4 discharge, either as part of the experimental design (i.e., Sacramento Regional Sanitation District WWTP, Parker et al. 2012b) or unintentionally (Suisun Bay stations USGS 6 and 7, Central Sanitation District of Contra Costa County WWTP, Wilkerson et al. 2006; Parker et al. 2012a). Parker et al. (2010) conducted a preliminary experiment testing the response of Sacramento River phytoplankton to additions of either NH_4Cl or a 24 hour composite of effluent-NH_4. This experiment revealed an unequivocal depression in phytoplankton C, NO_3 and NH_4 uptake for samples exposed to effluent-NH_4, but an unclear response of NH_4Cl additions. Follow-up experiments by Travis (2015) and Travis et al. (in prep) and Parker et al. (in prep) indicate negative impacts of effluent-NH_4 on phytoplankton but not NH_4Cl at variable concentrations (ranging between 20 to 50 μmol N L^{-1});. This variability may reflect variation in contaminant concentrations that reach wastewater treatment facilities. Similar experiments using advanced secondary (i.e., NO_3) effluent have not been conducted, but Kress et al. (2012) found a similar depression in phytoplankton abundance and C and N uptake immediately downstream of the Stockton Municipal WWTP, an advanced secondary discharger releasing NO_3

Key next steps are to test impacts of known contaminant mixtures on phytoplankton rates, including C and N uptake. Future experiments should include investigation of wastewater effluent from around the Bay, including those with different treatment practices (e.g., secondary versus advanced secondary treatment) as well as different contaminant profiles

5.4.4. Benthic supply of NH_4 and benthic contribution to productivity

Work to date on the Ammonium Paradox has focused on WWTPs as the major source of the NH_4 load to the water column and our work has explored the role of freshwater flow on the resulting concentration of the WWTP NH_4 load to Suisun Bay. Efforts to understand additional fluxes, including benthic fluxes, of NH_4 to Suisun Bay and incorporate these fluxes into nutrient models is a necessary next step. Another benthic uncertainty is the role of the microphytobenthos, the layer of superficial or benthic autotrophs and bacteria that can compete for nutrients with the phytoplankton. As with the other competing autotrophs, i.e. submerged aquatic vegetation there is little known or published about their nutrient metabolism or even their occurrence in SFE and possible role in trophodynamics.

Generally, benthic/pelagic coupling of biogeochemical cycles is understudied in the SFE (except for Caffrey 1995; Kubawara et al. 2009) and these linkages with the Ammonium Paradox are likely underappreciated, despite that the benthos can be the most nutrient and chlorophyll dense region of an estuary where the microbial communities may control nutrient flux from benthic to pelagic zones (Tyler et al. 2003). Cornwell et al. (2014) made benthic sediment nutrient rate flux measurements of nitrogen (from September 2011 to March 2014 from confluence to Suisun Bay)

and showed *sedimentary production of NH_4.* NH_4 flux was net positive across all sites in September.

NH_4 is released from the rich porewater concentrations, that in Suisun Bay reached 400 µmol L^{-1} by 3 cm depth (Cornwell et al. 2014, Glibert chapter this volume). When cores were treated with elevated salinity, efflux of NH_4 was increased from 50 µmol L^{-1} to >150 µmol $L^{-1.}$ Additionally denitrification (NO_3 reduction to NO_2 and N_2) and/or dissimilatory NO_3 reduction to NH_4 (DNRA) can occur in the sediment and change the DIN conditions. Denitrification rates measured by Cornwell et al. (2014) based on the N_2:Ar ratio approach were between 0.6 and 1.0 mmol m^{-2} d^{-1}, similar to other mesotrophic estuarine sediments.

The microphytobenthos have been estimated to be a major autochthonous source of organic carbon to the food web (Jassby et al. 1993, MacIntyre et al. 1996), although estimates of their primary productivity in the Bay Delta are uncommon. Cohen et al. (2014) found that microphytobenthos had higher chlorophyll (5.8 to >60 times greater) content than phytoplankton in several BayDelta wetlands, but contributed less to primary production. Despite this observation, it has been noted that the benthic microalgae are responsible for contributing heavily to the grazer food web in estuaries (Alpine and Cloern 1992). Cornwell et al (2014) estimated surprisingly high benthic microalgal productivity rates in Delta sediments and also suggested microphytobenthos may represent a major source of labile carbon to this ecosystem. They may also process the NH_4 in the rivers: Cornwell et al. (2014) suggest that benthic uptake of NH_4 could contribute up to 30% of the processing of the N discharged at Sacramento Regional Sanitation District WWTP (see also Glibert chapter this volume).

Another aspect of the benthic ecosystem to be explored is the contribution of the benthic grazers to the NH_4 part of the DIN pool. Kleckner (2009) measured NH_4 average excretion rate of *P. amurensis* of 5.05 µmol NH_4 h^{-1} g ash free dry weight $(AFDW)^{-1}$, consistent with the value reported by Glibert (this chapter), of 1-12 µmol NH_4 h^{-1} g $AFDW^{-1}$ based on nutrient flux measurements from cores containing clams. So the clam has the possibility of decreasing blooms both by closing the NH_4 gate and by grazing any chlorophyll produced. Dugdale et al. (in review) added a benthic grazer clam component to the NAMFLOW NH_4 model and incorporated NH_4 excretion and showed it to be an important parameter in delaying the uptake of NO3 by the phytoplankton. The balance between grazing by clams of phytoplankton, as production by clams of NH_4 and the benthic microbial N fluxes must be understood and placed into the context of processes in the pelagic zone.

Key next steps include continued measurements of benthic flux. Refinement of our understanding of benthic grazing and NH_4 regeneration by the benthos in the SFE. Characterizing benthic algal contribution to overall system productivity and susceptibility to the Ammonium Paradox.

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5.4.5. The role of NH_4 in phytoplankton community composition shifts.

Although not a focus of our research, we have speculated that the Ammonium Paradox is likely to differentially affect diatoms compared to other phytoplankton functional groups (Dugdale et al. 2007) and analysis of long-term monitoring data by others (Brown 2009, 2010; Glibert et al. 2011) indicate that a shift in phytoplankton taxa from diatoms to flagellates and cyanobacteria has occurred since the 1970s while NH_4 loading to the nSFE increased some three-fold (Jassby 2008).. Recent work (Lee et al. 2015) has shown that the toxigenic, colonial cyanobacteria *Microcystis spp.* collected from the SFE Delta displays N kinetics that favor the use of NH_4 over other forms of N. This is consistent with other analysis indicating that *Microcystis* in the Central SFE Delta overwhelmingly use NH_4 to support cellular N demand (Lehman et al. 2014). Lee et al. (2015) also showed that *Microcystis* in culture is less sensitive to NH_4 inhibition of NO_3 uptake. Finally, although diatoms are typically NO_3 opportunists it may be that some are able to handle high NH_4 (e.g. *Entomoneis,* see Lidstrom 2009) and some work has shown diatoms to grow with increased water residence time on wastewater NH_4.

Key next steps include experiments and models that can test hypotheses about phytoplankton community shifts due to alteration of N form should be carried out.

5.4.6. How N:P stoichiometry impacts phytoplankton blooms and community composition

It has been recognized that ecological stoichiometry, specifically N:P relationships, play an important role in determining whether seasonal blooms may occur and what algal species may be successful (Glibert et al. 2011; Glibert this volume) and the associated food web members (e.g. Bentley et al. in review). Inverse correlations have been reported for the long-term changes in chlorophyll a and DIN:DIP ratios, but this relationship is much stronger for the fall than the spring (Glibert this chapter). Direct experiments need to be conducted to test the strength of NH_4 inhibition on species under a range of temperature conditions and under a range of variable N:P stoichiometry. The effect of variable stoichiometry should be tested for conditions that are both limiting and non-limiting.

Key next steps are outlined in the Glibert chapter of this volume

5.4.7. Link to the coastal ocean and how climate at basin scales in the Pacific may impact biogeochemical processes in SFE?

Another unknown is how the coastal ocean influences biogeochemical processes in the SFE. It is clear that exchange of nutrients occurs, with export of nutrients at the Golden Gate. For example Wilkerson et al. (2006) showed that both SFE derived NH_4 and $Si(OH)_4$ along with unused NO_3

were observed in the lower salinity buoyant plume that moves northward along the coast. The $Si(OH)_4$ can only be from the SFE since surface concentrations above 40 μmol L^{-1} cannot be sourced from the Pacific Ocean. Wilkerson et al. (2002) using $Si(OH)_4$ to track SFE nutrients showed the interaction of basin scale climate and the exchange of nutrients (Figure 33). Data available for January of 1997, 1998 and 1999 showed that SFE was a source of $Si(OH)_4$ and NO_3 to the neighboring Gulf of the Farallones in neutral/normal conditions (i.e. 1997), became a major source during El Niño conditions (1998), but had little influence during the La Niña conditions of 1999.

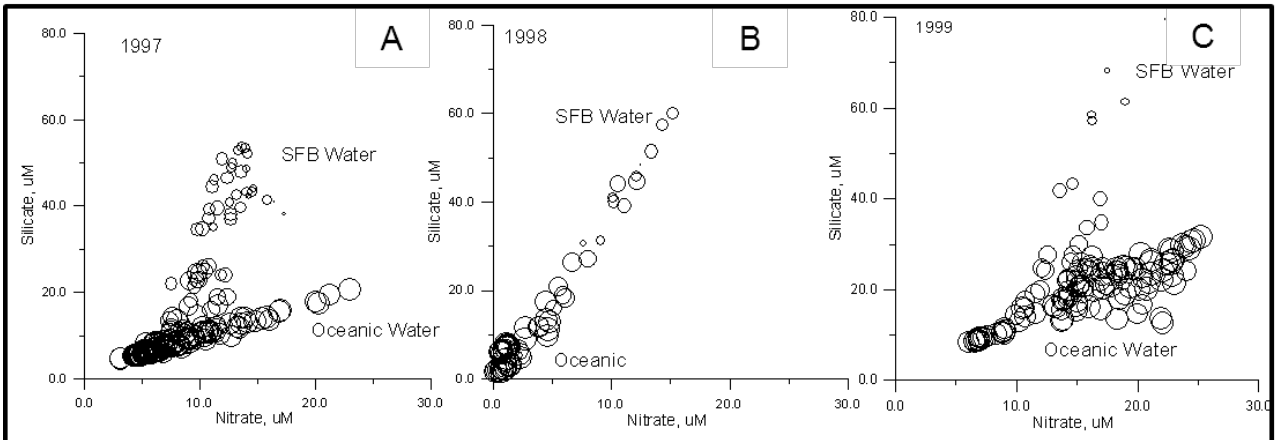

Figure 33 Silicate vs NO_3 plots showing how SFE water contributed in January to water masses in the Gulf of the Farallones during different climatic conditions, A) "normal", B) 1998-El Niño and C) 1999 La Niña. Circle size represents salinity (from Wilkerson et al. 2002).

Cloern et al. (2007) revealed "a previously unrecognized mechanism of ocean–estuary connectivity" to explain the appearance of phytoplankton blooms in SFE in 1999. The phytoplankton increase was consistent with a trophic cascade resulting from heightened predation on bivalves (and suppression of their filtration control on phytoplankton) by bivalve predators that accompanied a "cold phase" of the East Pacific. They noted the phytoplankton increase was paradoxical because it occurred in an era of decreasing wastewater nutrient inputs and reduced N and P. However the reduced wastewater nutrients would allow the NH_4 gate to open and we would predict more chlorophyll with less wastewater nutrient input.

A later paper (Cloern et al. 2010) showed the populations of demersal fish, crabs and shrimp to covary with the Pacific Decadal Oscillation (PDO) and North Pacific Gyre Oscillation (NPGO), both of which reversed signs in 1999. Interestingly the following paper (Cloern et al. 2011) projected the impact of climate change on California's SF Bay Delta-River system but the ocean changes were not included in the modeling effort. However the authors did point to uncertainty about how the SFE will evolve in future climate scenarios.

Key next steps are more studies measuring nutrients and phytoplankton rate processes inside and outside the Golden Gate at similar time and space scales. GCM-based models connecting the ocean, SFE and Delta that include biogeochemical and biological components are required.

5.4.8. Future –changes to the major WWTP influencing the nSFE

By 2021, the Sacramento Regional Sanitation WWTP plans to reduce NH_4 discharge into the Sacramento River by ~90%, and total DIN by 20-30% based on new permit regulations (SWTP Order R5-2013-0124). This means the future river will likely receive more NO_3 in addition to upstream sources (the SWTF permit maximum ~51 μmol L^{-1} NO_3), and the NH_4-plume in the river below the discharge site will likely be ~5 μmol L^{-1} NH_4 average (permit maximum, 7-12 μmol L^{-1}). This suggests that post-2021, NO_3 uptake will take place in the river. More importantly, as we have described in this chapter, biogeochemical fluxes in the river are low often limited by residence time and since the river acts more as a pipeline this low NH_4 but elevated NO_3 will be supplied to Suisun and San Pablo Bays. With reduced NH_4, the NH_4 gate will open and spring phytoplankton blooms are more likely to occur. The change at the Sacramento Regional Sanitation WWTP will have repercussions downstream.

Another management change that may be made by the WWTP in response to the extreme drought conditions occurring in California (that started in 2012) is to divert all the effluent in the summer months to use in agriculture. This would reduce the total DIN and NH4 in a similar way to that described for the 2021 scenario. Presumably the nutrients in the river and downstream would be those entering the river above the WWTP and the nonpoint sources below the WWTP.

It would be prudent to take ***next steps*** of monitoring the outcomes of such changes at the nutrient, biogeochemical flux and lower trophic levels. Our models (Dugdale et al. 2012, 2013, in review) should be improved and used to make "experiments" to evaluate the response.

5.4.9. Final Thoughts

Our research has provided a solid base of observation, experimental and process data to evaluate the role of nutrients as a contributing factor to the oligotrophic condition of nSFE. The recent interest in exploring the many potential roles that inorganic nutrients play in the ecology of the San Francisco Estuary opens up new areas for the development of conceptual models, hypothesis testing, and ultimately new approaches for estuarine management. Renewed interest in challenging the idea that *"nutrients don't matter"* is a positive step in the management of the SFE. Moving forward, it is critical that the SFE scientific community be open to new ways of thinking about nutrient regulation of phytoplankton community, and consider specifically the potential that nutrients play a regulatory role even at "saturating" concentrations.

It is our view that in the recent dialog about nutrients in the SFE, the importance of placing nutrient regulation into the context of multiple drivers has been lost, much as occurred previously with discussions about benthic control of phytoplankton biomass. We have attempted here to provide the evidence from both observational and experimental work that shows nutrient impacts of phytoplankton physiology, along with a mechanistic understanding of key processes. The gatekeeper conceptual model serves as a means to evaluate the Ammonium Paradox in the context of multiple ecological drivers that shape system responses to nutrients. The final section of uncertainties and next steps provides a synopsis of areas that we would prioritize future nutrient-related research for the SFE.

Our long-term estuarine data set includes both rate process and biogeochemical data and will compliment other long-term monitoring programs that exist for the Bay and Delta. Integration of our results into the Ocean-Bay-River models that are coming on line that combine hydrology, climate, biogeochemistry and productivity should aid in resolving nutrient - phytoplankton interactions. The underlying mechanistic data about nutrient processing by phytoplankton in nSFE available in our dataset is required for effective numerical nutrient endpoint decision making and for evaluating the eutrophication status of the nSFE.

Our physiological and flow models (e.g., Parker et al. 2012a; Dugdale et al. 2012, 2013) and uptake kinetics (e.g., Lee et al. 2015) need to be incorporated into these bigger models. A next step should be to incorporate a dynamic kinetic approach (e.g. Smith et al. 2009) as well as multiple currencies (C, N and P). Real progress in the prediction and management of the Bay-Delta ecosystem awaits the integration of validated submodels (for nutrients, phytoplankton growth and grazing) with validated 3-D open source hydrodynamic models, coupled with coastal ROMS models. These should all be integrated in turn with a monitoring program designed to serve both management and model operation. The California Coastal ROMS biogeochemical model is operational, i.e. run daily to make predictions of surface conditions, and is coupled with the SCHISM biochemical model of the Bay Delta. The aim should be to bring the coupled coastal estuary model to an operational level within two years.

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Table of Frequently used Abbreviations and Definitions

nSFE	northern San Francisco Estuary
WWTP	waste water treatment plant
LSZ	low salinity zone
NH_4	ammonium
NO_3	nitrate
DIN	dissolved inorganic nitrogen
NR	nitrate reductase
NRT	nitrate transporter
AMT	ammonium transporter
f-ratio	proportion of NO_3 uptake to total DIN uptake
NH_4 inhibition	NH_4 repression
NO_3 shift-up	acceleration of uptake
Ammonium Paradox	concept in which NH_4 prevents phytoplankton from being able to access the larger pool of nitrogen, NO_3, resulting in persistent low chlorophyll, lack of blooms and export of unused NO_3

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Section 3

Re-examining the paradigm of lack of nutrient regulation of primary productivity and trophodynamics of the San Francisco Bay Delta:
The view beyond classic nutrient limitation and the importance of dynamic metabolic regulation, the "paradox of enrichment," and ecological stoichiometry

Patricia M. Glibert
University of Maryland Center for Environmental Science
Horn Point Laboratory
PO Box 775
Cambridge, MD 21613

6.1. Introduction: The classic *vs* revised nutrient paradigm

It has long been known that nutrient availability (mainly nitrogen, N and phosphorus, P) regulates phytoplankton growth. Too little and growth is slowed; too much and excessive growth may be the outcome, with resulting eutrophication, hypoxia and associated adverse effects. From this it has been interpreted that if sufficient nutrients are available, as often measured by determining water column concentrations, then something else, either light or grazing, must be limiting phytoplankton growth, and by extension it is inferred that the nutrients in excess do not play a regulatory role with respect to productivity or trophic structure. This is the dogma that has been etched in the minds of students, aquatic scientists, and ecosystem managers alike. And this is the paradigm of the Bay Delta. Since nutrients are considered to be non-limiting, they are not thought to be regulating phytoplankton abundance or composition or trophodynamics, and instead light and grazing are considered to be the major regulatory factors of phytoplankton biomass and productivity (Cole and Cloern 1984, Alpine and Cloern 1992, Kimmerer 2004, Jassby 2008, Cloern and Jassby 2012, Kimmerer and Thompson 2014). Moreover, it is also interpreted from the perception of lack of limitation by nutrients that their differing proportions will have no effect on metabolism and growth or community composition. This idea, particularly with respect to phytoplankton, was articulated by Reynolds (1999, p. 31) who said, "...there should be no selective effect, consequential upon different affinities of storage capabilities for a nutrient resource, that might distinguish between the potential performances of any pair of planktonic algae, so long as the resource concentrations are able to saturate the growth demand. If that is true, then the ratio between the (saturating) concentration of any of the resources also fails to exert any regulatory significance." However, as will be shown herein, the control that nutrients impart on ecosystems - from phytoplankton metabolism to trophodynamic structure - is far more complicated than this simple perception.

In contrast to this classic view of nutrient regulation, a revised perspective of dynamic nutrient regulation and ecological stoichiometry incorporates our growing understanding that nutrient availability and the proportions of major nutrients have metabolic and ecosystem effects along the continuum from limiting to super-saturation, effects that are manifested at all levels of the food web. Nutrient form and proportion have consequences for ecosystem structure and function, *whether nutrients are limiting or not*. This paper describes the underlying mechanisms of dynamic nutrient regulation and how this has affected phytoplankton metabolism and community structure at all trophic levels. At the scale of metabolism and growth of primary producers, nutrient form and concentration affect the cell physiological processes of uptake and assimilation and, in turn, regulate the internal nutrient concentrations that determine growth and biomass yield (e.g., Hecky and Kilham 1988). Interactions between forms of the same nutrient also occur at the metabolic scale, affecting uptake and growth. Physiological preferences for, and metabolic differences in, assimilation of different nutrients lead to changes in phytoplankton community structure over time when nutrient availability changes. Moreover, the total amount of nutrients and their proportions also has implications for trophodynamics and broader ecosystem biogeochemistry, affecting the pathways and fluxes by which nutrients are recycled both in the water column and the sediment. Such pathways and fluxes further affect the quality of nutrients for primary producers and ultimately for the food web. It will be shown herein that in the Bay Delta nutrient forms have not only changed with time, the relative proportion of different nutrients have also changed with time, and these factors together have played major roles in the shift in species that has occurred, even if the system has been seemingly immune from classic eutrophication. Thus, the relevance of nutrients and their proportions to the shifting aquatic community of the Bay Delta should no longer be dismissed. It is time to replace the classic perception with contemporary perspectives of *dynamic metabolic, stoichiometric, and biogeochemical regulation*.

This paper is comprised of four parts. The first part reviews the effects of nutrient availability and composition on phytoplankton metabolism and community structure, with an emphasis on different forms of N, their interactions, as well as the relationships between uptake of different nutrients and between nutrient uptake and photosynthesis under dynamic conditions. The second part examines how nutrient content of grazers is regulated differently from that of primary producers and how nutrient content of prey differentially affects their grazers. The third part examines the effects of changing nutrient loads and biogeochemical processes and implications for changes in the stability of ecosystems. Each of these sections begins with a literature or theoretical overview and ends with relevance to the Bay Delta ecosystem. Where available, both experimental data on the metabolic or organismal level in response to nutrient changes are presented along with spatial or temporal changes in nutrients and how they relate to organismal or process-level responses for the Bay Delta. Finally, the fourth part of this paper synthesizes the analysis, examines overall implications for nutrient management, and provides some suggested next steps.

6.2. Nutrient availability, composition and algal metabolism

6.2.1. Classic *vs* dynamic nutrient kinetics

The role of limiting nutrients on algal growth is perhaps one of the most well known concepts in phytoplankton ecology. The concept of limiting nutrients was initially developed by Liebig (1855) for terrestrial plant systems and states that the nutrient in least availability relative to the needs of the organisms will limit total production. In classic phytoplankton physiology parlance, the kinetics of nutrient uptake and growth are defined analogously to the relationship describing enzyme-substrate kinetics, with two parameters, the half-saturation constant for uptake or growth, Ks (for uptake) or Kμ (for growth), and the maximal uptake or growth potential, Vmax (for uptake) or μmax (for growth), typically considered to be constants (Fig. 1). Nutrients are therefore assumed to be limiting if they fall below a measured or assumed Ks (or Kμ) value and sufficient if concentrations exceed such a value. This 'fixed' kinetic model is implicit in all assumptions that nutrients are non-limiting (and therefore non-regulating) in the Bay Delta, and has been applied in modeling studies that have been taken to show that dissolved nutrients play, at most, a minor role in primary productivity compared to light (Cloern et al. 1995, Cloern 1999, Jassby et al. 2002).

However, while this kinetic approach may be useful for defining nutrients when they are "limiting", such an approach fails to recognize the complexity of regulation that occurs throughout the range of substrate availability (Fig. 2a-d). Uptake or transport rates are generally assumed to be constant for a given process because they are enzymatic reactions, but there is, in fact, a wide range of variability in uptake kinetics even for a given substrate and species or functional group (reviewed by Goldman and Glibert 1983, Litchman et al. 2006). For example, the rate of nutrient uptake as a function of external nutrient availability can be highly variable depending on the physiological state of the cells, the time of exposure to the limiting nutrient, and other environmental factors (Goldman and Glibert 1983, Wheeler et al. 1982, Smith et al. 2009, Glibert et al. 2013 and references therein). Furthermore, measurements made over different periods of incubation or with different competing substrates have long been known to complicate interpretation of *in situ* rates or uptake kinetics (Dugdale and Wilkerson 1986; Gandi et al. 2011). Whereas short-term experiments measure uptake, longer scale experiments are skewed towards measurement of assimilation or growth (Wheeler et al. 1982; Goldman and Glibert 1983). Different species have vastly different capabilities for taking up nutrients and storing them in excess of their growth capabilities (e.g., Goldman and Glibert 1982; Flynn et al. 1999). When cells are not in steady-state the relationship between uptake and growth becomes uncoupled (Goldman and Glibert 1983; Fig. 2e). Most parameterizations of rate processes as a function of substrate assume a steady-state condition for the cell and such is the case with application of kinetic concepts to models of productivity in the Bay Delta; this wide plasticity in

nutrient uptake kinetics among species and within species under varying growth conditions (e.g., Rhee 1973, Burmaster and Chisholm 1979, Gotham and Rhee 1981, Goldman and Glibert, 1982, 1983, Morel 1987) has not been considered.

As is the case with photoacclimation, which is recognized to depend on the dynamic balance of energy flow through the entire photosynthetic apparatus and cell, rather than as a fixed response to an absolute irradiance (e.g., Kana et al. 1997, Geider et al. 1996, 1997, 1998), nutrient assimilation should be recognized to depend on the balance of nutrient acquisition at the cell surface and the maximal rate at which these nutrients can be assimilated within the cell, a balance between surface uptake sites and internal enzymes (Smith et al. 2009). *This dynamic balance approach recognizes that even at the level of "saturation" the cell continues to regulate its nutrient metabolism through processes of internal feedbacks and controls.* Such a suite of feedbacks may result in considerable adjustment of nutrient uptake in the region where nutrients are normally considered "saturating". Such adjustments may take the form of continued increase in uptake, leading to biphasic kinetics (Fig. 2c) or may reveal inhibition (Fig. 2d), comparable to a photosynthetic-irradiance curve at very high levels of irradiance. In fact, kinetic relationships should be viewed as continually varying within the bounds of a response surface and deviations from a single, classically defined kinetic relationship should be viewed as the norm rather than the exception (Goldman and Glibert 1983, Smith et al. 2009, Glibert et al. 2013; Figs. 2a-e).

The implications of a dynamically varying, rather than fixed kinetic model are important. On the one hand, nutrient stress can develop before nutrient availability declines below the defined half saturation value (and bearing in mind how poorly this value is typically known), while on the other hand, regulation of nutrient uptake does not cease when availability of nutrient reaches values defined as "saturating". Furthermore, regulation of nutrient uptake along this continuum may differ for different nutrients- or for different forms of the same nutrient. Importantly not only are there differences in cellular nutrient content between taxa, but within taxa at any given time there are differences in the plasticity or flexibility in nutrient content. Such regulation is fine-tuned and balanced at steady state. However, natural communities are rarely growing at steady state under single nutrient sources and fixed concentrations and are composed typically of numerous taxa. Conceptualizing the relationships between physiological processes and growth as dynamic rather than as fixed kinetic relationships, and understanding how this regulation may differ for different nutrients, has further implications for cell properties and ultimately for ecosystem metabolism. Emergent properties of cells in response to this dynamic balance include the relative proportions of ribosomes, enzyme activities, gene regulation, cellular pigmentation complement, and ultimately the cell elemental composition (e.g., Glibert et al. 2013). At the broader scale, the wide plasticity of cell composition in algae under both nutrient-limited and nutrient-saturated conditions alters the

elemental *quality* of the algal food available to grazers (Sterner and Elser 2002, Glibert et al., 2011), affecting their metabolism and emergent properties such as growth rate, fecundity, and ultimately the success of different populations of upper trophic levels (e.g., Jeyasingh and Weider, 2005, 2007, Boersma and Elser 2006).

6.2.2. Uptake of different forms of N- individually and when co-provided

One of the important metabolic processes of nutrient regulation for a phytoplankton cell is the assimilation of different forms of N. At any given time a phytoplankton cell (unless growing in highly controlled laboratory conditions) is regulating the acquisition of many different forms of the same nutrient. For N, the cell is generally balancing the uptake of both oxidized (e.g., nitrate, NO_3^-) and reduced (e.g., ammonium, NH_4^+) forms. In many cases, the cell may also be balancing the uptake of organic forms of N as well (Bronk et al. 2007 and references therein), however for the purpose of this discussion only the major inorganic N forms NO_3^- and NH_4^+ will be emphasized. Preferences for - and interactions between - one form of N *vs* another, especially when N is in "excess" relative to nutritional demands, have important metabolic consequences.

A central tenet of N metabolism of phytoplankton is that NH_4^+ is the preferred form of N relative to NO_3^- (e.g., McCarthy 1981, Raven et al. 1992 and references therein). The assimilation of NH_4^+ is considered to be less energy expensive for the cell, and NH_4^+ is more easily transported across the cell membrane than NO_3^- under balanced growth and N limited conditions. With a redox state of -3 for NH_4^+ and +5 for NO_3^-, it takes 8 electrons to reduce NO_3^- to NH_4^+ in the cell. Preferential uptake or use is defined in a number of ways in the literature, generally involving a comparison of 1) rates of draw-down of one substrate relative to another, 2) uptake affinity, 3) maximal or *in situ* rate of uptake, or 4) an index of relative preference (RPI) for different N forms. Although there is some debate as to the value of each of these indices, the general pattern of most reports is the same: NH_4^+ is typically preferentially used. Evidence for NH_4^+ preference is several-fold, much of this understanding grounded in the classical physiological literature, and, importantly, in studies where N was the limiting nutrient. Some of the first observations that NH_4^+ is assimilated by algae first, and only then does NO_3^- get assimilated, were from batch culture experiments in the 1930's, 40's and 50's (e.g. Ludwig 1938, Harvey 1953). McCarthy (1981, p. 224) stated, "...it is clear from many batch culture studies that there is little evidence to suggest that NO_3^- in the growth medium will be utilized when the available NH_4^+ is sufficient to meet the N growth requirement (cf. Syrett 1962, Morris and Syrett 1963, Eppley et al. 1969, Thacker and Syrett 1972)". In culture experiments where both NH_4^+ and NO_3^- are supplied together, as well in experimental mesocosm experiments in which both substrates are available, the typical pattern is for NH_4^+ to be drawn down first, and only then is NO_3^- used in any substantial way, as evidenced by the relative rates of

disappearance of the different N substrates from the water or media provided. Some of the early field demonstrations of this phenomenon were by MacIsaac and Dugdale (1969, 1972), followed by research in the Chesapeake Bay by McCarthy et al. (1975, 1977). Such a pattern has also been well documented in the Bay Delta from experimental mesocosms (Dugdale et al. 2007).

Uptake of the different forms of N are not equally substitutable, nor do they necessarily provide the same total N for the cell, even when provided in the same concentration (e.g., Glibert et al. 2014a, in review). There are many important differences in the transport and assimilation of NO_3^- and NH_4^+ by phytoplankton in addition to their redox state (Glibert et al. in review and references therein). As an example, NO_3^- transporters are induced by the presence of their substrate (NO_3^-), whereas NH_4^+ transporters are induced by the absence of their substrate, NH_4^+ (Clarkson and Luttge 1991, Navarro et al. 1996, Crawford and Glass 1998). Such a phenomenon for NO_3^- is seen as an acceleration of NO_3^- uptake in field measurements and has been termed "shift-up" (e.g., Dugdale et al. 1981, 2015 and references therein). Such a shift up in cellular machinery to process NO_3^- has been well documented for NO_3^- but not for NH_4^+ (e.g., Smith et al. 1992, Berges et al. 2004, Lomas 2004, Allen et al. 2006). In contrast, NH_4^+ (or, as described below, its assimilation products) acts as a repressor of NH_4^+ transport and assimilation. Consequently relationships such as biphasic or linear nutrient kinetics (Fig. 2c) are more common for NO_3^-, while saturating or inhibition kinetics (Fig. 2d) are more common for NH_4^+.

The seeming favorability for NH_4^+ by phytoplankton is actually a function of both the preferential use of NH_4^+ due to its favorable energetics and the inhibitory or repressive effect of NH_4^+ on NO_3^- uptake and assimilation (Dortch 1990, Glibert et al. in review and references therein). In addition to the repressive effect of NH_4^+ on NO_3^- metabolism, at very high concentrations - generally considered in the mM range, both NH_4^+ and the unionized form, NH_3, can be directly toxic due to destabilization of membranes and other redox effects (e.g., Britto and Kronzucker 2002, Glibert et al. in review; Fig. 3). However, under typical natural conditions, most of the repressive effect of NH_4^+ is due to the pronated form (NH_4^+), not the unionized form (NH_3) and direct toxicity from NH_3 is not likely to be significant except in very localized conditions (Glibert et al. in review). Collectively, these observations have led to NH_4^+ being characterized as a "paradoxical" nutrient, being preferentially used at the low end of the availability spectrum while considered inhibitory or toxic at the elevated end of the concentration spectrum (Britto and Kronzucker 2002, Dugdale et al. 2012, Glibert et al. in review; Fig. 3).

Interactions of NO_3^- and NH_4^+ have long been known, and while the complexity of the metabolic interactions continue to be unraveled, in general it is well understood that increasing cellular NH_4^+ has a repressive effect on NO_3^- uptake and its assimilation, while NH_4^+ uptake is

relatively unaffected by the availability of NO_3^- (Clarkson and Luttge 1991, Navarro et al. 1996, Crawford and Glass 1998, Flynn et al. 1997, Daniel-Vedele et al. 1998; Fig. 4). The typical response of NO_3^- uptake in the presence of increasing NH_4^+ concentrations is a near complete repression of NO_3^- uptake and examples of this relationship in the literature have long been reported (e.g. Caperon and Ziemann 1976, Collos 1989, Dortch et al. 1991, Lomas and Glibert 1999a,b, Maguer et al. 2007, L'Helguen et al. 1993; e.g., Figs. 4,5). Increasing concentrations of NH_4^+ serve to not only decrease the number of NO_3^- transporters for the cell, but they also down-regulate the activity of NO_3^- reductase (NR), the enzyme catalyzing the reduction of NO_3^- to NO_2^-, which is subsequently reduced to NH_4^+ via the enzyme nitrite reductase (NiR, e.g., Vergera et al. 1998). As a generality, the response by NR to any stress factor is an alteration in the structure of the enzyme, shifting the enzyme toward polyfunctionality, thereby increasing its reactivity with other substrates (Morozkina and Zvyagilskaya 2007). While the relationships between NH_4^+ availability and repression of NO_3^- uptake and assimilation are robust, it is now recognized that down-stream metabolites, not NH_4^+ *per se*, are responsible for this down-regulation (Flynn et al. 1997, Krapp et al. 1998, Lejay et al. 1999, Flynn et al. 1997). In most algae, the regulation is via the size of the glutamine pool (e.g., Flynn et al. 1997, Flynn and Fasham 1997) although in cyanobacteria and some other taxa, the metabolite 2-oxoglutarate also serves this regulatory function (Tanigawa et al. 2002; Muro-Pastor et al. 2001, 2005, Ohashi et al. 2011, Post et al. 2012). When glutamine levels are high, NR activity levels are "throttled" back (Flynn et al. 1994, Campbell 1999), and conversely if the supply of NH_4^+ is too low to maintain a high internal N-status (Flynn et al. 1989, 1994), then the ability to transport and use NO_3^- is up-regulated.

However, even though the biochemical and physiological regulation of NH_4^+ (or its metabolites) on NO_3^- transport and assimilation are well described, it is also known that not all phytoplankton at all growth conditions are equally susceptible to NO_3^- repression by the same amount of NH_4^+ or the metabolites of its assimilation. The threshold concentrations of NH_4^+ required to repress NO_3^- uptake or assimilation have been shown to depend on the species present, their physiological status (Dortch and Conway 1984, Dortch et al. 1991, Maguer et al, 2007), and the environmental conditions to which they have been exposed (e.g., Bates 1976, Harrison et al. 1996, Lomas and Glibert 1999a,b). Repression of NO_3^- uptake by NH_4^+ has been shown to occur at NH_4^+ concentrations as low as a few μM (e.g., Eppley et al. 1969, Bates 1976, Lund 1987, Dortch and Conway 1984, Cochlan and Harrison 1991, L'Helguen et al. 2008 among others; Fig. 5). From work in the subarctic Pacific, Wheeler and Kokkinakis (1990) even suggested that concentrations of NH_4^+ between 0.1 and 0.3 μM caused complete repression of NO_3^- assimilation, and L'Helguen et al. (2008) reported that similar concentrations of caused repression of NO_3^- uptake in the oligotrophic Atlantic. In the Bay Delta, much higher concentrations of NH_4^+, 4-10 μM, have been associated with repression of NO_3^- uptake by NH_4^+ based on direct measurements (e.g., Dugdale et al. 2007, Glibert et al. 2014b), and

similar concentrations were found to repress NO_3^- uptake in laboratory cultures of diatoms and dinoflagellates (Lomas et al. 2000; Fig. 5). Cells growing on highly elevated NO_3^- availability, as in the case of a nutrient rich environment may require considerably more NH_4^+ to repress cellular NO_3^- activity than is the case for cell with a low cellular NO_3^- content, as is the case in oligotrophic environments.

The dynamic regulation of nutrient acquisition and assimilation, along with the interactions of nutrient forms, leads to considerable disparity in reports with respect to these effects on growth. While several studies have shown that some phytoplankton species grown on NH_4^+ or urea may have higher growth rates than on NO_3^- (Wood and Flynn 1995, Herndon and Cochlan 2007, Solomon et al. 2010 and references therein), and such a pattern would be consistent with the observation of preferential uptake and assimilation of NH_4^+ over that of NO_3^-, in sharp contrast it has also been documented that under conditions of highly elevated NH_4^+, typically exceeding several tens to hundreds of μM, both the total N taken up and overall growth with NH_4^+ enrichment can be depressed rather than enhanced (e.g., Dasgenais-Bellefueille and Morse 2013 and references therein, Glibert et al. 2014a, in review). This latter observation is consistent with the repression of NO_3^- uptake and assimilation and its resulting effects on overall growth. Note that many comparisons of growth rates on one N form *vs* another are based on laboratory studies in which only a single N form was provided, and therefore the metabolic consequences of interactions between N forms were not expressed. Self-regulating feedbacks serve to alter both the transport and assimilation of different forms of N in the cell. When these feedbacks are overtaxed, as is the case in excessive NH_4^+, it may alter the ability of the cell to produce new biomass. As discussed below, such feedbacks may be under the same biochemical regulation but may be expressed differently in different phytoplankton taxa.

6.2.3. Nitrogen specialists within the phytoplankton and effects on community structure

It is well known that different classes of phytoplankton, and even different species of phytoplankton within the same classes have different eco-physiological characteristics with respect to their nutrient requirements. With a size range that spans many orders of magnitude in terms of cell volume, from <2 μm to 2000 μm, there should be no question that physiological processes such as affinity for nutrient, maximal uptake rates and other aspects of metabolism, should vary widely (Chisholm 1992, Finkel et al. 2010 and references therein- Fig. 6). Cell size alone sets many biophysical constraints on many aspects of physiology, including nutrient transport and assimilation (e.g., Finkel et al. 2010, Wu et al. 2014).

Although the biochemical and physiological preference for NH_4^+, and the effect of repression

of NH_4^+ (or its metabolic products) on NO_3^- transport and assimilation in phytoplankton are well understood, it is also increasingly recognized that not all function groups of phytoplankton express these effects to the same degree. A substantial body of literature suggests that diatoms are NO_3^- specialists (e.g., Lomas and Glibert 1999a,b, Figueras et al. 2002, Kudela et al. 2005), while cyanobacteria, especially picocyanobacteria and many chlorophytes and dinoflagellates, may be better adapted to use of NH_4^+ and such differences are consistent with differing evolutionary lineages of these groups (Glibert et al. in review and references therein). In fact, a broad survey of algae grown in culture suggested differences in NH_4^+ tolerance, with chlorophytes being most tolerant, and cyanobacteria and dinoflagellates being more tolerant than diatoms or raphidophytes (Collos and Harrison 2014). Such a spectrum of responses is consistent with emerging understanding of the differences in physiology between and among different functional groups (Wilhelm et al. 2006, Glibert et al. in review and references therein). For example, many cyanobacteria have constitutive expression of high affinity NH_4^+ transporters (Wilhelm et al. 2006 and references therein), while in general diatoms tend to have more copies of high affinity NO_3^- transporters (e.g. Armbrust et al. 2004). Molecular phylogenies of both NO_3^- and NH_4^+ transporters confirm that there are clear differences between those of diatoms and of other major algal groups (Song and Ward 2007, Chan et al. 2011, Kang et al. 2011, Kang and Chan 2014; Fig. 7). There are also differences in the regulation of transporters between centric and pennate diatoms (Bender et al. 2014). Additionally, Lomas and Glibert (2000) reported, from a comparison of 9 species grown under the same conditions, that diatoms had significantly higher cell-specific rates of NR activity than did the tested flagellates.

The different patterns of uptake of NO_3^- and NH_4^+ are embodied in the classic oceanographic paradigm of new and regenerated production (Dugdale and Goering 1967). This paradigm recognizes the distinction between production resulting from those reduced N forms, primarily NH_4^+ and urea, that are regenerated *in situ* (from zooplankton excretion or bacterial remineralization in the water column or sediment) and production resulting from the use of oxidized N forms, primarily NO_3^-, resulting from allochthonous inputs to a system. Marine pelagic ecosystems with predominantly NO_3^- sources are often dominated by diatoms (e.g., Kudela et al. 1997, Kudela and Dugdale 2000, Wilkerson et al. 2000) and typically have short, efficient food webs at the base of major natural fisheries (e.g., coastal Peru; Ryther 1969) and high rates of export of organic matter from the photic zone (e.g. Eppley and Peterson 1979). A proportionately greater flow of organic material through the microbial loop generally occurs when systems are more enriched with chemically reduced N forms, NH_4^+ and urea, and the resulting community composition is often dominated by mixotrophic dinoflagellates or (pico)cyanobacteria as well as bacteria (Eppley and Peterson 1979, Legendre and Rassoulzadegan 1995, Berg et al. 1997, 2003, LaRoche et al. 1997, Lomas et al. 2001, Glibert 1998, Glibert et al. 2001, 2006, 2010a). Bloom-forming diatoms are

often considered to be "r" selected species, with rapid growth rates, whereas K-strategists dominate in more nutrient-poor, "mature" systems, typified by dinoflagellates (Flynn et al. 2013). As will be described in more detail below, there is also evidence that the production of chlorophyll *a* on NO_3^- growth may be greater than that on NH_4^+ growth, at least under some conditions (Glibert et al. 2014a). Interestingly, in terrestrial systems, a similar pattern of species and growth is observed when soils are enriched with NO_3^- compared to NH_4^+: soil enrichment with NO_3^- often leads to early successional species with faster growth rates, while enrichment with NH_4^+ leads to later successional species (Britto and Krunzucker 2002 and references therein).

Large-scale nutrient manipulation experiments have provided strong confirmatory evidence that when natural phytoplankton communities are exposed to different N forms, the end result is changes in phytoplankton community structure over time consistent with physiological preferences or tolerances for specific N forms. Early mesocosm experiments by Glibert (1998) showed that different size classes of phytoplankton develop when the proportion of NO_3^-: NH_4^+ varies: a doubling in the ratio of the ambient NO_3^-:NH_4^+ resulted in a nearly 50% increase in the ratio of >10 μm:<10 μm sized biomass. In laboratory mesocosm experiments conducted with nutrient-rich water from the Choptank River (a tributary of Chesapeake Bay), Glibert and Berg (2009) showed that NO_3^- uptake was directly related to the fraction of the community as diatoms, while the proportion of NH_4^+ uptake was directly proportional to the fraction of the community as cyanobacteria. In experiments conducted in hypereutrophic Wascana Lake, Saskatchewan, Canada, in which N enrichments were made on a weekly basis, significant differences were observed between the NH_4^+ and NO_3^- enriched mesocosms in terms of overall biomass and composition (Donald et al. 2011, 2013). They found that NO_3^- enrichment led to a proportionately greater increase in chlorophyll *a* (relative to total wet weight algal biomass) and a greater initial response by diatoms, while NH_4^+ enrichment lead to a proportionately greater increase in cyanobacteria (Fig. 8). Domingues et al. (2011) also showed that enrichment by NH_4^+ in a freshwater tidal estuary favored chlorophytes and cyanobacteria whereas diatoms were favored under NO_3^- enrichment. As described in more detail below, similar patterns of response were also found in experiments conducted in the Bay Delta (Glibert et al. 2014a). Toxic cyanobacterial species also appear to be favored over diatoms when N is supplied in chemically-reduced relative to oxidized forms in the hypereutrophic Lakes Taihu, China, and Okeechobee, Florida (McCarthy et al. 2009). Additionally, there are also similar reports from field studies showing that dinoflagellates, many of which are harmful algal bloom (HAB) formers, are also associated with increased proportion of N in chemically-reduced rather than oxidized form (e.g., Berg et al. 2003, Glibert et al. 2006, Heil et al. 2007). Such growth responses by different species groups under different N forms are not just a function of the relative availability of each form; the physiological basis for such differences determines their relative success when one form of N dominates over the other. Yoshiyama and Sharp (2006) summarized decades of data

from the Delaware Bay and observed that the primary productivity rate per unit chlorophyll *a* declined exponentially with increasing NH_4^+ concentration (most of the change occurring on the <10 μM NH_4^+ range) and classified these systems as High-Nutrient, Low-Growth (HNLG; Sharp 2001).

6.2.4. Balancing nitrogen metabolism with other cellular demands

At any given time a cell is regulating not only the acquisition of different forms of the same nutrient (e.g., NH_4^+ and NO_3^-), but it is also balancing the uptake and metabolism of other nutrients (e.g., N and P), along with cell energy demands, reductant and cell redox state. Well recognized within the phytoplankton ecology literature is the importance of N:P stoichiometry. Codified by Redfield (1958), phytoplankton have often been reported to have an elemental composition of 16:1 in terms of molar N:P (= ~7 on a wt:wt basis; Redfield 1934, Falkowski 2000, Arrigo 2005). However, wide ranges in this ratio have been reported (e.g., Klausmeier et al. 2004, Finkel et al. 2010, Hillebrand et al. 2013) and there is a growing appreciation that different taxa have different elemental composition under ideal growth conditions (Geider and LaRoche 2002, Ho et al. 2003, Klausmeier et al. 2004) and that there is an evolutionary basis for elemental stoichiometry in phytoplankton (Quigg et al. 2003). Importantly there is accumulating evidence that a species' elemental stoichiometry varies with environmental conditions (Leonardos and Geider 2004a,b, Cullen and Sherrell, 2005, Finkel et al., 2006, 2007, Glibert et al. 2013). Phytoplankton stoichiometry is a function of taxon-specific differences on the one hand, and their flexibility in stoichiometry through luxury uptake and storage, on the other. In a meta-analysis of both fresh and marine studies of phytoplankton stoichiometry, Hillebrand et al. (2013) confirmed that phytoplankton N:P varied with growth rate, increasing under N limitation, and decreasing under P limitation (Fig. 9). But, in addition to having a higher P content, fast-growing phytoplankton were more constrained in their stoichiometry. Hillebrand et al. (2013) also found that the weighted molar averages for optimal N:P (defined as the ratio of minimal cell quota for N and P when available ratios exactly match uptake and growth demands and there is no luxury uptake or storage) were lowest for diatoms (14.9) and increased for dinoflagellates (15.1) and even more for cyanobacteria (25.8) and chlorophytes (27.0; Fig. 9). Their findings lend support to the idea that not only does phytoplankton N:P become more restricted and is lower with increasing growth rate, but that at maximum growth rate, N:P converges to an optimal ratio (or a more narrowly defined range) that is different for species and phylogenetic groups. As will be developed below, the relevance to Bay Delta of the fact that different species have varied elemental stoichiometry is that the availability of N:P has changed considerably over time, with availability of P declining while N has increased. The extent to which different species can adapt to this changing nutrient availability has consequences for changes in species dominance over time.

Taxonomic differences can be significant in terms of elemental composition due to biochemical differences in allocation of biomolecules, including proteins, carbohydrates, lipids, nucleic acids and storage molecules, such as polyphosphates (Geider and LaRoche 2002, Sterner and Elser 2002). The growth rate hypothesis (GRH) suggests that interspecific variations in P content relate to differences in maximal growth rates due to differences in P-rich ribosomal RNA, such that faster growing organisms require an overall greater P content associated with more rRNA and increased capacity for protein synthesis (Elser et al. 1996, 2000). Indeed, strong couplings between growth rate, RNA, and/or P content have been documented for some algae and cyanobacteria (e.g., Rhee 1978, Lepp and Schmidt 1998), but the capacity of many algae to store large amounts of P complicates this relationship. In fact, for P uptake the enhanced capacity for uptake relative to growth may, for some algae, be counterproductive when provided at high concentrations, resulting in reduced rather than enhanced growth (e.g., Reef et al. 2012). Thus, elevated levels of P have been shown to have metabolic costs in terms of growth; this is another example of metabolic regulation across the entire spectrum of substrate availability.

Looking specifically at the types of adaptations that phytoplankton have for nutrient acquisition, there are a number of specific physiological strategies that allow certain types of algae to thrive under conditions of variable P relative to N (Glibert and Burkholder 2011). For conditions when P is reduced relative to N cellular demands, one strategy is that of having a lower overall requirement. Very small cells, such as picocyanobacteria, have a lower requirement for P due to the smaller need for structural components in the cell (Finkel et al. 2010). Alternatively, species that may thrive under such conditions may have the ability to "make do with less" by physiological substitution of a P- containing compounds with a non-P- containing compound, as in the case of substitution of a P-containing lipid with a non-P- containing lipid (sulfolipid) and many cyanobacteria do appear to have this capability (Van Mooy 2009). Thus the cellular C:P content of *Synechococcus* for example, is about 100, whereas that of a typical diatom is about 50 (Finkel et al. 2010). Many algae also have well developed capability for acquisition of P via alkaline phosphatase, and therefore they can use organic forms of P when other cells cannot. Thus, as nutrient availability changes, species with different cellular nutrient content may either be physiological stressed or may be favored.

Another important process that phytoplankton cells must balance with their nutrient acquisition is that of C assimilation, or photosynthesis. The balance of C and P in the cell is important especially with respect to energy (ATP) and redox balance (NAD(P)H) for the cell, while that of C and N is more directly linked in the assimilation of new biomass. The assimilation of N and of C are linked in multiple biochemical pathways and thus C and N metabolites have various "cross-talk" in the cell (Glibert et al. in review). Regulation of the balance between C and N acquisition and metabolism is especially challenging and the cell has various coping mechanisms to ensure that a

balance can be maintained. Fundamentally there are two mechanisms to adjust imbalances in substrate (or energy) availability: up-regulate the pathways for acquisition of the constituent that is in least supply, or down-regulate the cellular constituent that is in over-supply. Up-regulation results in enhanced or accelerated assimilation (and therefore growth potential), while down-regulation results in a slowing of assimilation (and growth potential). Up-regulation is only possible when there is sufficient (but not excessive) supply of all necessary elements. Cellular energy balance as been termed the "broker" of coordinated regulation between N and C interactions (Foyer et al. 2011). Through signaling pathways that sense a change in cellular metabolites, internal N or C pools, or redox state of the cell, a change in the regulation of N uptake or metabolism occurs through changes in gene and enzyme expression and activity.

Some of the well-documented processes by which a cell down-regulates the photosynthetic process include non-photochemical quenching mechanisms such as xanthophyll cycling, dissipation of heat and Mehler activity (e.g., Müller et al. 2001; Fig. 10). These processes allow cells to rebalance their excess electron energy that may derive from photosynthesis during periods of non-steady-state growth and are important in the continual dynamic regulation of metabolism. However, such pathways and metabolic feedbacks may be disrupted or overwhelmed when the cell is subjected to stress, including a change in the redox state of the N compound on which they are growing. Nonphotochemical quenching, for example, may be greater when the cell is growing on NH_4^+ than on NO_3^- (Shi et al. in press, Glibert et al., in review; Fig. 11a).

Recent physiological and molecular studies especially of diatoms have revealed much insight into feedback mechanisms of N and C assimilation and the role of pathways that serve as release pressure values when metabolism is stressed, including elevated concentrations of NH_4^+ as a stress. An important pathway regulating overall cellular energy balance in algae, especially diatoms, is the reduction of NO_3^- and NO_2^- via NR and NiR in a nonassimilatory mode that complements such reduction in N assimilation (e.g., Lomas and Glibert 1999a,b, Parker and Armbrust 2005, Rosenwasser et al. 2014; Fig. 10). The importance of this pathway relates to the identification of diatoms as NO_3^- specialists, but also to their susceptibility to repression by increasing NH_4^+. Diatoms appear to use NO_3^- as an oxidant to dissipate the periodic overflow of electron energy through the activities of NR and NiR as an additional pathway to their use of NO_3^- as an N nutrient. The reduction of NO_2^- to NH_4^+ in the chloroplast uses the reducing power of the ferredoxin (Fd) system, and there it can serve as a sink for excess reductant, derived from the splitting of water (in photosynthesis), that may develop when photochemistry exceeds assimilatory capacity, as might be observed during conditions of high light but cool temperatures (i.e., $<18^oC$; Glibert et al. in review). This process is particularly important at comparatively cool temperatures because the biophysical light reactions of photosynthesis are relatively temperature-insensitive, but the biochemical

reactions (e.g., Calvin Cycle reactions) are temperature-sensitive, leading to a reduction in the rate of the reactions of C assimilation. This process can thus protect the chloroplast electron transport chain from over-reduction and may be characterized as a ‘‘futile cycle’’ (Lomas and Glibert, 1999b). In order for such a dissimilatory pathway to function, release of N in a more reduced state should be observed. In fact, release of NO_2^- by diatoms has also been commonly observed during NO_3^- uptake (e.g., Serra et al. 1978, Anderson and Roels 1981, Collos 1982, Ward et al. 1984), and there are also numerous reports of release of NH_4^+, as well as release of DON, from both field and laboratory cultures utilizing NO_3^- (Lomas et al. 2000 and references therein). Clearly an important criterion for such pathways to function is the availability of NO_3^- or NO_2^- in the cell and associated enzymes to carry out reduction. Without these substrates the options for redox homeostasis are more limited. If NO_3^- uptake and assimilation are repressed by sufficiently elevated concentrations of NH_4^+, then this important mechanism of energy balance cannot function in the cell and stress results.

The interaction of NH_4^+ and NO_3^- also appears to play an important role in the balance of C assimilation and photorespiration (Parker and Ambrust 2005, Shi et al. in press, Glibert et al. in review). Pathways of C and N assimilation are linked via their requirements for ATP and NAD(P) that are most efficiently generated though the light reactions of photosynthesis and therefore via the activity of the C-assimilating enzyme, Rubisco. This is an enigmatic enzyme, as it has dual catalytic reactions with both CO_2 and O_2 (Fig. 10). It has been characterized as “hamstrung by slow catalysis and confusion between CO_2 and O_2 as substrates, an ‘abominably perplexing’ puzzle” (Tcherkez et al. 2006, p. 7246). Arguably one the most important reactions in cellular redox homeostasis is photorespiration, initiated by O_2 consumption via the oxygenase reaction of Rubisco (Fig. 10). Photorespiration provides no net gain in C or energy for the cell (i.e., no net growth) and it imposes other cellular costs in terms of the repair, quenching, and other functions impeded by increased oxygenase activity (Raven 2011, Voss et al. 2013, Raven et al. 2014). Although not a N-dependent reaction, photorespiration is a critical part of the C-N cross-talk of cells (e.g., Parker and Armbrust 2005, Prihoda et al. 2012). Up-regulation of the genes associated with photorespiration have been seen in diatoms in response to increased irradiance (Parker et al. 2004) and growth on NH_4^+ compared to growth on NO_3^- (Parker and Armbrust 2005, Shi et al. in press; Fig. 11b) and other plants (Guo et al. 2007a,b). The difference in photorespiration under NH_4^+ *vs* NO_3^- consumption is related to the differences in photo-energy consumption and reductant supply. When NO_3^- is comparatively unavailable to the cell, the sink for NADPH consumption via NR reduction is not available, and photorespiration becomes the alternative electron sink (Keys and Leegood 2004, Guo et al. 2007a,b, Nunes-Nesi et al. 2010). When photorespiration increases, growth inevitably is stressed because C assimilation is reduced.

In all, nutrient regulation is a dynamic process, and these dynamics are not captured in fixed kinetic models. The mechanisms of nutrient acquisition, cellular energy and redox balance described herein suggest a suite of responses by phytoplankton to variable NH_4^+ and NO_3^-, N and P, and to N metabolism in relation to C assimilation. All of these interactions depend on the extent of substrate availability (nutrients or light), from limiting to supersaturating, the taxonomic group, and specific metabolic adaptations. Regulation of elemental acquisition for the cell in natural environments is more challenging than in the steady-state conditions cells may experience in the laboratory. Thus, while NH_4^+ may be preferentially taken up at the low end of the substrate availability spectrum when cells are N deficient, as NH_4^+ availability increases, and as its availability increases in proportion to NO_3^-, the potential for growth suppression increases. Similarly, potential for suppression of growth exists when excess of P is provided. Growth suppression occurs when the normal metabolic feedback processes are inhibited or repressed.

The wealth of data on dichotomous phytoplankton community response to different forms of N, and to variable nutrient stoichiometry, combined with the increasing knowledge of taxon-specific differences in nutrient metabolism, lead to hypothesis that phytoplankton in the Bay Delta should respond to changes in both changes in N form and to changes in N:P availability and these responses may change with environmental conditions. The important ecological questions are: *to what extent are these effects observed in the Bay Delta? Is there evidence that phytoplankton respond metabolically to changes in nutrient form or ratio, whether limiting or not, and are differences in phytoplankton composition in space or time consistent with differences in nutrient availability and known metabolic differences between and among taxonomic groups?* These questions are addressed below. Representative experimental evidence is first brought to bear, and then both spatial and temporal differences are discussed.

6.2.5. Relevance to Bay Delta phytoplankton dynamics

6.2.5.1. Effects of N form: experimental evidence at the metabolic scale

Numerous direct measurements of interactions of NH_4^+ on NO_3^- metabolism have been made on phytoplankton from the Bay Delta (e.g., Wilkerson et al. 2006, Dugdale e al. 2007, Parker et al. 2012, Glibert et al. 2014a,b). If NH_4^+ is repressing NO_3^- uptake, then such effects should be seen in direct measurements of NO_3^- uptake with and without NH_4^+ additions. In addition to the grow-out or mesocosm experiments described by Dugdale et al. (2007, 2015), specific experiments have been conducted to examine 1) variability in the kinetics of NO_3^- uptake with and without additions of saturating pulses of NH_4^+ and 2) variability in the saturating rates of NO_3^- uptake along a continuum of additions of concentrations of NH_4^+.

Samples were taken from the Sacramento River in September 2011, above the influence of the Sacramento Regional wastewater treatment plant (WWTP). With the addition of 20 μM NH_4^+, the kinetics of NO_3^- uptake were repressed at all concentrations of NO_3^- (Fig. 12a). These results are similar to those reported by Flynn et al. (1999; Fig. 4 above). Experiments exploring the effect of variable concentrations of NH_4^+ on the uptake rate of NO_3^- at V*max* (Fig 12b) illustrate that additions of 10 μM resulted in approximately 50% repression in uptake relative to no supplemental NH_4^+, and additions of 80 μM resulted in an order of magnitude repression. These results are consistent with literature findings (e.g., Lomas et al. 2000, L'Helguen et al. 2008) illustrating the repressive effect of NH_4^+ additions on NO_3^- uptake after short-term (1 to several hours) exposure.

Experimental evidence also supports the notion of community compositional changes with exposure to different N forms. Enrichment experiments with different N forms were conducted during different seasons over 2 years for samples collected from the upper Sacramento River and Suisun Bay. In each experiment, the response by different phytoplankton groups over the scale of several days to additions of either 30 or 40 μM NH_4^+ and NO_3^- was determined both under high light and reduced light incubation treatments (Glibert et al. 2014a). All samples were prescreened to remove large zooplankton so any differences were largely due to differences in production rather than to differences in grazing. Combining all results for all seasons and sites, it was found that twice the chlorophyll *a* was produced per unit N taken up in enclosures enriched with NO_3^- under low light incubations than in treatments with the same total N enrichment as NH_4^+ under either light treatment, consistent with the notion that enrichment with NH_4^+ can lead to suppression of productivity. The enclosure with greater chlorophyll *a* (the NO_3^- treatments held under reduced irradiance) also had proportionately more fucoxanthin (generally indicative of diatoms). In contrast, with NH_4^+ enrichment and higher light exposure, proportionately more chlorophyll *b* (generally indicative of chlorophytes or green algae) and zeaxanthin (generally indicative of cyanobacteria) were produced (Glibert et al. 2014a; Fig. 13). Moreover, from subsamples collected from these same enrichment treatments on which the rate of N uptake was measured using isotope tracer techniques, it was found that the summed rate of uptake of NO_3^-, NH_4^+ and urea was always higher for experiments initially enriched with NO_3^- compared to those initially enriched with NH_4^+ (Glibert et al. 2014a). These data provide direct experimental evidence that the rates of N productivity differed, and that dichotomous phytoplankton communities developed, when samples collected from the same location were enriched with different N forms but in the same absolute concentration, even for samples for which the initial concentrations prior to N enrichment appeared to be non-limiting. It is of note that the Cloern-Jassby model of productivity, which is the basis of the contention that 'nutrients don't matter', would predict no change in productivity or ecological effect because these experiments were all conducted at nutrient saturation. Clearly that model would

not capture these dynamics empirically measured.

6.2.5.2. Phytoplankton composition changes at the spatial scale

If the Bay Delta phytoplankton community responds to differences in N composition in a manner predicted by the differences in physiology of dominant phytoplankton groups, and in the manner demonstrated experimentally, then differences in the phytoplankton community should be observed spatially along the longitudinal axes from the Sacramento and San Joaquin Rivers to Suisun Bay as the composition of the N pool changes. The Sacramento River is generally dominated by NH_4^+ relative to NO_3^-, whereas that of the San Joaquin is dominated by NO_3^- relative to NH_4^+ (Fig. 14a,b)

Such a comparison was undertaken during 2010 and 2011, prior to the current major drought (Kress 2012). Under "normal" precipitation and flow conditions (spring, summer 2010) the San Joaquin River with high concentrations of NO_3^- had greater phytoplankton abundance (as chlorophyll *a* and number of fluorescing cells) and productivity rates, and was dominated by diatoms in the spring and by chlorophytes in the summer. In contrast, the Sacramento River, with much higher concentrations of NH_4^+ had lower phytoplankton biomass and productivity and was usually dominated by flagellates (Fig. 14c,d). Strikingly different results emerged during the "above-normal" precipitation period of spring 2011, with elevated river flow for both rivers (not shown). During April 2011, compared to the previous low-flow April, the concentrations of NH_4^+ in the Sacramento River were about half, and the dominant form of N shifted to NO_3^-, whereas in the San Joaquin the concentrations of NO_3^- were about one-quarter what they were the previous year. Consequently, the Sacramento River had higher phytoplankton abundance and productivity than in 2010 and it shifted to a diatom-dominated system, while the San Joaquin River had less productivity and phytoplankton abundance and it was composed of cryptophytes and fewer diatoms (Kress 2012).

The spatial gradient in NH_4^+ along the Sacramento River also has been shown to have a direct effect on phytoplankton community composition and physiological state. A comparison of transects made during 3 springs (March 2011, 2012, 2013) suggests a strongly inhibitory effect of NH_4^+ on diatom abundance (Fig. 15). In March 2012, a year of comparatively normal-to-high flow conditions, the NH_4^+ concentration along the transect from above the Sacramento Regional WWTP to Suisun Bay showed concentrations in excess of 5 μM and a progressive decrease in diatoms (as measured by its fucoxanthin pigmentation) into Suisun Bay. The following two springs had much lower flow, concentrations of NH_4^+ in the Sacramento River were comparatively much higher, in fact >70 μM in March 2014, but were comparatively lower in Suisun Bay. The concentration of

diatoms (based on pigment signatures) declined sharply several stations below the peak in NH_4^+ in both cases. Chlorophytes, in contrast, showed a near mirror-image response, with increases in virtually all of the regions where diatoms decreased (Fig. 15).

During the most recent of these transects, spring 2014, additional measurements were made of phytoplankton physiological "health" using the variable fluorescence parameter Fv/Fm, and the phaeophytin/chlorophyll *a* ratio (Glibert et al. 2014b; Fig. 16). The 2014 spring transect was undertaken during a period of low flow (the ongoing drought) and there was a comparatively large phytoplankton bloom, with total chlorophyll *a* exceeding 20 $\mu g\ l^{-1}$ at both the upper-most site (above the Sacramento Regional wastewater treatment plant, WWTP) and in the region from the lower Sacramento River to the upper Suisun Bay. In the mid-reach of the Sacramento River (sites GRC to US655), chlorophyll *a* values were substantially depressed relative to values above and below these sites. They declined from 14.5 $\mu g\ l^{-1}$ at GRC to 1.6 $\mu g\ l^{-1}$ at US655. Photosynthetic efficiency was also depressed relative to values above and below these sites, with Fv/Fm declining by about half, from 0.6 to 0.3 and phaeophytin/chlorophyll *a* ratios reached 0.79, indicative of cell stress (Glibert et al. 2014b). This stress response is consistent with NH_4^+ inhibition or repression. Note that during this period of low flow, the concentrations of NH_4^+ in the upper Sacramento River were about double those of the previous spring and several-fold higher compared to those in 2011 when flow was higher. Low flow would result in less dilution of the point-source discharge.

Collectively, these results, both from the river-to-river and the spring-to-spring comparisons conducted in years of differing water flow conditions, suggest that nutrients together with flow are critical for phytoplankton success- or, conversely, the degree to which phytoplankton may be stressed by nutrients. River flow interacts with nutrients by increasing dilution on the one hand, and altering the time for biogeochemical processing on the other. During periods of normal to high flow, the effect of any inhibiting concentration of nutrient may be seen over a greater longitudinal extent. This was the case, for example along the transect seen in spring 2012 (Fig. 17). Interestingly, under extreme low flow conditions, as was seen in spring 2014, for example, the concentration of the inhibiting substrate, i.e. NH_4^+, may accumulate to much higher levels, but its aerial reach may be less. The effect of these very elevated concentrations of NH_4^+, >70 μM, in the upper Sacramento, had noticeable effects in terms of phytoplankton physiology and abundance: photosynthesis was depressed, and cells were highly stressed, as evident from both Fv/Fm and phaeophytin/chl *a* ratios. Yet, longer residence times may have had a non-intuitive positive effect: the extent of nitrification was apparently elevated, resulting in increased NO_3^- downstream and a freshwater diatom bloom was able to thrive in the section of the river between the inhibiting concentrations of NH_4^+ and the stress of increased salinity that intruded into Suisun Bay (Glibert et al. 2014b; Fig. 17). Increased flow and reduced residence time appear to tip the system into a low biomass state, and one that

cannot sustain diatom blooms, as growth is both inhibited by elevated NH_4^+ and short residence time, resulting in a condition of overall poor primary production. High flow also reduces the opportunity for in-river nitrification and therefore dilution/reduction of NH_4^+ levels that may result in repression of NO_3^- uptake and assimilation. This creates a "squeeze play" for phytoplankton growth rather than a "window of opportunity". For phytoplankton growth that does occur under conditions of higher flow, smaller, non-diatom taxa are favored. Increased flow thus reduces chlorophyll *a* accumulation and also results in transport of phytoplankton and unassimilated nutrients out of the Bay; the low salinity zone is maintained in a phytoplankton-poor condition due to imbalanced nutrient availability and depressed growth (Glibert et al. 2014b). Low flow and associated higher residence time restrict the effects of NH_4^+ repression, while creating conditions conducive for N biogeochemical transformations.

This conceptual model is consistent with the N-based model developed by Dugdale et al. (2012, 2013) that predicts two states for particulate N (as a proxy for phytoplankton) as a function of flow and NH_4^+ concentration in the inflowing water. One state is a high-biomass, high-productivity, NO_3^--based system that occurs at low flow and a large range in NH_4^+, illustrated by the development of the unusual phytoplankton bloom of spring 2014 (e.g., Glibert et al. 2014b) and the second state is illustrated the high flow period of spring 2012 during which phytoplankton biomass of the Sacramento River was reduced. With this model in mind, it may now be clear as to why different concentrations of NH_4^+ may repress NO_3^- uptake under different conditions. Dugdale et al. (2007) have suggested that a concentrations of $\geq$ ~4 μM-N may repress NO_3^- metabolism in Suisun Bay phytoplankton under many environmental conditions. In what may seem like a substantially different finding, Glibert et al. (2014b) reported concentrations closer to 10 μM as a repressive concentration during the spring 2014 diatom bloom. This value, however, did fall within the flow-NH_4^+ space defined by Dugdale et al. (2013) critical for the high-biomass state. As described above, the absolute concentration required to repress NO_3^- uptake and assimilation is not a fixed entity, but rather a complex function of species, their physiological state, including their prior NO_3^- exposure history, which affects their cellular NO_3^- content.

6.2.5.3. Phytoplankton changes at the interannual scale

If the Bay Delta phytoplankton community responds to differences in N composition in a manner predicted by the differences in physiology of dominant phytoplankton groups, and in the manner demonstrated experimentally, then differences in the phytoplankton community over time should be consistent with the long-term changes in nutrient loading.

Several analyses, using long-term, publically available monitoring data have documented that the concentrations of N and P have changed significantly over time (Van Nieuwenhuyse 2007, Glibert 2010, Glibert et al. 2011). The extent and timing of the changes between N and P differ, but so too do the changes in forms of N. Summarizing the trends from 1975-2005, Glibert et al. (2011) showed that for Suisun Bay, NH_4^+ concentrations increased significantly, as did the ratio of inorganic N:total P (DIN:TP). Herein, the time series for several parameters have been re-examined, (now including data from 1979- 2011), and were explored seasonally (spring and fall), by Bay Delta region, and in relation to changes in conductivity (indicative of changes in flow). Consistent with the previous analysis, the overwhelming and significant trend has been a decline in TP in all regions examined and in both spring and fall (Fig. 18). With respect to N, while the changes in both NH_4^+ and NO_3^- varied with season and Bay region (Fig. 19), both forms of N generally increased, together resulting in DIN:TP ratios that have climbed steadily and significantly with time in all segments of the Bay Delta (Fig. 20). The trend is particularly strong during late summer/fall. Interestingly, although TP and NO_3^- in both spring and fall increased significantly with increasing conductivity (inversely related to flow), NH_4^+ increased significantly only in spring with conductivity, and DIN:TP did not track conductivity in either season (Fig. 21).

In relation to these changes in nutrients, chlorophyll *a* was inversely related to NH_4^+ in Suisun Bay during both spring and late summer/fall, but the relationship was stronger during the spring months (Fig. 22a-d). Inverse correlations were also noted between chlorophyll *a* and DIN:TP, but this relationship was stronger during the late summer/fall (Fig. 22e-h). Exploring this latter relationship more closely, it can be seen that not only chlorophyll *a*, but also the abundance of centric diatoms was strongly inversely related to DIN:TP during the late summer/fall months and this relationship was observed for both Suisun Bay and Chipp's Island subregions (Fig. 23). Of particular note is the fact that this relationship is apparent for the years prior to the invasion of *Potomocorbula* clams. Clearly biomass on average is lowest in the post-clam era, but the decline began years before the clam invasion. Increased N:P ratios should lead to lower growth rates (Sterner and Elser, 2002) and selection for species with lower P cellular demands. Diatoms generally have higher growth rates than dinoflagellates. Diatoms, being generally considered an "r" selected group, and with their comparatively low optimal N:P ratio (Hillebrand et al. 2013), would be expected to be outcompeted by species with higher optimal N:P (e.g., chlorophytes, cyanobacteria, dinoflagellates) if N:P in the environment increases. When these trends for chlorophyll *a* and centric diatoms are compared with concentrations in NH_4^+, the correlations are stronger in the spring months for chlorophyll *a* than in the summer/fall (Fig. 24).

In all, from the short-term experiments in Bay Delta water, in which different forms of N were manipulated and phytoplankton uptake rates and compositional changes tracked, to spatial

differences in nutrient and phytoplankton composition along both major rivers within and between seasons, to changes in nutrient availability with time and concomitant observed changes in phytoplankton abundance, the patterns are consistent with our modern understanding of nutrient effects. With increases in NH_4^+, a loss of diatoms and an increase in chlorophytes was observed both in short-term experiments and in riverine transects. Similarly, with increases in DIN:TP (Glibert et al. 2011), loss of diatoms relative to other functional groups has been observed. An emerging pattern is that the strength of NH_4^+ repression of productivity is greater in the cool, spring months when diatoms dominate, while N:P stoichiometry plays a greater regulatory role during the warmer, late summer/fall (see also Dugdale et al. 2015). This seasonal difference is consistent with our physiological understanding of the different enzyme activities, the coupling of C and N, and the relative strength of different dissipatory strategies under different temperatures and other non-steady-state conditions. The importance of these seasonal and temperature differences would help to explain why suppressive effects by NH_4^+ were not seen in the summer sewage-enrichment experiment undertaken in Suisun Bay by Esparza et al. (2014).

It is important to underscore that the relationships shown above using experimental and riverine observational data illustrate a phytoplankton compositional shift as nutrient form and ratio changes that supplement the patterns shown in the long-term time series. Although there is some disagreement as to long-term changes in phytoplankton taxa due to analytical reasons (see Malkassian et al., 2015), there is no question or disagreement that diatom abundance has declined over time. While analyses by Brown (2010) and Glibert et al. (2011) suggest an increase in flagellates over time, and in the more recent decade, blooms of the toxic cyanobacterium *Microcystis* have increased (e.g., Lehman et al. 2005, 2008, 2010), such data were not used herein. It is of note however, that trends in cyanobacterial blooms over time would be consistent with the previously described higher optimal N:P ratios in cyanobacteria (Hillebrand et al. 2013).

6.3. Nutrient effects through the food web

6.3.1. Ecological stoichiometry: An emerging concept with historical roots

Ecological stoichiometry is a relatively recent conceptual framework for understanding the interactions of organisms in relation to energy and elemental flow (Sterner and Elser 2002). It builds on classical concepts of Leibig's Law of the Minimum relating to nutrient limitation (Leibig 1855), Lotka's (Lotka and Dublin 1925) understanding of the dynamics of predators and prey, Lindeman's understanding of trophic dynamics (Lindeman 1942) and Redfield's (1934) concept of balanced proportions of elements in the ocean. It brings these concepts together by recognizing that different organisms both within and between trophic groups have fundamentally different elemental

requirements, that food web structure is a function not only of food quantity but food quality, and that these interactions result in a complex suite of feedbacks that shape community composition. Ecological stoichiometry (*sensu* Sterner and Elser 2002) thus provides "an integrated framework for merging perspectives across individual, population, community, and ecosystem levels." The stoichiometric framework recognizes that changes in the proportions of dissolved nutrients in the environment have profound effects on food webs *even when the availability of these elements are not in limiting proportions*, with the potential of transforming ecosystems to new stable states. Just as phytoplankton continually regulate their capability for nutrient assimilation along a continuum of substrate availability and other environmental factors, grazers together with primary producers operate in a dynamic balance with respect to nutrient composition and availability (e.g., Glibert 1998, Boersma and Elser 2006, Meunier et al. 2014).

In a nutshell, the concept of ecological stoichiometry predicts that while the total nutrient load of a system may set the amount of biomass that can be supported, the composition, both in form of nutrients and the proportion of different nutrient elements, affects the composition of the community, from autotrophs to heterotrophs and throughout the food web. Quite simply, ecological stoichiometry suggests that food webs are not merely as a summation of a series of rate processes (and kinetic curves), or food quantity (as C, for example), but are an outcome of both the quantity and quality of the substrate (or food) provided and the balance of nutrients therein. Ecological stoichiometry views food quality from the nutritional element perspective. Within this framework, the Growth Rate Hypothesis (GRH) suggests that P is a particularly important element for setting growth rates, as the availability of P sets the limit on allocation of P to P-rich ribosomes needed for growth (Elser et al. 1996, 2000; Fig. 25). This biochemical investment therefore affects body stoichiometry and sets constraints on growth, resource competition, and, in the case of animals, trophic efficiency, and nutrient recycling.

Unlike phytoplankton, which have considerable plasticity in their C:N:P stoichiometry, grazers are generally more constrained in stoichiometry and there may be a mismatch between the stoichiometry of grazers and their food (Sterner and Elser 2002). Grazers may 1) reflect the stoichiometry of their prey (within reasonable limits), 2) be somewhat more restrictive in their stoichiometry than their prey, or 3) be highly constrained in their stoichiometry (Sterner and Elser 2002). Although phytoplankton do have metabolic dissipatory pathways to regulate nutrients in excess of the nutritional demands (e.g., xanthophyll cycling, Mehler activity, dissimilatory NO_3^- reduction), grazers have especially well developed pathways of release and excretion to eliminate nutrients acquired in excess (Sterner and Elser 2002; Fig. 26a,b). Grazers are thus affected by food quality and they, in turn, affect food quality by altering the composition of nutrients available to them through multiple processes. Grazers can sustain a mismatch in the stoichiometry of their food

because they can retain what they need and release what they do not need. For example, in principle, grazers that are stoichiometrically balanced with strict stoichiometry feeding on phytoplankton that are N-rich will excrete proportionately more N than those grazing on phytoplankton that are more balanced in their N:P or N:C ratio (Fig. 26c,d). By excreting more N, a condition of excess N is, in turn, maintained or even amplified for the phytoplankton *(*Sterner and Elser 2002). Consumer-driven changes in stoichiometry can thus maintain and may even amplify nutrient changes for producers (e.g., Schindler and Eby 1997, Loladze et al. 2000). Imbalances in the N:P of excretion products presents further cellular challenges for producers to regulate uptake of both the limiting and saturating nutrient cellular levels. Ecological stoichiometry principles would predict that the dominant predator, if its biomass N:P ratio is tightly constrained, can maintain a biomass nutrient ratio that is inversely related to the nutrient ratio of its food, and homeostasis from nutrient recycling will drive the nutrient balance of the system to be self-sustaining (Fig. 26c,d).

Ultimately, species of grazers that can sequester the nutrient in least supply relative to their needs, while releasing what they do not need, should become the dominant (and, in some cases, the keystone) species by outcompeting grazers that cannot effectively acquire what they need and/or dissipate what they do not. As an example of the effect of biomass retention and excretion stoichiometry, it has been shown that copepods with their comparatively higher body N:P generally dominate under lower water column N:P conditions, while *Daphnia*, with their lower body N:P generally dominate under comparatively higher water column N:P, assuming all other conditions for growth such as salinity regime are met (Sterner and Elser 2002, Cease and Elser 2013; Fig. 27). Such stoichiometric regulation means that as a nutrient resource is diminished, those organisms with a higher demand for that resource should thrive.

Analogous to the conceptual relationship of phytoplankton productivity or growth as a function of nutrient availability, the rate of ingestion by grazers has also been conceptualized as a hyperbolic function of increasing food (e.g., Holling 1959; Hansen et al. 1997; Fig. 28). However, as is the case with phytoplankton and nutrients, the saturating hyperbolic function belies the complexity of regulation at all substrate (here: prey nutrient content) levels. Satisfying a higher demand for a particular resource may come with the capacity for compensatory feeding (Fig. 28). In kinetic terms this would mean organisms with a higher efficiency for acquisition of the limiting nutrient (a lower "K*s*") and/or a higher maximal ingestion rate (I*max*) should dominate. Ultimately, ecological stoichiometric principles suggest that changes in biodiversity can occur as a consequence of stoichiometry, and that populations should self-stabilize as a result of stoichiometric constraints.

The ecological stoichiometric perspective contrasts with the classical perception of the Bay Delta in terms of the links of phytoplankton to the grazer community. This conventional approach

for determining the amount of energy (as reduced C) that would be available to upper trophic levels unfortunately ignores the transfer of elements other than C. In marked contrast, and as developed herein, ecological stoichiometry dictates that it is the processing and transfer of all elements, especially N and P, through the phytoplankton assemblage that drives the fitness of species at higher trophic levels (Sterner and Elser 2002, Allen and Gillooly 2009, Schoo et al. 2010, Malzahn et al. 2010). No insight into these aspects of community response can be drawn from the existing C-based primary production models.

6.3.2. Consumer stoichiometry: regulation above and below the "stoichiometric knife edge"

Grazers, like algae, are continually challenged with maintaining their nutrient and energy balance. This fine-tuning of the stoichiometric needs of grazers has been termed a "stoichiometric knife edge" (Plath and Boersma 2001, Boersma and Elser 2006, Laspoumaderes et al. 2015): there is an optimum nutrient proportion. As an example, increasing amounts of dietary P have a positive effect on *Daphnia* growth to a point, but as concentrations increase further beyond the optimum, reductions in growth rate occur (Fig. 29). The concept of the "stoichiometric knife edge" is thus the grazer analogy of the concept of dynamic regulatory balance of C:N:P, and specifically the "paradox" of NH_4^+ for phytoplankton (Britto and Kronzucker 2002, Dugdale et al. 2012, Reef et al. 2012, Glibert et al. 2013). In fact, Naddafi et al. (2009), in studying the invasion of zebra mussels in lakes, referred to nutrient enrichment that can destabilize the dynamics of consumers as the "paradox of enrichment" and suggested this could be lead to invasional success of alien species or the extinction of others. Importantly, not only can the metabolic processes of release by the consumers affect nutrient availability for phytoplankton, but changes in the external balance of nutrients can affect higher trophic levels by changing the relative elemental composition of the prey on which consumers depend (Glibert et al. 2013, Laspoumaderes et al. 2015).

For all organisms there is an optimal balance of nutrients, and importantly both above as well as below this optimum (or knife edge) there is a metabolic cost and there can be compensatory mechanisms. For example, if grazers are nutritionally deficient, they may undertake compensatory feeding. A compensatory feeding response is analogous to rapid uptake of nutrients by phytoplankton when they are nutrient deficient (e.g., McCarthy and Goldman 1979, Glibert et al. 1981, Glibert et al. in review), but such a response requires energy and may not fully compensate the deficiency. Specifically, increased intake without increased gut passage time may mean that there is less efficiency in the extraction of nutrients within the gut (Boersma and Elser 2006). Grazers may also be selective feeders, but selective feeding can only be effective within narrow limits and certainly within the limits of availability of the favored food (e.g., Meunier et al. 2015).

Moreover, an animal may respond to the nutritional demand for one nutrient (e.g., C), and in so doing obtain an excess of another nutrient (e.g., P) requiring an investment of energy to release this excess. The consumption by grazers of food with excess nutrient comes at a cost and may impair many metabolic functions with a consequence of reduced growth (Boersma and Elser 2006, Peace et al. 2014; Fig 29). Peace et al. (2013) have offered several possible mechanisms by which high food content may reduce growth: reduction in feeding, reduction in C absorption sites should excess P hinder C absorption, or increase in respiration due to the costs of egestion, metabolism or excretion. Furthermore, these relationships may change with temperature, with higher temperatures leading to higher rates of respiration and increased sensitivity to nutrient, especially P, limitation. This "stoichiometric knife edge" may change with temperature simply due to the effect of temperature on metabolic rates (e.g., Cross et al. 2015; Fig. 30).

For grazers, the challenge of acquiring the requisite nutrients and balancing biomass stoichiometry is also compounded by the fact that stoichiometry affects various life stages of the predator differently (Moe et al., 2005). As a specific example, there is a greater need for C, N, and P for developing copepod juveniles, but at a later stage, while C is still needed for metabolism, more P is generally allocated to eggs; thus, P-poor food can disproportionately affect egg production while not affecting adult survival (Faerovig and Hessen 2003, Laspoumaderes et al. 2010). Just as nutrient uptake and growth (or photosynthesis and growth) can be uncoupled, so too is the case for food ingestion and assimilation by zooplankton. Due to the need for P in eggs, food P content should affect both egg production and viability. Boersma et al. (2008, p. 484) specifically noted the potential mismatch between food quality and larval growth and how this can be affected by differential nutritional quality of prey should the timing of spring blooms vary: "Larval fish growth typically follows the population increase of herbivorous zooplankton, which succeeds the spring bloom of phytoplankton...if for some reason the tight coupling of these dynamics becomes less...it could well be that the larval fish is faced with herbivorous zooplankton that is feeding on late-bloom phytoplankters rather than early bloom ones. Feeding on late-bloom algae automatically implies that the nutrient conditions of these algae are more depleted with respect to phosphorus and nitrogen and thus these zooplankters are a food source of suboptimal quality for larval fish." This idea may be of importance with respect to the Bay Delta as the assemblages of both phytoplankton and zooplankton have not only changed over time, but the timing of their abundances have changed seasonally as well.

Finally, properties such as lipid composition, toxin production, cell membrane thickness and other chemical constituents that are also, at least in part, functions of cellular elemental availability, can also alter the quality of food for consumers, in some cases turning "good food to bad" (e.g.,

Mitra and Flynn 2005). Clearly, food quality as measured as C intake alone is an inadequate measure of nutritional demands. This is a particularly salient point with respect to the Cloern-Jassby productivity model that is strictly C-based in terms of output.

6.3.3. Effects of variable prey stoichiometry on zooplankton and higher trophic levels

The effect of varying N:P stoichiometry of food on copepods has been quantified for a number of different grazers. Villar-Argaiz et al. (2012) found that P-enrichment in food explained between 60-74% of the total variance in zooplankton growth in a series of bioassays in which natural rotifers, *Keratella cochlearis*, copepods, *Mixodiaptomus laciniatus*, and cladocera, *Daphnia pulicaria*, from a freshwater lake were studied. The recent work of Malzahn and Boersma (2012) convincingly demonstrated that the copepod *Acartia tonsa* reared on P-poor diets had lasting effects on copepod growth that were non-reversible even when the animals were subsequently exposed to more nutritious food and that the effect on growth was related to the duration of exposure to the low quality food. Furthermore, Shoo et al. (2012) showed that poor quality food for copepods has effects higher in the food web as the resulting nutritionally poor copepods themselves are grazed. The decline of copepod populations due to poor quality food have far-reaching effects and may lead to the collapse of higher trophic levels that depend on these copepods as a food source (Malzahn et al. 2010, Winder and Jassby 2011, Hessen et al. 2013). As evidence, Nobili et al. (2013) showed that changes in the quality and quantity of phytoplankton resulting from changing nutrient ratios in the North Pacific Subtropical Gyre have resulted in changes in zooplankton structure; as larger zooplankton decline, the biomass of smaller zooplankton have increased, in turn affecting energy transfer and productivity at higher tropic levels (Fig. 31), and Beaugrand et al. (2003) showed than declines in abundance of *Calanus* zooplankton in the North Sea have resulted in long-term declines in cod.

Several recent reviews have addressed the stoichiometry of higher aquatic food webs, namely fish (Sterner and George 2000, Hendrixson et al. 2007, McIntyre and Flecker 2010). Studies to date have shown that there is considerable variation in body P among fish species and there is consistency in this variation in taxonomic organizational levels. Whereas whole-fish N content generally varies across a relatively small range (8-11%), whole-fish P content tends to vary many-fold (Sterner and George 2000, Hendrixson et al. 2007; Fig 32). Vanni et al. (2002) examined the stoichiometry of 28 species of fish and amphibians, and their data suggested that elemental stoichiometric controls were strongest when consumers ingested nutrient-poor items such as nutrient-limited algae or detritus. Accordingly, from a stoichiometric perspective, detritus, high in C, may result in high metabolic costs to consumers, including altered metabolic rate and growth rate (Plath and Boersma 2001, Hessen and Andersen 2008). Detritivores consume the least nutritionally

balanced foods and thus have lower growth rates than their planktivorous or piscivorous counterparts (Sterner and Elser 2002). The effects of stocihiometric regulation were weaker when consumers ingested multiple food items including other animals that were apparently more nutrient-rich. For fish, the most important determinant of stoichiometry is structural demand; growth demands appear to be secondary (McIntyre and Flecker 2010). A clear trend is that, "as one ascends the pelagic food web...trophic groups grow increasingly nutrient and especially P rich..." (Sterner and Elser 2002, p. 254) because there is a greater need for P in skeleton and bone than in skin, heart, kidney, muscle or brain. The latter tissues and organs all have a relatively high N content (Sterner and Elser, 2002). In aquatic food webs, small fish that have a higher muscle:skeleton ratio than large fish thus tend to have a higher biomass N:P ratio. Planktivorous fish accordingly have a lower P content than omnivores, insectivores or piscivores, which have more bone and skeleton (Sterner and Elser 2002, Hendrixson et al. 2007, Fig. 32).

The net effect of the stoichiometric regulation of consumers together with different nutrient requirements of phytoplankton, is that not only are grazers affected by food quality, they, in turn, affect food quality by altering the composition of nutrients available to them, and this process of stoichiometric interaction is seen at all levels of the food web, leading to a complex interaction of stoichiometry and trophic cascades (Fig. 33). Ecological stoichiometric theory predicts that systems that undergo a shift in availability of nutrient resources (bottom up control) should experience shifts in dominant species at all levels of the food web such that 1) consumers that successfully sequester the nutrient in least supply relative to their needs should dominate and, in so doing, should stabilize at a new stable state, and 2) with reduction in relative availability of P, there should be a shift from planktivore to piscivores or omnivores (Sterner et al. 1992, Sterner and Elser 2002; Elser and Hamilton 2007). This implies that an inverse relationship will be seen between the stoichiometry of dissolved nutrients and that available in food, or between that in prey and that in biomass of grazers higher in the food web (Fig. 33). Indeed, lake studies have shown that where there are four dominant trophic levels, with piscivorous fish at the top, P-rich crustacean (*Daphnia*) and large-bodied fish dominate, whereas where there are three trophic groups, low-P copepods dominate and there are no piscivores. Interactions between alterations in food web structure cause the zooplankton communities to recycle N and P differently (Sterner et al. 1992), in turn modifying the nutrient availability for phytoplankton. So too can anthropogenic changes in nutrients affect the nutrient content of the prey food. As well summarized by McIntyre and Flecker (2010, p. 539) nutrient stoichiometry "… can either constrain trophic cascades by diminishing the chances of success of key species, or be a critical aspect of spectacular trophic cascades with large shifts in primary producer species and major shifts in ecosystem nutrient cycling."

The stoichiometric perspective, therefore, contrasts with the viewpoint that large shifts in species dominance in the Bay Delta are a function of the somewhat random nature of invasive species and other non-nutrient-related factors. While increased propagule pressure may increase the possibility of success of an invading species, success is also a function of the environment to which the organism has been introduced, the physiological strategies of the invading organisms and the how they relate to those of the native fauna or flora (Glibert 2015 and references therein). When food quality changes, it can disrupt normal predator-prey interactions, enhancing the likelihood of success of some species at the expense of others. It can thus destabilize established trophic relationships, increasing susceptibility for additional invasions to occur (Simberloff and Von Holle 1999, Glibert 2012, 2015). As argued herein, for the Bay Delta, the potential is high for nutrient changes to be, and to have been, a destabilizing mechanism for changes in upper trophic level community composition.

The questions for the Bay Delta are: *Do changes in nutrient stoichiometry in this system, achieved through both external forces (altered land-based nutrient loads) and internal, organism-driven, assimilative and dissimilative processes, relate to upper-trophic level community compositional changes? Are such shifts mediated by changes in homeostatic mechanisms at the organismal level, including changes in egg production?* The metabolic responses of copepods to changes in N:P in their diet and many of the shifts in upper level taxa over time in relation to N:P availability in the Bay Delta are indeed consistent with ecological stoichiometric principles and those findings are summarized next.

6.3.4. Relevance to Bay Delta trophic dynamics

6.3.4.1. Food quality experiments at the metabolic scale

Recently food quality experiments were undertaken for two contrasting copepods that are either common in the Bay Delta or conspecific with species found in the Bay Delta, *Acaria tonsa* and *Eurytemora carolleeae*, the former a broadcast spawner and the latter a brood spawner. These copepods were fed constant and saturating amounts of food (prey) as measured by C content, but the prey nutritional quality varied as defined by N:P ratio (Bentley et al. in review). Varying nutritional quality was accomplished by varying the P content in the media of the diatom prey, while holding N and C content constant. When these copepods ate P-rich food, both copepods responded by increasing PO_4^{3-} excretion as predicted by the hypothesis that a grazer will excrete the nutrient in excess in its food (Urabe 1993, Sterner and Elser 2002). Summarizing these findings, *E. carolleeae* was the more P-rich copepod, and it excreted P at a higher rate than *A. tonsa* when both were eating the same food. Differences were also found in nutrient allocation to eggs between the

two copepods: *E. carolleeae*, the more nutrient-rich copepod, had comparatively nutrient-poor eggs, while *A. tonsa* had more variable C, N and P content in the eggs. Egg viability was also found to vary with N:P of the food for both of these copepods. In both copepod species, grazing on P-poor food led to reduced egg viability. In the case of *E. carolleeae*, egg viability dropped to near 0% as prey N:P increased to 15 on a molar basis (Fig. 34). The comparatively lower P content in the prey may have prevented copepods from obtaining sufficient P to produce viable eggs. Although the environmental factors selecting for varying copepods and their population success are many, these results add weight to the growing body of evidence that nutrient content of the prey may be important for copepod growth and ultimately population success.

6.3.4.2. Long term changes in macrozooplankton in relation to nutrient stoichiometry

Long-term changes in zooplankton in the Bay Delta have been well documented (e.g., Kimmerer 2004, Bouley and Kimmerer 2006, Glibert et al. 2011). Decreases in the calanoid copepods *Eurytemora*, *Sinocalanus*, *Acartia*, and harpacticoid copepods occurred from roughly the start of the available data in the long-term time series (mid-1970s) to the early to mid-1990s, although the decline in *Acartia* mostly occurred in the mid- to late 1990s. The decline in these species, especially *Eurytemora*, has been interpreted to be a consequence of increased grazing after the invasive clam *Potomocorbula* became established (e.g., Alpine and Cloern 1992, Kimmerer 2004). Abundance of the cyclopoid copepod, *Limnoithona tetraspina*, increased significantly during the mid-1990s (Bouley and Kimmerer 2006). The latter is considered an invasive species (Kimmerer 2004), with its population increasing several orders of magnitude since its introduction also in the mid 1980s.

An alternative explanation to the interpretation that these species shifts occurred simply due to clam grazing and invasional success of new species is that these species shifts occurred consistent with ecological stoichiometric principles and changes in nutrient availability over time. Total biomass may have been reduced due to enhanced grazing, but proportional biomass changes may have been due to stoichiometric and other nutrient changes. When zooplankton abundances were examined in relation to DIN:TP ratios many of the relationships were highly significant. Of particular note are the overall significant declines in *Eurytemora, Acartia*, *Pseudodiaptomis,* and *Neomysis* in relation to increasing DIN:TP ratios as well as the increases in *Limnoithona*; such trends were reported by Glibert et al. (2011) and the changes in *Eurytemora, Acartia,* rotifers and *Limnoithona* are substantiated herein using time series data that have been extended through 2011 (Fig. 35). Comparisons of the ratio of *Eurytemora*, the dominant copepod (a calanoid) of the 1970s-1980s, to that of *Limnoithona*, the current dominant copepod (a cyclopoid) over time in relation to changes in DIN:TP over time are highly significant (Fig. 36). Calanoid copepods generally have a

high N:P ratio of their biomass, ~20-35 by atoms, whereas cyclopoid copepods have N:P ratios much closer to Redfield atomic ratios (Walve and Larsson 1999, Sterner and Elser 2002). Such a comparsion is not unlike that of calanoid copepods and Daphnia (Fig. 27). Calanoid copepods thus generally retain N, while excreting nutrients in a lower N:P ratio than their biomass (i.e., they release proportionately more P), while cyclopoid copepods and cladocerans have a high P requirement in biomass, and therefore excrete nutrients in a higher N:P ratio than their biomass (i.e., they release proportionately more N; Hessen et al. 1997, Sterner and Elser 2002, Cease and Elser 2010). These patterns in the proportions of grazer nutrient excretion would help to contribute to increasing N:P availability over time as *Limnoithona* abundance increased. Such a decline in *Eurytemora* and *Acartia* over time would also be consistent with the pattern seen at the experimental scale where loss of egg viability was found for both species (but of even greater magnitude for *Eurytemora*) as the N:P of prey increased (Bentley et al. in review).

6.3.4.3. Long term changes in fish in relation to nutrient stoichiometry

Changes in fish abundance in the Bay Delta have also been well documented and the associated proposed reasons for such changes have been complex, including, to varying degrees flow, invasive species, climate changes, pollutants, predation and water exports (Kimmerer 2002, Sommer et al. 2007 and references therein). Among those identified as invasive are "largemouth bass, white and black crappie, bluegill, threadfin shad, striped bass, inland silversides, white catfish, black and brown bullhead, and common carp" (Moyle 2002, p.31). Food limitation has been invoked by numerous researchers as key to the decline in the Bay Delta food web over time (Bennett and Moyle 1996, Jassby et al. 2002, 2003). Among the trends of most concern for management in the Bay Delta are the significant population declines in delta smelt, along with longfin smelt, threadfin shad (*Dorosoma petenense*) and young-of-the-year striped bass (*Morone saxatilis*; Rosenfield and Baxter 2007, Sommer et al. 2007, Baxter et al. 2010). Delta smelt (estimated from both summer townet (STN) or fall midwater trawl (FMWT) indices), as well as longfin smelt, began to decline in ~1982, but their declines accelerated beginning in ~1999, a period referred to as the pelagic organism decline, or POD (Sommer et al. 2007, Baxter et al. 2010). In contrast, other fish species increased in numbers over the time series, especially those considered invasive, the silversides, largemouth bass (*Micropterus salmoides*), and sunfish (*Lepomis* spp.).

As with the change in zooplankton, the change in fish can also be interpreted from an ecological stoichiometric viewpoint. Ecological stoichiometry theory predicts that systems that shift from low to high N:P ratios should sustain shifts from planktivores to piscivores or omnivores (Sterner and Elser, 2002; Fig. 33). Several of the changes in abundance of these and other fishes have been found to be directly and significantly correlated with NH_4^+, TP concentrations, or

DIN:TP when the original and the detrended data are compared (Glibert et al. 2011). Specifically for the previously reported time series analysis encompassing 1975-2005, delta smelt (STN index) was reported to be positively correlated with TP, while abundances of longfin smelt, crappie, sunfish, and largemouth bass were significantly negatively correlated with TP, and these fish thus showed the opposite trends with DIN:TP (Glibert et al. 2011). Herein, such relationships for DIN:TP were re-examined for several species, now through the year 2011, and trends were substantiated or even strengthened (Table 1; Fig. 37). Moreover, as was the case with the ratio of the dominant copepod of decades past and the current dominant copepod, the change in the ratio of longfin smelt to silversides, a fish occupying a similar trophic niche, in relation to the change in DIN:TP over time was highly significant (Fig. 38). The inverse relationship between longfin:silversides and DIN:TP is consistent with substitution of the higher-P containing fish (silversides) as DIN:TP increased; both fish share similar habitat and prey items such as copepods and cladocera (e.g., Bennett et al. 1995). Additionally, as shown in the Glibert et al. (2011) analysis, in this updated analysis, the abundances of longfin smelt and striped bass were significantly negatively correlated with NH_4^+ concentrations, while those of inland silversides, sunfish and largemouth bass were positively correlated (Table 1).

The relationships including the more recent data between fish abundance and conductivity in virtually all cases were far weaker than relationships with DIN:TP (Fig. 39; Table 2). When relationships between delta smelt, longfin smelt, age 0 striped bass, sunfish, largemouth bass and silversides were examined with respect to nutrients (as ratios, as DIN, TP, or as N derived from the Sacramento River WWTP loads), and abiotic parameters (X2, secchi depth, temperature and water exports), the strongest and most consistent results were those of nutrient ratios (Table 2), substantiating the broader annual analyses conducted by Glibert (2010) and Glibert et al. (2011). As predicted by stoichiometric principles, the more P-rich fish, e.g., silversides, sunfish, and largemouth bass, were negatively related to TP and positively related to DIN:TP, while the more N-rich fish were negatively related to DIN and N from WWTP loads, and to DIN:TP.

These trends also support the premise that nutrient stoichiometry has effects that propagate up the food chain (c.f., Malzahn et al. 2007, 2010, Boersma et al. 2008). The fish presumably efficiently acquired and retained the nutrient (P or N) most abundant in their biomass, disproportionately releasing the other, perpetuating the imbalance between nutrient availability, food, and grazer biomass (Fig. 33). The omnivores or piscivores (e.g., sunfish, largemouth bass) that have a higher P demand and that can seemingly can sequester this nutrient more efficiently or acquire it more efficiently (Fig. 28) became more abundant. They increased inversely with changes in TP (Table 2). The planktivores, with a lower P demand, are apparently less efficient at sequestering P and generally showed either no relationship with P or evidence of a positive

relationship with P, especially in the latter years of the time series. They were negatively related to DIN (Table 2). Planktivorous fish and calanoid copepods have similar relationships with N:P ratios, whereas omnivorous fish have relationships with N:P ratios that are more similar to those of cyclopoid copepods. Sequestration of P in the biomass of the omnivorous fish (with more skeleton and bones) together with more efficient or compensatory feeding would lead to them being proportionately more abundant when P is less available in the water column. The relationships between major fish species and nutrients reported for the Bay Delta are thus consistent with Hendrixson et al. (2007) who showed that the plankivorous fish, less capable of sequestering P, were the most susceptible to P limitation. These trends also support the conceptual model of the relationships with time that have occurred in the food chain in relation to changes in nutrients and phytoplankton dominance suggested by Glibert (2010, Fig. 40). In fact, the trajectory of food web changes in the Bay Delta is analogous to many other systems having undergone nutrient changes leading to stoichiometric imbalance (Glibert et al. 2011, Glibert 2012).

6.4. Changing nutrient loads and biogeochemical processes

6.4.1. Biotic and abiotic nitrogen and phosphorus fluxes

In addition to the myriad of dynamics affecting nutrient forms and their proportions just described, sediments represent enormous stores of N and P. Sediments are key components of shallow water estuarine ecosystems, both responding to changing environmental conditions and at the same time, modifying the chemistry of the overlying water column. Processes such as denitrification, anaerobic ammonium oxidation (anammox), dissimilatory NO_3^- reduction to NH_4^+ (DNRA), and abiotic retention and release of PO_4^{3-} all alter the proportion of fixed N and P in the system. Years of nutrient loading may result in large nutrient reservoirs in the sediment that may continue to exchange with the water column years after the rate of nutrient loading may be reduced.

Sediments can be sinks for nutrients via burial, sources of remineralized nutrients to support water column primary production, sinks via microbial denitrification, and sites of nutrient assimilation into benthic algae and rooted macrophytes (Joye and Anderson 2008, Lehtoranta et al. 2009). Sediments recycle fixed N and P to the water column at rates that do not necessarily reflect their rates of deposition. Remineralized N can be returned to the water column as fixed N (NH_4^+ or NO_x), or as N_2 after the process of denitrification. Phosphorus can be returned to the water column as soluble reactive P (SRP, essentially equivalent to PO_4^{3-}), or retained within the sediments as both inorganic (mineral or adsorbed) and organic P. Rates of sediment-water exchange of nutrients and oxygen (O_2) can be affected by a large number of factors, including input rates of labile C, N and P, the concentration of dissolved O_2 in the overlying water (Mortimer 1971, Rysgaard et al. 1994,

Kemp et al. 2005), salinity (Caraco et al. 1990, Gardner et al. 1991), availability of terminal electron acceptors (NO_3^-, Mn(IV), Fe(III), SO_4^{2-}) (Anderson 1982, Cornwell et al. 1999, Lehtoranta et al. 2009), and the presence and activity of bioturbating and bioirrigating animals (Aller 1980, Pelegri et al. 1994); and pH (Seitzinger 1991, Glibert et al. 2011, Gao et al. 2012). The relative importance of sediment processes to the biogeochemistry of coastal systems is inversely related to the depth of the water column and directly related to the residence time of the water within the system (Nixon et al. 1996). Sediment nutrient concentrations and fluxes in the Bay Delta would thus be expected to be high and to show variable effects with season, with salinity and with concentration of animals. Sediment nutrient fluxes would not be expected to be in Redfieldian stoichiometric proportion.

The questions for the Bay Delta are: *what are the rates of fluxes of nutrients to/from the sediment to the water column and how do they vary with salinity and presence of invasive clams? Do fluxes change spatially and what is the stoichiometric balance of these fluxes?* Recent measurements of sediment fluxes along the Bay Delta spatial gradient and experimental manipulations thereof provide insight into these questions.

6.4.2. Relevance to Bay Delta sediment fluxes

Rates of sediment nutrient fluxes in the Bay Delta are comparatively poorly known, the measurements of sediment nutrient dynamics in the Bay Delta have been minimal (Caffrey 1995, Kuwabara et al. 2009), but a suite of such measurements were made from September 2011 to March 2014 across a gradient from above the confluence of the Sacramento and San Joaquin Rivers to Suisun Bay (Cornwell et al. 2014). Benthic chlorophyll *a* and estimated rates of productivity were determined. Pore water concentrations of NH_4^+ were measured. Flux rates were measured under illuminated and dark conditions for those sites normally receiving light to the bottom, while only dark rates were measured for those sites normally not receiving light to the bottom. In addition, experiments were conducted to assess the effects of salinity and variable clam abundance on rates of NH_4^+ fluxes.

6.4.2.1. Spatial gradients in sediment chlorophyll a and flux rates

The concentrations of sediment chlorophyll *a* were significantly higher in Delta sites (Mildred Island, Frank's Tract, Big Break, Sherman Island) than Bay sediments (Brown, Honker, Grizzly and Suisun Bay; Fig. 41). Individual sediment chlorophyll *a* concentrations ranged from < 1 to 91 mg m^{-2}. Estimated benthic microalgal productivity was surprisingly high in Delta sediments, with a large range in both biomass and productivity. The median estimated rates of O_2-based

photosynthesis were 795 and 395 μmol O_2 m^{-2} h^{-1} for September and March respectively; these data are equivalent to 119 and 57 mg C m^{-2} d^{-1} for the day length at each sample period. While these rates are estimates and more detailed measured as a function of irradiance are required, these rates are similar to benthic microalgal photosynthetic rates observed in a wide range of coastal environments (MacIntyre et al. 1996). Guarini et al. (2002) examined potential productivity in San Francisco Bay tidal environments using resuspended benthic algae and a modeling extrapolation, concluding that spatial variation in benthic productivity would be large, but measured no *in situ* or *ex situ* benthic microalgal production.

Concentrations of NH_4^+ with depth in the sediment were measured in March and July at Big Break, Mildred Island, Honker, Grizzly, Suisun Bay and Montezuma Slough. Concentrations were elevated with depth in all sites measured, but the concentrations from cores in Suisun Bay were significantly higher than any other site, reaching 400 μM by 3 cm depth (Fig. 42a). The Suisun pore water concentrations of SO_4 (Fig. 42b) were also highest of these sites measured, suggesting potential for hypoxia in these sediments.

Fluxes of NH_4^+ (Fig. 43) were generally ≥ 50 μmol m^{-2} h^{-1} in the September relative to the March sampling (Mildred's Island, Franks Tract I, Big Break, Brown, Suisun Bay), but lower flux rates (i.e., high rates of NH_4^+ uptake) were measured under illuminated conditions in both September (Sherman Island) and March (Franks Tract, Big Break). Under dark conditions, 8 of the 12 measured sites appeared to be important sources of NH_4^+, with a substantial amount of benthic microalgal attenuation of NH_4^+ effluxes at 4 sites. Fluxes of NO_x (NO_3^- + NO_2^-) showed considerable site-to-site variability, and overall trends differed considerably from those of NH_4^+ (Fig. 43). Fluxes of NO_x were directed both into and out of the sediments as a result of nitrification, assimilation, denitrification and possible DNRA (dissimilatory reduction of NO_3^- to NH_4^+; Burgin and Hamilton 2007). Whereas the highest NH_4^+ efflux rates were measured at the Delta sites, the same sites yielded the highest rates of NO_x uptake. These rates were, in fact, higher during the spring sampling than the late summer sampling. The highest effluxes of NO_x were observed at Sherman Island. Thus, there appeared to be a spatial difference in the fluxes of NH_4^+ and NO_x, with the flux of NH_4^+ highest in the Delta and in Suisun, but higher NO_x fluxes in the region from Mildred Island to Big Break; NO_x uptake was highest in the lower salinity sites (Mildred Island to Big Break, Fig. 43), switching to dominantly NO_x release starting at Sherman Island.

Net effluxes of N_2-N (denitrification) were measured during 23 of 24 dark incubations and 7 of 11 illuminated incubations (Fig. 43). The dark N_2-N fluxes averaged 34±30 μmol m^{-2} h^{-1} with a median of 32 μmol m^{-2} h^{-1} in September and 48±31 μmol m^{-2} h^{-1} with a median rate of 48 μmol m^{-2} h^{-1} in March. Despite the decrease in temperature and decreased O_2 uptake, denitrification rates were

~50% higher in March. This likely occurred because of the much more widespread high overlying water NO_x concentrations and perhaps more efficient nitrification of remineralized NH_4^+. The production of benthic microalgae generally attenuates denitrification (Cornwell et al. 1999; Risgaard-Petersen 2003), but, in contrast to this general observation, increases in denitrification with light were measured at Mildred's Island and at Big Break.

Sediment-water exchange rates of SRP (essentially equivalent to PO_4^{3-}) were variable, but the variability appeared to be more a function of illuminated versus dark conditions than of site-to-site variability (Fig. 44a). The dark SRP flux rates for September were generally low, with most sites having a net SRP uptake (except Honker II and Suisun I and II). In contrast to the September dark data, the September light data showed modest effluxes of SRP under illumination for Delta sites (Mildred – Sherman Island). In March, however, SRP fluxes under illumination were directed into the sediment, with high (>10 μmol m^{-2} h^{-1}) rates at Mildred Island, Franks Tract II, Big Break and Sherman I and II. Moreover, in March, dark SRP fluxes had a distinct spatial pattern, with moderate to large effluxes in Mildred to Big Break, large rates of uptake in Sherman Island, moderate efflux at Brown, and little flux at Honker, Grizzly and Suisun sites.

In general, higher flux N:P ratios were observed than expected by Redfield stoichiometry (Fig. 44b,c). Deviations from Redfield proportions were driven by processes such as denitrification, variable light/dark uptake of nutrients by microalgae, and adsorption of SRP. Overall, three Bay sites had significant P retention relative to DIN, while all the Delta sites from the light experiments had excess P release relative to N. Furthermore, the tendency for flux rates to show a trend toward excess N relative to P was accentuated in the fall experiments compared to those conducted in the spring (Fig. 44c). This seasonal pattern is consistent, at least in direction, with the stronger fall trend in DIN:TP shown earlier (Fig. 20). Large differences along the spatial (salinity) gradient were also observed in the form of N flux, i.e., the ratio of NO_x:NH_4^+.

Rates of the processes measured here fell largely within the broad range of rates for such processes reported worldwide. Average September rates of DIN (NO_3^-, NO_2^-, NH_4^+) flux were net positive across all sites, while March DIN flux indicated net uptake of DIN at some sites. Denitrification rates were between 0.6 and 1.0 mmol m^{-2} d^{-1}, similar to other mesotrophic estuarine sediments. Coupled nitrification-denitrification was the dominant denitrification pathway in September, with higher overlying water nitrate concentrations in March resulting in denitrification driven by NO_3^- flux into the sediments. Estimated benthic microalgal productivity was variable and surprisingly high in Delta sediments and may represent a major source of labile C to this ecosystem. The daily average N_2-N flux rate calculated over 24 hours of light/dark conditions from this study ranged from 0.7 to 0.9 mmol m^{-2} d^{-1}; when extrapolated to the whole Delta area of $2.3 \cdot 10^8$ m^2

(Jassby et al. 2002), 2.2-2.9 tonnes N d^{-1} N would be removed via denitrification. For comparison, recognizing that such estimates at the current time are approximate, the ~2.5 tons N removed via denitrification is roughly 1/6 of the ~15 tonnes N d^{-1} of the wastewater load from the Sacramento Regional WWTP. Using the integrative estimates from the Lower Sacramento and San Joaquin Rivers provided by Sobota et al. (2009), denitrification would represent 10% of the total daily net N yields of these two rivers. Using the benthic microalgal requirement for N based on Redfield stoichiometry, a maximum uptake estimate of 2-5 tonnes N d^{-1} within this community is calculated. Depending on the area of benthic photosynthetic uptake, this suggests that an amount up to ~20% of the inputs could either be removed via denitrification or taken up by benthic microalgal photosynthesis (Cornwell et al. 2014).

The benthic microalgal requirement for P would be equivalent to 0.3-0.7 tonnes P d^{-1}, with wastewater loading rates from the Sacramento WWTP of 1-2 tonnes P d^{-1}. Similarly, using the integrative estimates of total P yields for these rivers provided by Sobota et al. (2011), the removal of P due to benthic uptake would be in the range of 10-100%. Although such budgetary estimates are broad and approximate, they suggest a greater potential for N relative to P to be exported downstream where it may support phytoplankton production displaced spatially from the upstream sources (Cornwell et al. 2014), but with further contributions to stoichiometric changes.

6.4.2.2. Biotic and abiotic controls on fluxes

The effect of *Potomocorbula amurensis* clams on rates of sediment efflux of NH_4^+ was tested in core experiments conducted with sediment collected from both Montezuma Slough (both spring and early fall) and Suisun Bay (late summer). Cores were collected, incubated following the protocols of Cornwell et al. (2014), and following the termination of the core, animals were sieved and counted. Rates of efflux were estimated based on changes in fluxes as a function of clam biomass and in relation to "control" cores that did not include these clams. The rate of NH_4^+ efflux was positively and linearly related to clam biomass (Fig. 45), but with different slopes of the relationship for different sites. Such differences may be a function of the different seasons during which the two sites were studied. Overall, the flux of NH_4^+ averaged 1-12 μM g^{-1} m^{-2} h^{-1} of clams (ash free dry weight). Note that this range is consistent with direct clam excretion rates of 5.05 μM g^{-1} m^{-2} h^{-1} of clams (ash free dry weight; Kleckner 2009, Dugdale et al. 2015). For comparison, the flux of PO_4^{3-} was variable and did not scale with organism number (not shown). At all Montezuma sites the flux of PO_4^{3-} was net negative, meaning retention of P by the organisms and associated sediments. At the Suisun site, clam excretion rates rates ranged from 0-3 μM g^{-1} m^{-2} h^{-1} of clams. Averaging all sites, the N:P ratio of excretion was far in excess of balanced stoichiometry (assuming minimal levels of detection for P when rates were negative).

As noted above, salinity intrusion is expected to enhance the efflux of both NH_4^+ and PO_4^{3-} through abiotic exchange (e.g., Seitzinger 1991, Glibert et al. 2011, Gao et al. 2012). In 2012 and 2013, cores were collected from Suisun Bay and were experimentally manipulated with respect to salinity, raising the salinity in the overlying water by 2 in order to simulate the effect of salinity intrusion (or a change in X2). Compared to control cores in which salinity was not altered, in 2012, the addition of salt increased NH_4^+ efflux from an average of 50 $\mu M\ l^{-1}\ h^{-1}$ to >150 $\mu M\ l^{-1}\ h^{-1}$ (Fig. 46a). In contrast, when such experiments were repeated in 2013, such an enhancement in salt-treated cores compared to controls was not observed (Fig. 46b). However, both the rates of control core efflux and experimentally manipulated cores were high, on par with rates of the salt-treated cores in 2012. The difference between these two years was that in 2013 the water was already of a higher salinity and thus further salinity did not result in further NH_4^+ availability; concentrations in pore water were already higher in 2013 prior to the salinity enrichment than initially in 2012.

Overall, these data reinforce the notion that there are many factors contributing to changes in the fluxes and concentrations of C, N and P, and Redfieldian proportions are not observed for a range of reasons. Nitrification and denitrification change dissolved inorganic N effluxes, even when N_2-N fluxes are considered. Sediment fluxes contribute to N disproportionately relative to P, especially in the Bay sites. Benthic microalgae can skew elemental ratios by "luxury" uptake during periods without light and by intercepting remineralized N and P. Stoichiometric relationships for SRP are affected by SRP retention/release on the surface of iron oxide minerals; the predominance of inorganic forms of P in these sediments (Nilsen and Delaney 2005) can result in both retention and possible release under low redox conditions (Lehtoranta et al. 2009), changes in salinity (Froelich 1988, Gardolinski et al. 2004), and changes in pH (Glibert et al. 2011, Gao et al. 2012). Extrapolating from both chlorophyll *a* and O_2 fluxes, the sediments may potentially have large impacts on estimates of total system productivity. While these data have provided the first such estimates of nutrient fluxes for this region of the Bay Delta, these rates are nevertheless limited in scope.

6.5. Summary implications for Bay Delta dynamic system changes

When viewed through the lens of classic eutrophication responses (high algal biomass, hypoxia), the Bay Delta has long been thought to be immune from nutrient effects; the prevailing view, consistent with Reynolds (1999) has been that since nutrients are "sufficient" they are not regulating phytoplankton growth and therefore not regulating any component of the food web (e.g., Cole and Cloern 1984, Alpine and Cloern 1992, Kimmerer 2004, Jassby 2008, Cloern and Jassby 2012, Kimmerer and Thompson 2014). The assumed flat response of the saturating portion of the

classic nutrient response curve (Fig. 1) has led to the incorrect assumption that all regulation ceases once nutrient concentrations are in this saturating range. The possibility of "bottom up control" by nutrients of fish populations has also been dismissed in favor of interpretations such as ecological invasions, food limitation and other "multiple stressors" (e.g. Sommer et al. 2007). However, there is no question that changes in nutrients have occurred, as has the food web. The regulatory impact of nutrients has been under appreciated far too long, derived from the flawed paradigm that lack of nutrient limitation equates to lack of nutrient regulation. The attention on NH_4^+ toxicity (and concomitant lack of recognition of the dual roles that NH_4^+ plays in the cell) and on defining absolute concentrations of NH_4^+ inhibition effects without understanding taxonomic and environmental effects on physiology has been disproportionate. NH_4^+ is a nutrient first, but its metabolic products may be inhibiting or repressive for the assimilation of other substrates, mainly NO_3^- when concentrations are elevated. Factors such as the relative availability of the N (P and C) substrates, the nutritional status of the component organisms, the number of trophic interactions, along with environmental parameters such as ambient light and temperature, all determine the extent of the dynamic metabolic balance in the use of one substrate *vs* another and, in turn, the relative success of (or not) of the primary producers and the consumers. Once the balance is tipped, a new dynamic emerges (Fig. 47). That nutrition plays a key role in this process should be obvious with our contemporary understanding of phytoplankton physiology and ecological stoichiometry; unraveling the multifaceted effects of nutrients in an imbalanced and otherwise dynamic system will continue to present challenges, however. Nutrients are metabolically different from toxicants and characterization of threshold values for metabolic suppression without consideration of the context of other environmental conditions will be erroneous at best.

An alternative viewpoint to the contention that nutrients are non-regulating, is that *metabolic dynamic regulation* and *ecological stoichiometry* combine as a significant contributor to the complexity of responses in the Bay Delta. Such an interpretation was proposed by Glibert (2010, 2012, Glibert et al. 2011) and has been reinforced though experimental measurements of the phytoplankton, zooplankton and sediment biogeochemistry, and through new analyses of time series that encompass more recent years of data. The stoichiometric perspective does not contradict the observation that phytoplankton biomass has declined over time (nor does it challenge the recent suggestion that the "resilience" of the bay to nutrients may be changing). The stoichiometric hypothesis does not negate the importance of ecological invasions, habitat changes, multiple stressors and food web complexities, but *adds a mechanism* to these factors through metabolism, organismal stoichiometry and biochemistry. As emphasized herein, *metabolism of nutrients operates dynamically from limitation to excess*. Metabolism of nutrients thus spans preferential use to inhibition, from growth enhancement to growth suppression. Through the "paradox of enrichment" and the "stoichiometric knife edge" of metabolic regulation, nutrients can enhance or

destabilize the dynamics of primary producers and consumers. The stoichiometric perspective contrasts with the prevailing view, and as stated by Reynolds (1999), that since nutrients are "sufficient" they not regulating for the phytoplankton and other ecosystem elements. Stoichiometric imbalances, even when nutrients are not "limiting", may promote transformations of nutrients or may alter the processes by which nutrients are cycled in the ecosystem and thus alter nutrient availability or form for primary producers (Elser and Hamilton 2007). The trends in the Bay Delta suggest that such effects have occurred, and are occurring, on a range of scales. It is time to lay to rest the notion that nutrients and nutrient stoichiometry are only regulatory for physiology at the limiting end of the spectrum. Recognizing stoichiometric and nutrient regulation does not mean or imply that all trends and all changes are solely related to nutrients, but it does mean that *nutrients are a key driver*. This is a concept that is directly testable, and relevant experiments to date are supportive.

To summarize the nutrient regulatory dynamic perspective, the inhibitory or repressive effect of NH_4^+, together with changing N:P, over time have contributed to the changes observed in the food web. The composition of the food web at all levels appears to have shifted to organisms that are either 1) increasingly NH_4^+ tolerant; and/or 2) increasingly efficient at obtaining their requisite P and releasing excess N; or 3) can make due with lower P availability. Representative "winners" include chlorophytes, toxic cyanobacteria, cyclopoid copepods, piscivorous fish, while those suffering losses are the diatoms, the calanoid copepods and the planktivores. Spring blooms appear to have been susceptible to NH_4^+ inhibition, especially when high flow leads to a combination of sustained elevated concentrations of NH_4^+ throughout the Bay Delta and low residence time, leading to phytoplankton "wash out". The spring is also a time when the physiological regulation of C and N metabolism by phytoplankton is especially challenged due to low temperatures. In contrast, during the warmer months, when overall rates of metabolism are higher, the balance of N:P seems to have a greater regulatory effect. This effect is seen at the phytoplankton level, but also at all levels of the food web. While the importance of N:P stoichiometry was previously suggested to be important in food web structure of the Bay Delta based on annualized data (Glibert 2010, 2012, Glibert et al. 2011), as shown here, when examined from a seasonal perspective, the trends are substantiated or strengthened.

Recently Cloern et al. (2015) reasserted that food quantity, not quality, is the issue of concern for the food web of the Bay Delta. There is no debate or disagreement over the fact that phytoplankton biomass (chlorophyll *a*) has declined over the past decades and that complex changes in the food web have occurred. The Cloern et al. (2015) paper restated that many factors contributed to these changes, including light limitation, grazing, and freshwater flow variability, especially low food quantity. They questioned whether phytoplankton compositional changes have indeed occurred

over the past several decades in the Bay Delta, suggesting there are no relationships with changes in nutrient loads. They have also questioned the quality of the long-term data on phytoplankton (see also Malkassian et al., 2015), suggesting that no statistical conclusions can be drawn from the low number of cells enumerated for each sample collected. In contrast, Glibert (2010, Glibert et al. 2011) suggested that the nutrients changed in form and composition over time, that phytoplankton compositional changes mirrored these nutrient changes in terms of the dominant taxa, that these changes follow that which would be expected based on phytoplankton physiology, and these changes had effects through the food web. As shown here, direct experiments on effects of changes in nutrient forms and ratios on phytoplankton composition, even when provided at 'saturating' levels, have provided results consistent with the hypotheses that nutrient form and stoichiometry are regulatory.

Cloern et al. (2015) also calculated an index of food quality for the food web, one that weighs the fatty acid contribution of different phytoplankton groups that were available to copepods over time, and their resulting calculations suggest there has been little to no change in food quality through time. They do not consider stoichiometric effects that alter metabolism and growth. Their analysis does not account for any nutrient effects that may propagate though the food web as described herein. Cloern et al. (2015) suggest that an interpretation of food web changes should depend on listening to what the estuary is telling us. In agreement, it is suggested here that we listen to the organisms and their metabolism and physiology, including their stoichiometric needs and differences. These are aspects of the system that have rarely been measured, and virtually all the "listening" to date has been through observations and enumerations, not process-based measurements. Numbers of organisms should not be the only measure of ecosystem or metabolic status. Further, as emphasized throughout this report, nutrient effects are also related to grazing and flow effects. And, not all fish have declined; there have been increases in the piscivorous fish, so food limitation cannot hold at all levels of the food web. Cloern et al. (2015) further suggest that accepting nutrients as a master variable poses the risk of missed opportunities to identify the root causes of fish decline. However, if indeed nutrient changes are the root cause of fish changes, then failure to examine nutrient relationships will indeed result in the very failure about which they are concerned: failure to identify the root cause of fish changes. Of course the risk always exists that the system may not respond as predicted - models may be wrong, but failure to consider the possibility of nutrients as an important determinant could mean that recovery of the ecosystem and planktivorous fish will remain elusive.

As shown above, changes in stoichiometry affect physiology and metabolism – at the phytoplankton level in terms of N acquisition and growth, at the zooplankton level in terms of excretion, egg production and viability, and finally at the level of fish through their strict

stoichiometric regulation. Ultimately effects on metabolism do relate to changes in community composition. This fundamental concept lies at the heart of the differences in "new" and "regenerated" production (sensu Dugdale and Goering 1967). It holds for the higher trophic levels as well. When metabolism is stressed due to nutritional constraints, secondary stressor effects may be enhanced. Indeed there are many stressors in the Bay Delta. Even if the quality of the long-term data set is less than ideal (Cloern et al. 2015), the trends nevertheless are consistent with those that would be expected from both physiology and stoichiometric regulation (e.g., Hillebrand et al. 2013).

Experimental results on physiology and metabolism conducted to date suggest that organisms of the Bay Delta are not unique in how they respond to nutrients, either in limitation or in excess. When viewed through the perspective of dynamic regulation of metabolism, phytoplankton compositional changes and trophic level effects, the long-term ecosystem changes in the Bay Delta follow that which would be predicted of a system that has undergone changes in the balance of N forms and of N:P. Imbalances in stoichiometry may destabilize the dynamics of consumers, shifting systems to new conditions. These imbalances are a function of many factors and processes. Anthropogenic loads of N have increased relative to those of P. Consumer-driver stoichiometry contributes to the maintenance of this imbalance. Sediment fluxes are also imbalanced with greater N relative to P efflux in the Bay sites studied. Together these trends lead to strong evidence for nutrient regulation (Table 2). Interaction of nutrient changes with other stresses of course cannot be ignored, but neither should nutrient changes in the context of other changes. Effects of contaminants may be related to the nutritional status of different organisms. Flow effects are related to nutrients; increased freshwater flow may dilute potentially inhibiting concentrations of NH_4^+, while reduced flow not only may enhance residence time allowing for increased nitrification, but sediment fluxes of NH_4^+ may also be increased. The trajectory of food web changes in the Bay Delta is analogous to many other systems having undergone nutrient changes leading to stoichiometric imbalance (Glibert et al. 2011, Glibert 2012). The changes expected in N loads and form in the coming years due to sewage treatment upgrades (Dugdale et al. 2015) will alter both the proportion of NH_4^+:NO_3^- and the stoichiometry of N:P. Even though the Bay Delta is a dynamic system, it is expected that the ecosystem will respond to these changes in a manner predicted by both metabolic regulation and by stoichiometric principles.

Several specific next steps are herein recommended. First, there is certainly much work to be done to understand physiological trade-offs at varying substrate levels (both nutrients and light) across functional groups of all trophic levels. Much needs to be done in parameterizing rates, characterizing traits, and how they are both externally driven and internally dynamically regulated. The ecology of the Bay Delta has, for too long, been interpreted based on statistical relations of

observational data. The underlying metabolic processes and physiology needs to be much better elucidated. Thus, phytoplankton culture studies that go beyond steady state and that expose cells to potentially stressful light and temperature conditions, or other conditions more representative of the dynamic and changing conditions of natural, N-enriched waters are needed to more fully disentangle the complexities of effects of N form at all growth conditions. Additional field-based experiments that test the effects of changes in nutrients need to be conducted. Relatedly, care must be taken in applying appropriate methods for understanding physiological regulation of metabolism. Progress has been significant but there is much ecophysiological work to do. Improved understanding of functional genetics, adaptive physiology, variable kinetic relationships, and experiments undertaken under non-steady state conditions are required. This should include studies that examine growth and metabolic responses when more than a single N substrate is provided. As noted by Allen and Polemine (2010), it is time to conduct the experimental work required at all scales that will "fully capture ecosystem dynamics...the physiology of the component organism, their behavioral traits and the interactions between them."

Second, more work is needed to understand rates of nutrient processing at the microbial level. In particular, a better understanding of nitrification, denitrification, and DNRA contributing to changes in the NO_3^- and NH_4^+ field is needed, along with better understanding of P uptake, sequestration, and release, from both water column and sediment (along with their resident organisms) is needed. Bacterial processes are important in both the sediments and the water column, but there is still much to be learned about their abundance and activity. Also, not all phytoplankton responses have been well characterized. As examples, the dynamic changes in, and the ecological roles of, picoplankton and of mixotrophs have been virtually ignored in this system. Moreover, at the microbial level, the composition, rates of grazing by, and variability in, microzooplankton are not well known.

Third, moving up the food web, food quality for grazers must be better understood. The notion of food quantity (as C) as the primary factor regulating upper trophic level biomass should be reconsidered in light of emerging understanding of food quality from a stoichiometric perspective. An appreciation of food quality beyond classic measures such as fatty acids must advance. The findings here suggest that strengthened insights with respect to changes in dominant upper trophic level species may be gained by use of additional denominators – that P and N "currency" yields insights not found with C "currency." While productivity is a function of C, community composition may be more strongly linked to N and P. Caloric intake is an insufficient metric of food quality; nutrient content does matter.

Fourth, the stoichiometric impact of invasive clams should be further examined. As shown here, there is evidence that their excretion further sustains an elevated DIN:TP balance. It is of interest that a comparable invasion by *Corbicula fluminea* occurred in the Potomac River, Chesapeake Bay, in the 1970s. Its abundance peaked in the late 1980s when N:P loads were similarly increasing. However, its abundance subsequently declined coincident with efforts to remove N from sewage effluent and a resulting decline in the N:P of the water column (Phelps 1994, Jaworski and Romano 1999, Cummins et al. 2010, Glibert et al. 2011). Estimating how clam biomass is affected by, and in turn affects, nutrient stoichiometry, may prove to be insightful in terms of understanding what may give them an ecological advantage or how they may respond to future nutrient changes.

Fifth, the recent drought has brought to the fore the importance of the interactions between nutrients and flow. The past ~3 years have seen spring blooms of a magnitude not seen in recent decades. This recent phenomenon should raise questions, and provide insight into, the relation between nutrients and flow. On the one hand, the drought has created conditions of less dilution of potentially inhibiting NH_4^+, and therefore higher concentrations, but has also constrained these effects to a smaller riverine region, while also creating conditions conducive for nitrification. Thus, flow, like nutrients, affects the ecosystem in a complex way: there is an optimum, and both too little flow and too much flow affect nutrient cycling and production in complex ways. The drought has provided an opportunity for "natural experiments" in understanding the interactions of nutrients and flow.

Sixth, the conceptual interpretation of disconnected multiple stressors, without the inclusion of nutrient changes, as the cause of fish declines should be revisited. While indeed there are multiple stressors in the system, the appreciation of their interactions with nutrients has been lacking. Stressors such as changes in freshwater flow, invasive species, and even climate change all affect nutrient fluxes in multiple ways, altering nutrient availability and/or form and/or stoichiometry. Relationships between fish and flow, for example, may actually be a proxy for relationships between fish and food of varying quality. The effects of contaminants in relation to stoichiometry should also be examined, as relationships between tolerance to contaminants and algal stoichiometry in some zooplankton are emerging. Further, when "top down" control is altered, due, for example, from changing abundance of grazers, as in the case of clam biomass, so too are nutrients and their stoichiometry altered by their rates of release and excretion. To further understand these effects, fish stoichiometry should be measured and effects of varied food stoichiometry should be studied experimentally.

Finally, a new emphasis on improved model formulations is needed. Efforts to incorporate

dynamic balance models for physiology and for trophodynamics need to be advanced (Glibert et al. 2013). The plasticity of nutritional pathways, as well as the plasticity of food web interactions, including grazing, allelopathy, symbioses and other interactions, creates immense challenges for model constructs. Monod and Michaelis-Menten kinetics which assume a fixed half-saturation constant and maximal rate are inadequate, and in most cases incorrect, to capture variable physiological processes. Even cellular Droop kinetic relationships (Droop 1973) do not classically capture regulation beyond saturation. A new generation of models is needed to capture stress at the supersaturating end of the spectrum as well as at the limiting end (Flynn 2010, Allen and Polemine 2010, Glibert et al. 2013). The dynamic regulatory modeling approach now being applied to both photosynthesis and nutrient uptake (e.g., Kana et al. 1997, Geider et al. 1996, 1997, 1998, Smith et al. 2009, Bonachela et al. 2011) is an important advance along these lines. In terms of modeling, multiple currency models (C, N, P) should be applied, as opposed to fixed Redfield constructs (Flynn 2010, Glibert et al. 2010b, Glibert et al. 2013). Multi-element descriptions also support bioenergetic descriptions, which may be important for predicting the survival of organisms under unfavorable conditions. Variable stoichiometric parameterizations in models must also begin to recognize that physiological processes and organismal stoichiometry can and do vary even at growth-saturating substrate concentrations. Positive feedback mechanisms exist between microbial processes, macrobenthos, macrophytes, pH, nutrient efflux, and other biogeochemical processes affecting stoichiometry, and in turn, food webs. Incorporating the full complexity of these interactions is an enormous challenge for modelers, but there are important steps being made in recognizing these complex interactions. Benthic nutrient fluxes, benthic productivity, and their dynamic interactions with the water column should also not be ignored in measurements or in models. Understanding and parameterizing these important feedbacks has implications for modeling current and projected changes in climate, nutrient loads, and land use, and has direct application in understanding thresholds of system response or altered stable states (*sensu* Scheffer et al. 1993).

The ecological impacts of NH_4^+ loading and the importance of changes in NO_3^-:NH_4^+ in phytoplankton succession also have important implications for nutrient criteria development, as criteria are typically based on *total* N or *total* P and *total* biomass measures such as chlorophyll *a* (e.g., Bricker et al. 2007, Harding et al. 2014). Moreover, single N recommendations that do not account for other factors in the environment, such as temperature variability and its relation to N uptake and growth of different phytoplankton groups, are particularly simplistic. Such an un-nuanced view fails to recognize that the excess of N loading, its redox state, and stoichiometric imbalances of C, N and P have consequences for not just the quantity, but also the quality, of primary producers and ultimately for higher trophic levels – and such relationships are modified by the interplay of multiple growth factors.

The ecology of the Bay Delta has, for too long, been interpreted based mainly on observational data. Such observational data is immensely valued, but emerging relationships are ever more powerful when directly tested in in experiments and process-based studies. Mechanistic and physiological understanding has lagged. The increasing nutrient loads to coastal systems, combined with their disproportionate composition in both space and time make the issue of stoichiometry ever more important (Seitzinger et al. 2002, 2005, Howarth et al. 2005, Glibert et al. 2006, 2014c). *Nutrients do matter, and their stoichiometry does matter, even when nutrients are non-limiting.* Disproportionate N and P loads globally are now recognized to have effects at all scales, from genomic to ecosystem that need further empirical resolution (Peñuelas et al. 2012). Even relatively small changes in nutrient supply are being shown to have large consequences on many important properties of the ecosystems (Nielsen 2003). Understanding the full suite of processes and factors that underlie variable stoichiometry at all scales – and for elements beyond N and P emphasized here – and the feedbacks between them is a grand challenge (Frigstad et al. 2011). Such a grand challenge has been recognized globally; Bay Delta nutrient management should meet this challenge.

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Table 1. Comparison of correlation coefficients (r) reported herein on a seasonal basis for values encompassing Suisun Bay, Chipp's Island and the lower Sacramento River for 1979-2011 (zooplankton and fish data log transformed), with results previously reported by Glibert et al. (2011) on an annual basis encompassing 1975-2005. Note that the Glibert et al. (2011) analysis took several approaches: (1) original data log transformed; (2) stationarized by trend (pre-whitened); (3) stationarized by first difference; and (4) smoothed using a three-year backward moving average. Only values with $p<0.10$ are shown. Values that are significant at $p<0.05$ are shown in bold font and those that significant at $p<0.01$ are shown in bold, italic font; values are red if negative, blue if positive. Note that herein the fish abundances were only correlated with fall values. N/A means the relationship was not calculated.

Relationship	Spring (this analysis)	Fall (this analysis)	Annual (Glibert et al. 2011) original data	Annual (Glibert et al. 2011) pre-whitened	Annual (Glibert et al. 2011) first-differenced	Annual (Glibert et al. 2011) 3-yr backward average
Phytoplankton and nutrients						
Chlorophyll and NH_4^+	***-0.64***	***-0.56***	**-0.43**	-0.34	***-0.45***	-0.37
Diatoms and NH_4^+	***-0.47***	***-0.59***	***-0.57***	***-0.60***	***-0.60***	***-0.54***
Chlorophyll and DIN:TP	***-0.61***	***-0.84***	***-0.76***	**-0.40**	***-0.57***	***-0.77***
Diatoms and DIN:TP	***-0.58***	***-0.66***	***-0.53***	**-0.58**	***-0.45***	***-0.93***
Zooplankton and nutrients						
Eurytemora and NH_4^+	***-0.74***	***-0.53***	**-0.37**		***-0.56***	**-0.40**
Limnoithona and NH_4^+	0.07	***0.57***	**0.46**			***0.54***
Eurytemora vs DIN:TP	***-0.67***	***-0.81***	***-0.75***	-0.34	***-0.55***	***-0.83***
Limnoithona and DIN:TP	***0.56***	***0.80***	***0.68***			***0.73***
Fish and nutrients						
Delta smelt and NH_4^+	N/A	-0.23				
Longfin smelt and NH_4^+	N/A	**-0.38**	***-0.64***	**-0.57**		***-0.52***
Striped bass age 0 and NH_4^+	N/A	***-0.53***	N/A	N/A	N/A	N/A
Silversides and NH_4^+	N/A	***0.52***	***0.48***	**0.44**		***0.52***
Sunfish and NH_4^+	N/A	**0.47**				**0.40**
LM Bass and NH_4^+	N/A	0.24			**-0.40**	0.35
Delta smelt and DIN:TP	N/A	***-0.53***	**-0.36**			-0.35
Longfin and DIN:TP	N/A	***-0.68***	***-0.65***	***-0.60***		***-0.64***
Striped bass age 0 and DIN:TP	N/A	***-0.71***	N/A	N/A	N/A	N/A
Silversides and DIN:TP	N/A	***0.62***	***0.54***		**-0.40**	***0.74***
Sunfish and DIN:TP	N/A	***0.71***	***0.63***			***0.77***
LM Bass and DIN:TP	N/A	***0.51***	**0.46**			***0.80***

Table 2. Table of correlation coefficients (r) for the fish and nutrient, chlorophyll *a* or abiotic parameter and duration of the time series indicated. Delta smelt, longfin smelt and striped bass age 0 are fall midwater trawl index (FMWT) values, while silversides, sunfish and largemouth bass values are from the beach seine surveys. The ratio of silversides/longfin is an arbitrary ratio of these fish with contrasting responses to N and P. All fish values were log transformed. The nutrient values are the averages of the years indicated for sites monitored in Suisun Bay, near Chipp's Island, and lower Sacramento River for the period of July-October. Nutrient values are in weight units and N:P is inorganic N:total P. The N loading values for the Sacramento Regional WWTP (NSacR) are the summed loading (metric tons) for the period from July through October. Exports are SWP-CVP export flow estimates (cfs) calculated for the months of July-October only. Values that are significant at $p<0.05$ are shown in enlarged bold font and those significant at $p<0.01$ are shown in bold, italic, font. Values are shown in red if negative, blue if positive. In the time series from 1979-1986 the values that are barely significant ($p=0.052$) are shown in colored, bold font but not enlarged.

	DIN:TP	TP	DIN	N SacR#	Chl	X2	Secchi	Temp	Exports
1979-1986									
Delta smelt	-0.61	**-0.75**	0.00	N/A	**0.79**	0.00	**-0.75**	-0.48	0.00
Longfin smelt	-0.26	0.00	-0.46	N/A	-0.46	-0.72	-0.61	0.00	-0.56
Striped bass age 0	0.42	-0.66	-0.39	N/A	-0.50	-0.70	0.20	**0.78**	-0.67
Silversides	0.66	**-0.82**	0.17	N/A	-0.67	-0.56	0.56	0.40	-0.47
Sunfish	0.52	-0.59	0.17	N/A	-0.44	**-0.85**	0.00	0.33	**-0.80**
LM bass	**0.82**	-0.73	0.44	N/A	**-0.79**	-0.56	0.64	0.28	-0.62
Silversides/Longfin	0.37	-0.32	0.22	N/A	**-0.75**	0.53	**0.77**	0.22	0.46
1987-1999									
Delta smelt	0.00	-0.20	-0.33	0.14	0.45	-0.39	-0.33	0.14	0.31
Longfin smelt	0.17	**-0.68**	***-0.77***	0.14	0.46	***-0.76***	**-0.59**	-0.30	**0.69**
Striped bass age 0	0.26	0.20	0.41	-0.32	0.00	0.17	0.24	0.22	**-0.59**
Silversides	0.24	-0.28	-0.17	0.00	-0.10	-0.22	0.20	0.00	-0.10
Sunfish	0.30	**-0.65**	-0.57	***0.72***	0.00	**-0.69**	-0.10	-0.43	***0.62***
LM bass	0.00	-0.14	-0.10	0.10	***-0.71***	0.14	***0.74***	0.20	-0.17
Silversides/Longfin	0.00	0.51	***0.72***	-0.10	-0.49	0.60	**0.62**	0.24	**-0.68**
2000-2011									
Delta smelt	-0.54	0.49	0.00	**-0.65**	0.33	0.00	0.00	0.00	0.10
Longfin smelt	**-0.68**	0.35	-0.26	-0.26	0.30	-0.48	-0.57	-0.26	0.26
Striped bass age 0	-0.10	0.24	0.14	**-0.66**	0.14	0.00	-0.36	0.10	-0.14
Silversides	-0.48	-0.20	**-0.65**	0.30	-0.33	**-0.63**	0.22	-0.10	0.49
Sunfish	0.54	-0.51	0.10	0.47	-0.10	0.24	0.50	0.20	0.00
LM bass	0.44	**-0.67**	-0.17	0.58	0.00	0.17	**0.65**	0.22	0.00
Silversides/Longfin	0.42	-0.57	0.00	0.43	-0.49	0.17	0.59	0.20	0.00
Overall 1979-2011									
Delta smelt	***-0.53***	**0.39**	-0.20	***-0.56***	0.28	-0.14	***-0.67***	-0.17	-0.08
Longfin smelt	***-0.68***	0.00	***-0.71***	-0.39	***0.66***	***-0.65***	***-0.65***	***-0.51***	0.00
Striped bass age 0	***-0.71***	0.28	***-0.47***	***-0.81***	***0.55***	0.28	**-0.41**	**-0.35**	***-0.52***
Silversides	***0.62***	***-0.46***	**0.37**	***0.66***	***-0.51***	-0.10	**0.43**	**0.37**	0.28
Sunfish	***0.71***	***-0.61***	0.32	***0.70***	***-0.50***	-0.30	**0.39**	0.32	0.33
LM bass	***0.51***	***-0.56***	0.24	**0.47**	**-0.36**	0.10	***0.73***	0.32	0.00
Silversides/Longfin	***0.75***	0.14	***0.69***	***0.63***	***-0.68***	***0.47***	***0.66***	***0.54***	0.13

[#] Values cannot be calculated for first time period since the WWTP came on line in 1982 and first data are available in 1984; overall trends for this relationship are for 1987-2011 only.

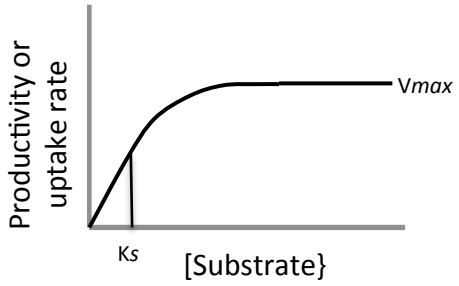


Figure 1. Classic depiction of uptake rate, productivity or growth as a function of substrate availability. K*s* is the half-saturation constant or the concentration at which the uptake rate is half maximal, or half of V*max*.

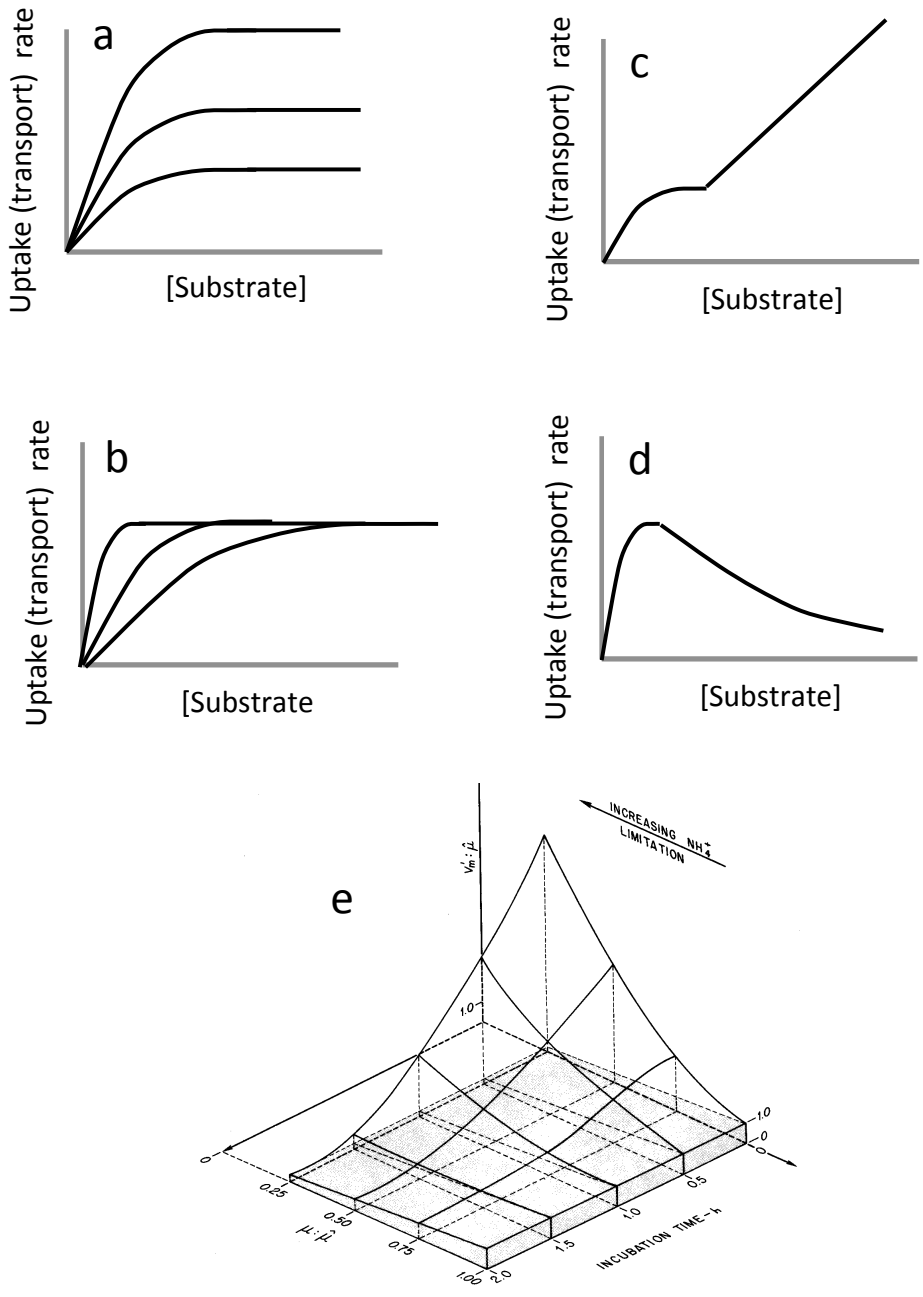


Figure 2. Variable responses in uptake (or transport) rate to substrate availability. The bottom panel is the conceptual relationship between transient NH_4^+ uptake, physiological state (as relative growth rate, μ:μmax) and duration of incubation. Lower panel reproduced from Goldman and Glibert (1983).

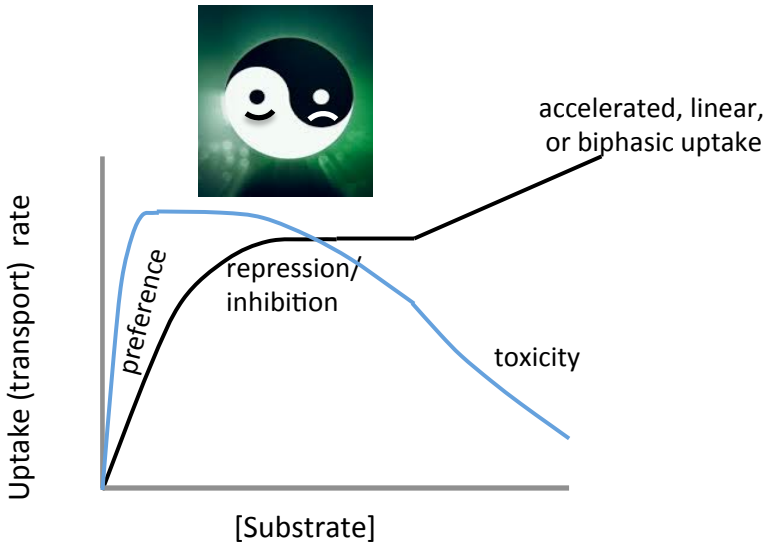


Figure 3. Summary conceptual relationship of uptake as a function of substrate concentration for two different substrates (blue-NH_4^+, black-NO_3^-), illustrating preference, repression/inhibition and toxicity. Note that at low concentrations of substrate, uptake of NH_4^+ may display not only a lower K*s*, but may also have a higher V*max*. The result is the paradoxical behavior of NH_4^+ kinetics relative to those of NO_3^-.

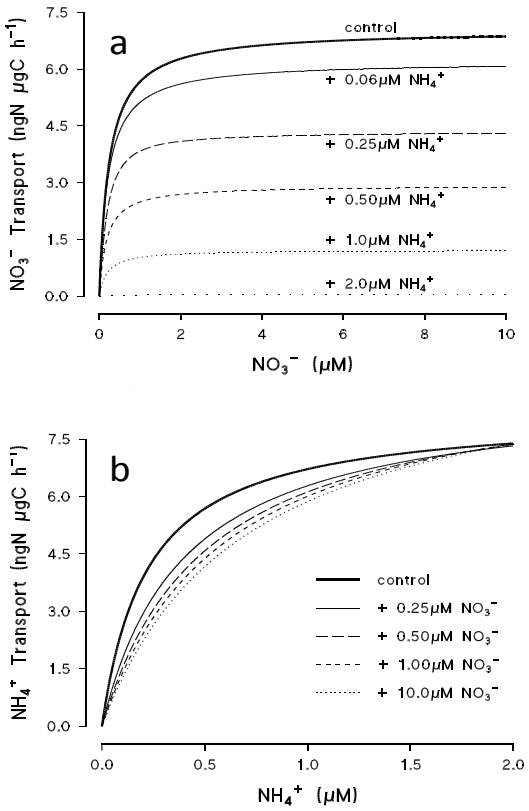


Figure 4. Effects of external concentrations of NH_4^+ on NO_3^- transport (a) and external NO_3^- concentrations on NH_4^+ transport (b) at steady state. Note that while NH_4^+ has a large effect on NO_3^- transport, NO_3^- has a minimal effect on NH_4^+ transport.
Reproduced from Flynn et al. (1997).

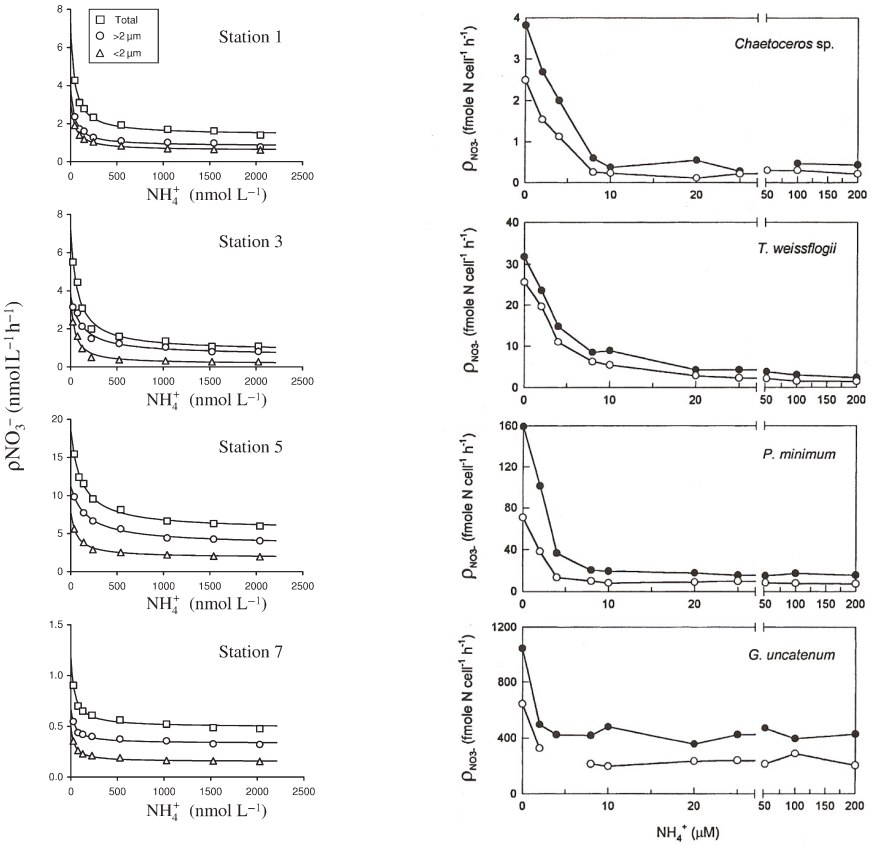


Figure 5. Two examples of inhibition of NO_3^- by NH_4^+. Left panels: NO_3^- uptake rates as a function of concentrations of NH_4^+ in the total (square), >2 μm (circle) and <2 μm (triangle) size fractions for stations sampled in the NW Atlantic. Solid lines were fit iteratively to the data according to the reverse Michaelis-Menten equation ($p<0.005$).
Right panels: NO_3^- uptake and assimilation rates as a function of NH_4^+ for the diatoms *Chaetoceros* Sp., *Thalasiosira weissflogii*, and the dinoflagellates *Prorocentrum minimum* and *Gyrodinium uncatenum* grown at 22ºC. Rates of uptake into the total cell (solid circle) and assimilation rates into the protein fraction (open circle) are shown. Note that the rates of NO_3^- uptake in the field experiments are reported on a volumetric basis whereas those of the culture experiments are reported on a cell basis; note also the differences in units of the NH_4^+concentrations.
Left figure reproduced from L'Helguen et al. (2008); right figure reproduced from Lomas et al. (2000).

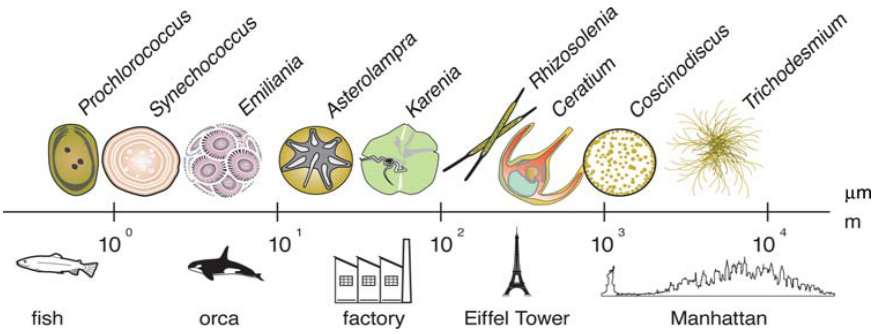


Figure 6. Schematic depiction of the range in cell size of various phytoplankton in relation to well recognized objects.
Figure reproduced from Finkel et al. (2010).

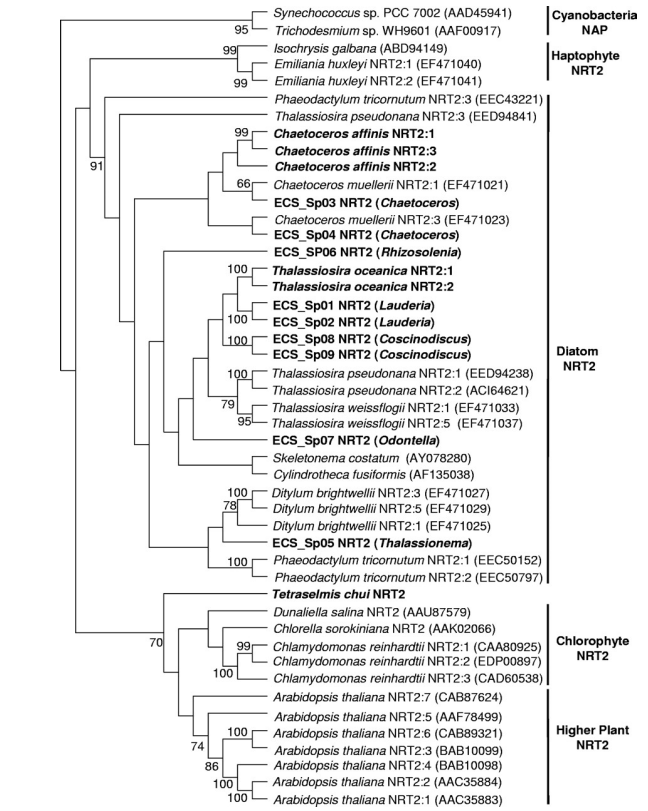
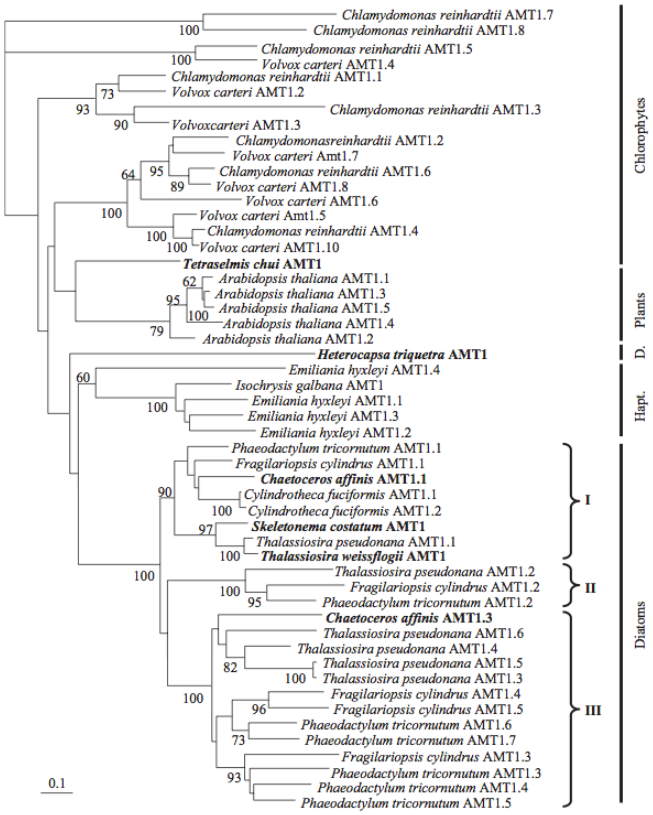


Figure 7. Upper panel: Phylogenetic tree of NO_3^- transporter (NRT2) sequences in cyanobacteria, eukaryotic phytoplankton, and higher plants. Results indicated that NRT2 sequences belonging to cyanobacteria, haptophytes, chlorophytes, and diatoms formed 4 distinctive clades at the phylum level. [Bold font indicates the NRT2 sequences obtained in the study from which this figure was reproduced]. Numbers at the nodes are the bootstrap values based on 1,000 resamplings, and only values that are >60% are shown. GenBank accession numbers are shown in parentheses.
Reproduced from Kang et al. (2011).



Lower panel: Phylogenetic tree of NH_4^+ transporter (AMT1) sequences in eukaryotic phytoplankton (diatoms, Hapt-haptophytes, D-dinoflagellates and chlorophytes) and higher plants. Results show that AMT1s of higher plants were most closely related to those in chlorophytes and that haptophyte and diatom AMT1s formed distinct monophyletic clades. Diatom AMT1s were further divided into 3 orthologous subclasses. [Bold font indicates the sequences obtained in the study from which this figure was reproduced]. Numbers at the nodes are bootstrap values based on 1000 resamplings, and only values of > 60% are shown. The scale bar represents an estimated number of amino acid substitutions per position. Accession numbers are provided in the original paper. Reproduced from Kang and Chang (2014).

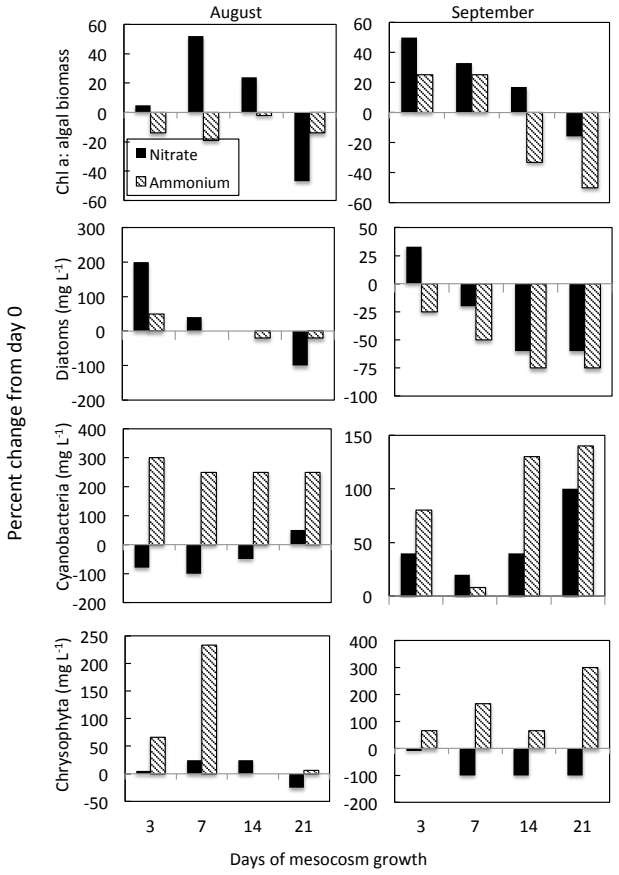


Figure 8. Comparison of phytoplankton community composition in mesocosm experiments from the Qu'Appelle Lakes, Canada, conducted during the summer months and enriched with NO_3^- or NH_4^+.
Data replotted from Donald et al. (2013).

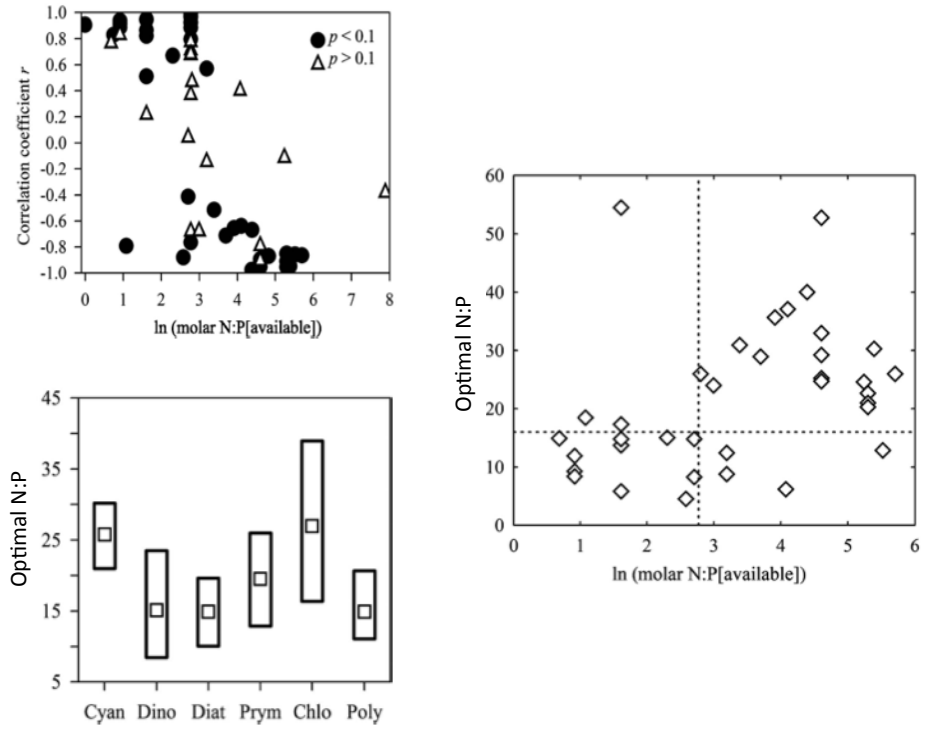


Figure 9. Left upper panel: correlation coefficients (r) between natural log-transformed N:P and growth rate for 55 data sets assembled in the meta-analysis conducted by Hillebrand et al. (2013).
Left lower panel: Average optimal N:P ratio (+95% confidence intervals) for different phytoplankton groups (Cyan: cyanobacteria; Dino: dinoflagellates; Diat: diatoms; Prym: prymnesiophytes; Chlo: chlorophytes; Poly: polycultures of multiple species.
Right panel: Relationship between optimal N:P ratio of phytoplankton and available N:P in the environment. Note that increasing N:P in the environment is associated with increasing optimal N:P (Spearman rank correlation, n=36; r=0.46, p=0.004).
All figures reproduced from Hillebrand et al. (2013).

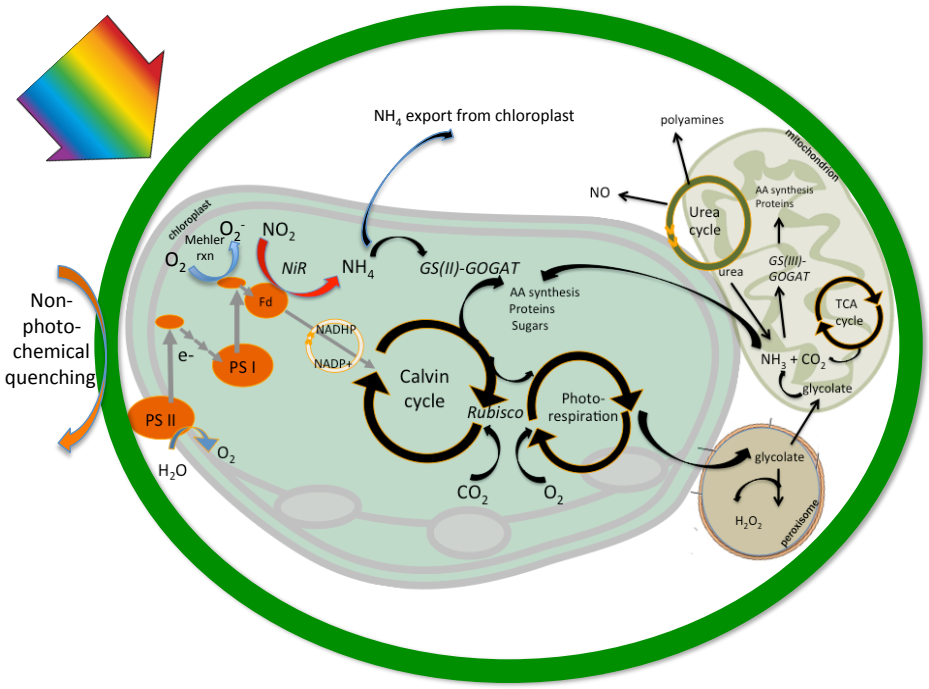


Figure 10. Conceptual schematic illustrating, for a generic algal cell, the electron transport of photosynthesis, the coupling to the Calvin cycle and N assimilation pathways, and the various mechanisms for energy and excess reductant dissipation. The dissipatory mechanisms shown include non-photochemical quenching, Mehler activity, dissimilatory NO_3^-/NO_2^- reduction to NH_4^+, and photorespiration. The dual catalytic reactions of Rubisco with CO_2 and O_2 are also shown.
Figure reproduced from Glibert et al. (in review).

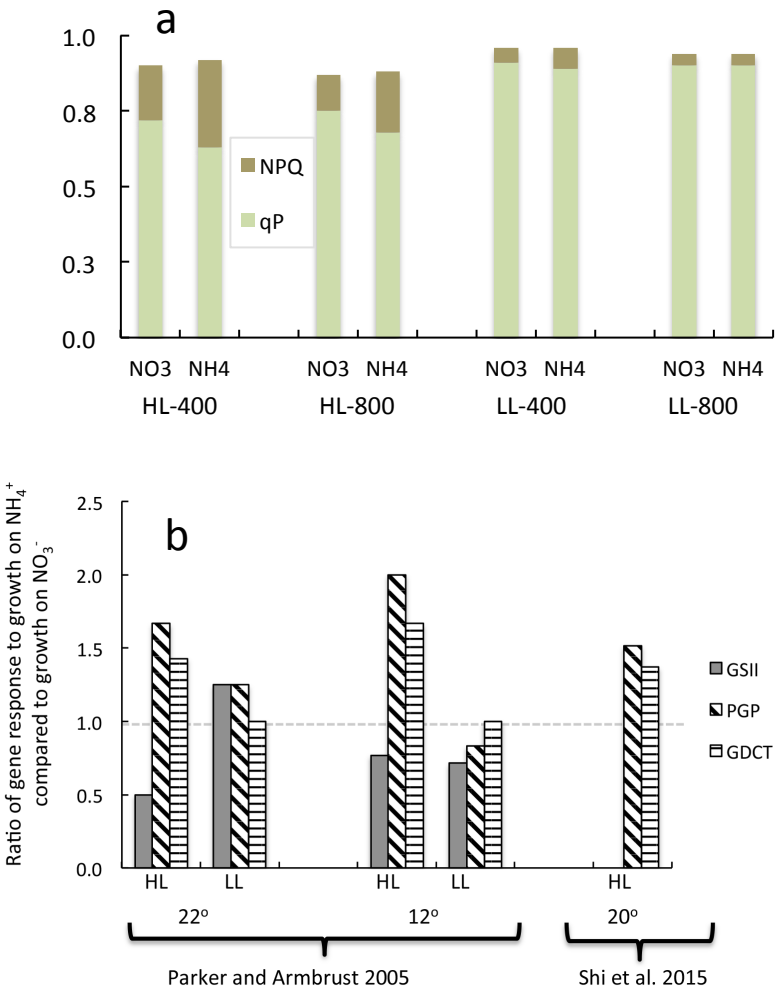


Figure 11. Panel a: Comparison of the estimates of photochemical quenching (qP) and non-photochemical quenching (NPQ; relative fluoresence units) in cultures of the diatom *Thalassiosira pseudonana* when grown on NO_3^- or NH_4^+, high light (HL) or low light (LL) and different concentrations of CO_2 (400 or 800 µatm pCO_2). Note the increase in NPQ under NH_4^+ growth especially under high light conditions.
Panel b: Comparison of response of different genes on NH_4^+ *vs* NO_3^- growth under different temperature and light conditions in the diatom *Thalassiosira pseudonana* from two independent studies, Parker and Armbrust (2005) and Shi et al. (2015). Note that the number of copies of glutamine synthetase II (GSII) was lower on NH_4^+ growth (ratio <1.0) in all cases except 22°C and low light (LL), and that the number of copies of the two genes involved in photorespiration, phosphoglycolate phosphatase (PGP) and T-protein subunit glycine decarboxylase (GDCT), were higher (ratio >1.0) in all cases under NH_4^+ growth, except low light, especially at 12°C.
Data were replotted from Shi et al. (in press, upper and lower panels) and Parker and Armbrust (2005, lower panel); panel b reproduced from Glibert et al. (in review).

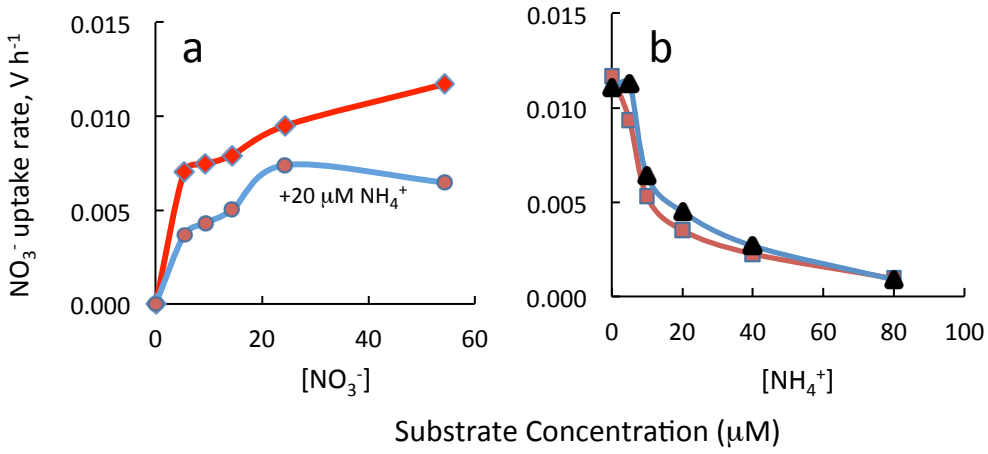


Figure 12. Two examples of effects of added NH_4^+ on rates of NO_3^- uptake by natural phytoplankton collected from the Sacramento River. Panel a: the change in kinetic relationships for NO_3^- uptake in the presence of an addition of 20 μM NH_4^+; panel b: the saturating rate of uptake of NO_3^- in the presence of increasing additions of NH_4^+ and incubated under high light (60% natural irradiance, triangles) and low light (15% natural irradiance, squares). All rates were determined using ^{15}N isotope tracer techniques.

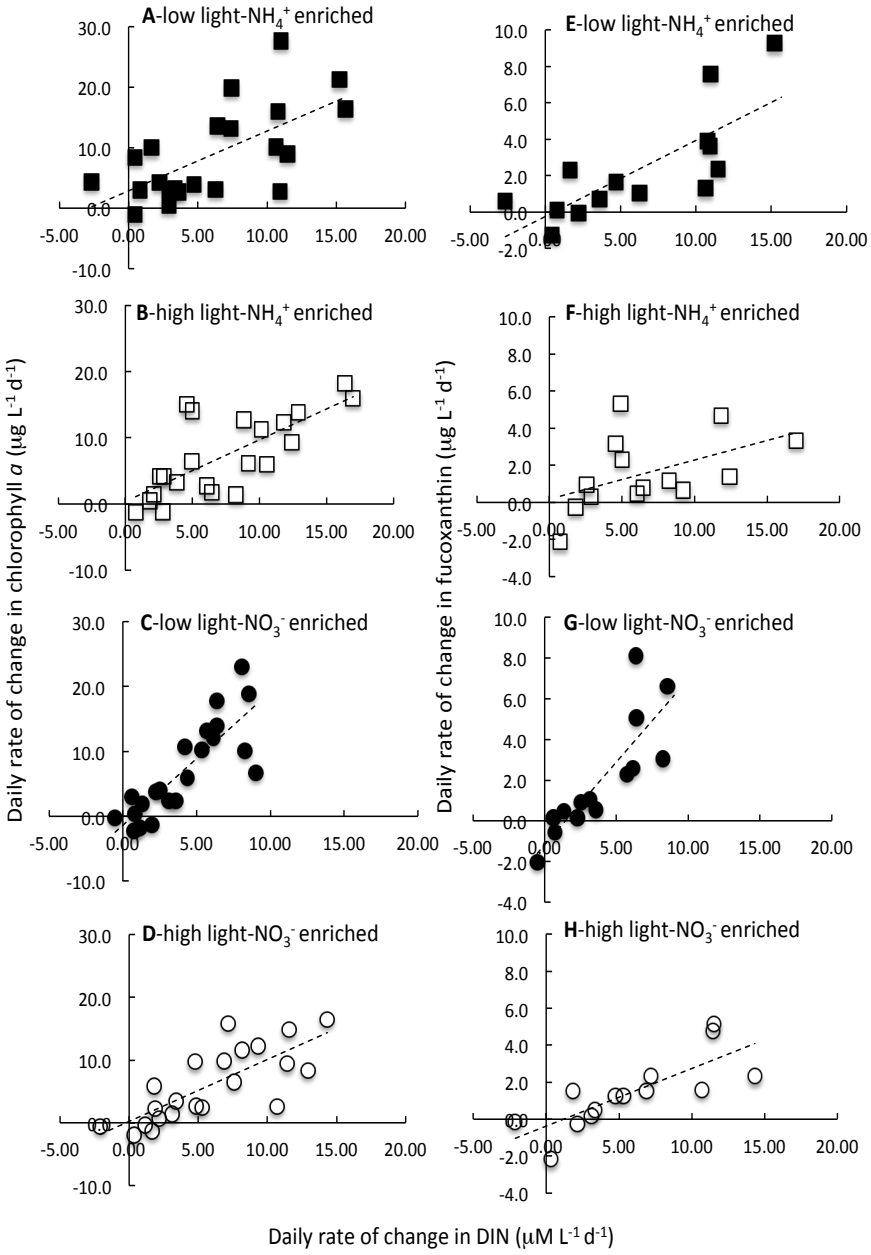


Figure 13. Daily rate of change in chlorophyll *a* (Panels A-D) and in fucoxanthin (panels E-H) in relation to daily rate of change in the dissolved inorganic nitrogen (DIN) concentration. Data are the composite of all N-enrichment experiments conducted on samples collected from the Sacramento River (Garcia Bend) and Suisun Bay (USGS4) in September 2011, 2012, and in March 2012 and 2013. Samples were enriched with NH_4^+ (panels A,B,E,F; squares) or NO_3^- (panels C,D,G,H; circles) and incubated at 15% of ambient irradiance (panels A,E,C,G; filled symbols) or 60% of surface irradiance (panels B,F,D,H; open symbols). Nitrogen enrichment levels and other experimental details are described in Glibert et al. (2014a). Lines represent linear regressions. Note that of all the pigments measured, only fucoxanthin is illustrated here.

Figure reproduced from Glibert et al. (2014a).

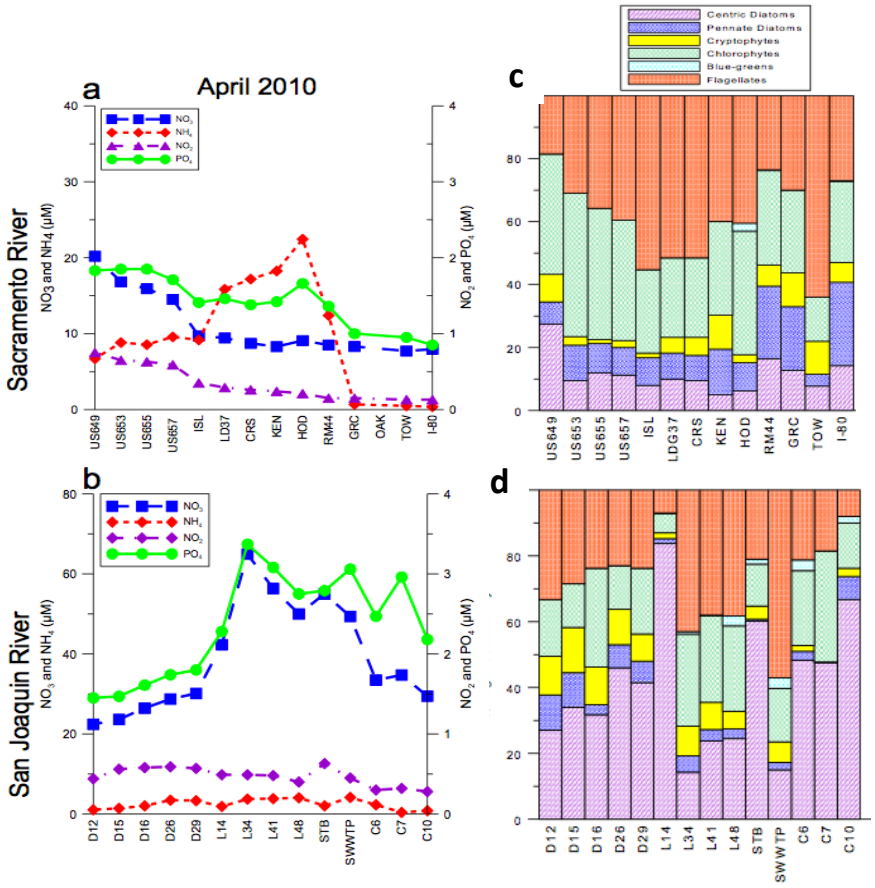


Figure 14. Concentrations of major inorganic nutrient concentrations along the Sacramento River (panel a), and the San Joaquin River (panel b) during April 2010, and the dominant phytoplankton groups along the same riverine transects (panels c, d). Note that the dominant form of dissolved inorganic nitrogen (DIN) in the Sacramento River was NH_4^+; in contrast NO_3^- dominated the San Joaquin River, with changing concentrations going downstream. Microscopic enumeration showed the Sacramento River community to be composed of 38% flagellates, 32% chlorophytes, 12% centric diatoms, 12% pennate diatoms (especially *Navicula*), 6% cryptophytes (dominated by Cryptomonas) and <0.5% blue-green algae. In contrast, centric diatoms (43%), including *Cyclotella* sp. and *Melosira* sp. dominated in the San Joaquin River followed by, flagellates (26%), chlorophytes (20%), cryptophytes (6%) pennate diatoms (4%) and 1% blue-green algae.
Figures reproduced from Kress (2012).

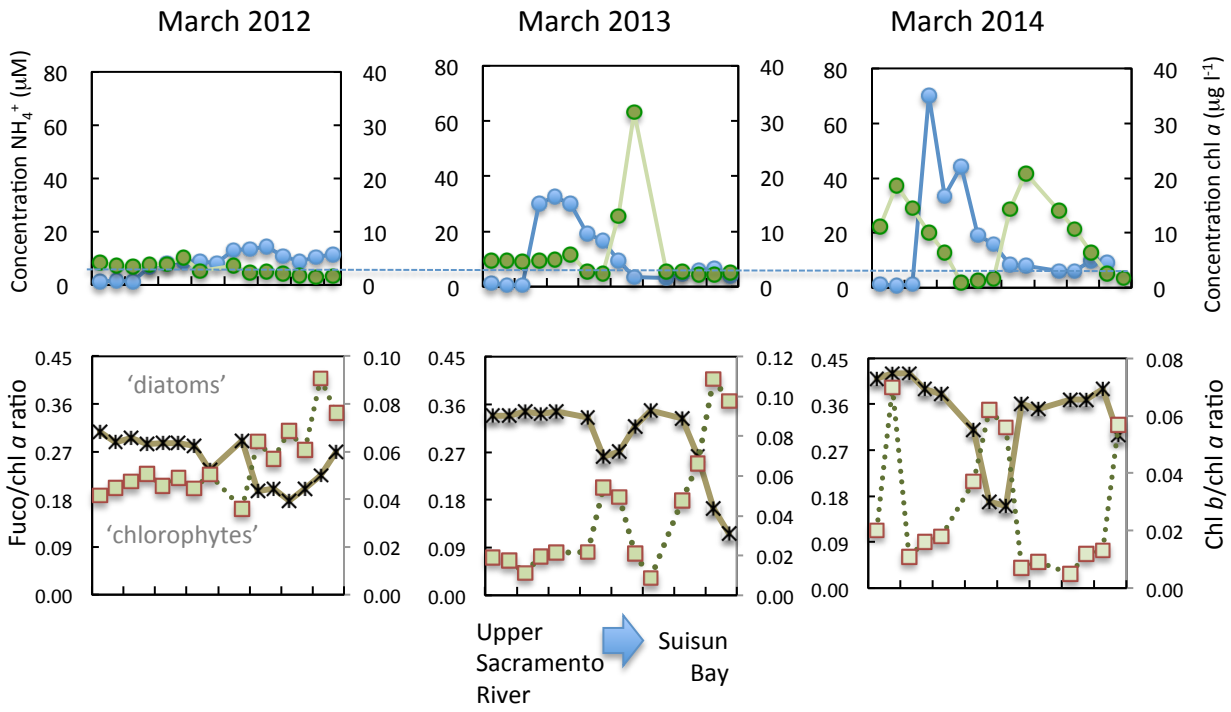


Figure 15. Upper panels: Comparison of the concentrations of NH_4^+ (blue circles) and chlorophyll *a* (green circles) along a transect from the upper Sacramento River (above the WWTP) to Suisun Bay for March 2012 (a high flow year), 2013 (a medium-low flow year) and 2014 (a very low flow year). Lower panels: corresponding estimates of diatom availability (crosses; as fucoxanthin/chlorophyll *a*) and chlorophyte availability (squares; chlorophyll *b*/chlorophyll *a*). Note the loss of diatoms several stations below the peak of the NH_4^+ suggestive of a time delay with respect to full repression. The horizontal line on the NH_4^+ panels represents ~4 µM, the value previously suggested by Dugdale et al. (2007) to be inhibitory for diatoms.

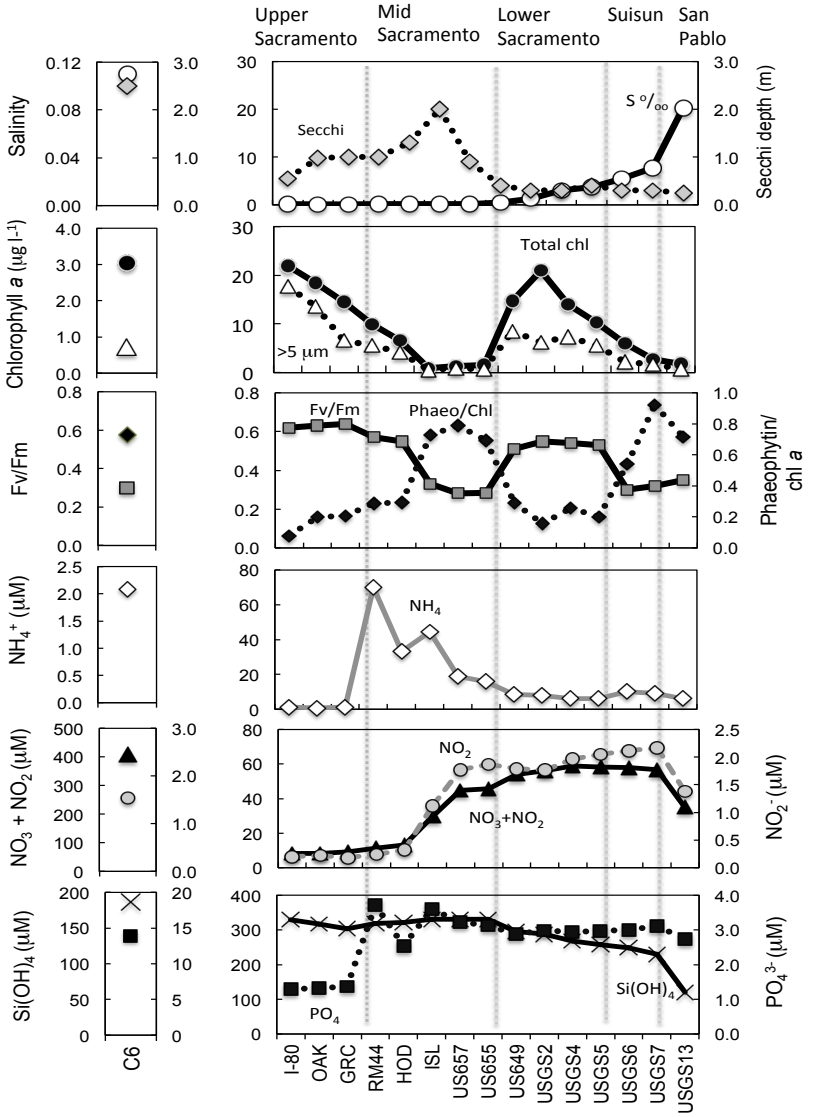


Figure 16. Measured parameters along a transect of the Sacramento-San Joaquin Bay Delta on March 24, 2014. Spatial changes in abiotic and biotic parameters measured in the San Joaquin River (site C6, small panels) and along a transect from the upper Sacramento River to San Pablo Bay (sites I-80 to USGS13) on March 24, 2014. Vertical dashed lines delineate various bay segments. Note the change in scales from the small panels depicting data for C6 and the larger panels depicting data for the other stations.
Figure reproduced from Glibert et al. (2014b).

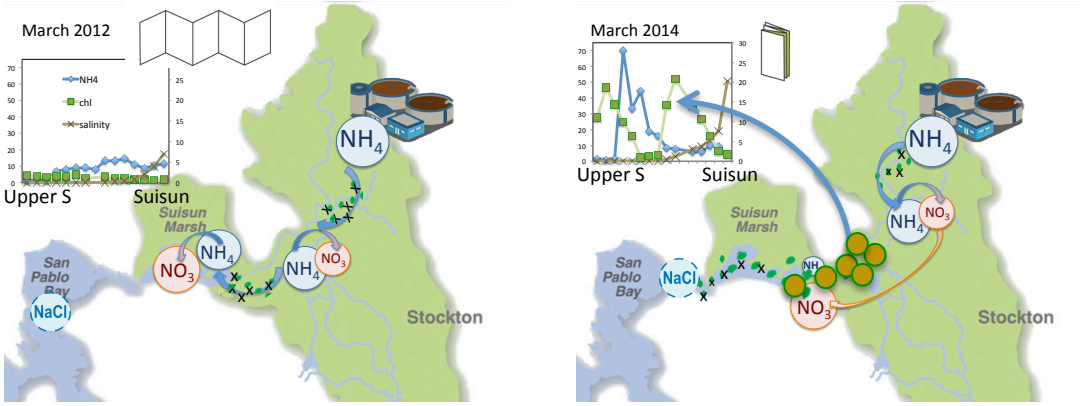


Figure 17. Comparison of the conceptual dynamics of the NH_4^+ and NO_3^- transformations along the spatial axis of the the river to bay. Note that during the comparatively high flow year (2012), when more dilution occurred, residence time was also low and a bloom was prevented, but during the very low flow year (2014), nitrification occurred further up the river and residence time was longer, so a bloom (shown by large circles) was able to be sustained in the lower Sacramento. Inset panels show the NH_4^+, chlorophyll *a* and salinity values along the transects from upper Sacramento River to Suisun Bay.
Right panel modified and reproduced from Glibert et al. (2014b).

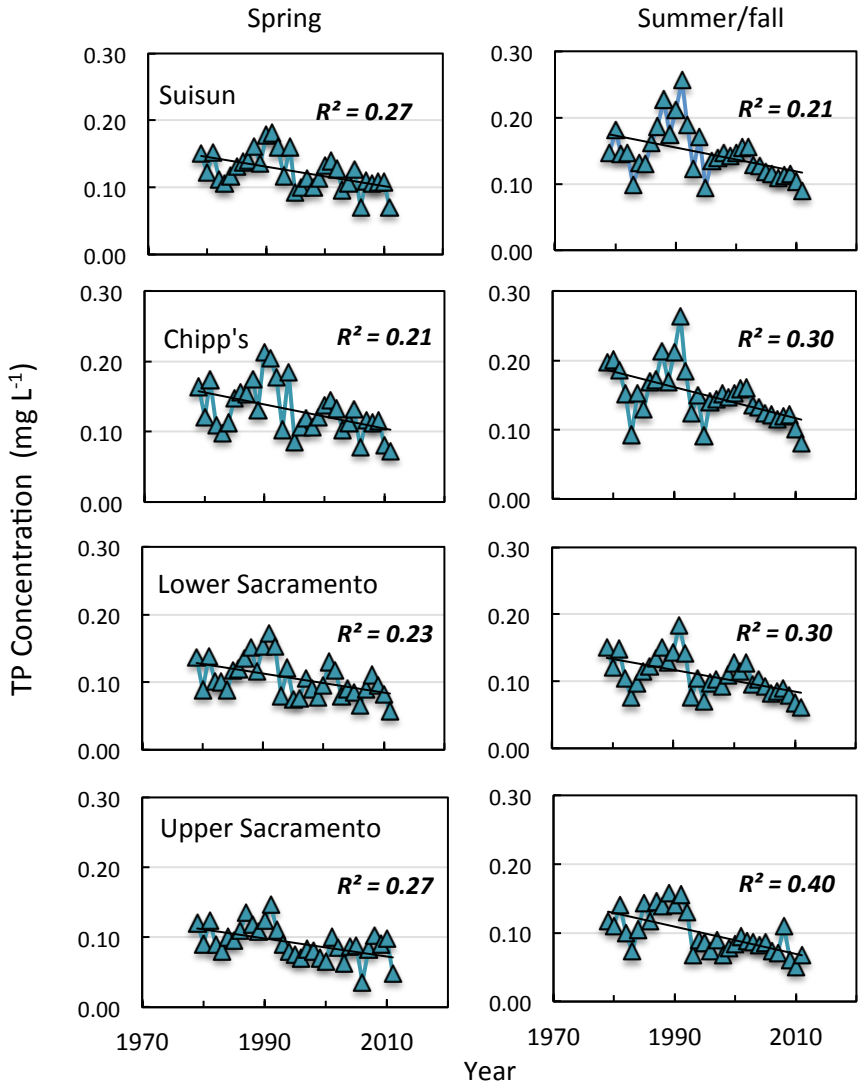


Figure 18. Concentrations of total P (TP) for Bay Delta regions indicated. Data are the average measured values for the months of March-June (left hand panels) and July-October (right hand panels). Coefficients of determination are given in the panels; all are significant at $p<0.01$ and are shown in bold, italic font.

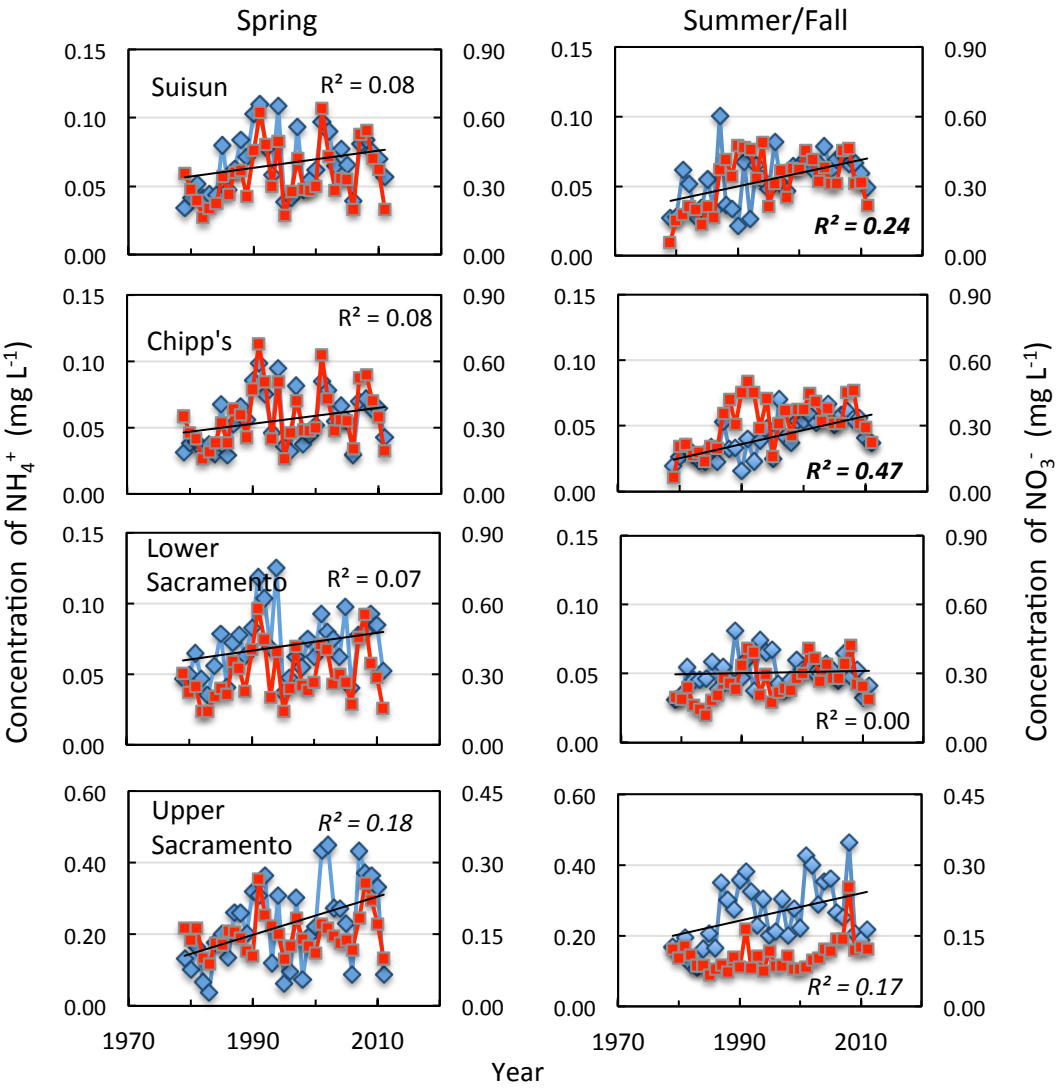


Figure 19. Concentrations of inorganic N (NH_4^+, blue diamonds; NO_3^-, red squares) for Bay Delta regions indicated. Note that the scale for NH_4^+ is the left axis and the scale for NO_3^- is the right axis and that the scale ranges change between panels. Data for spring are the average measured values for the months of March-June (left hand panels) and for summer/fall are July-October (right hand panels). Coefficients of determination for NH_4^+ with time are given in the panels; those that are significant at $p<0.05$ are shown in italic and those that are significant at $p<0.01$ are shown in bold, italic font. Note also that concentrations of NH_4^+ were generally higher in spring than in summer/fall.

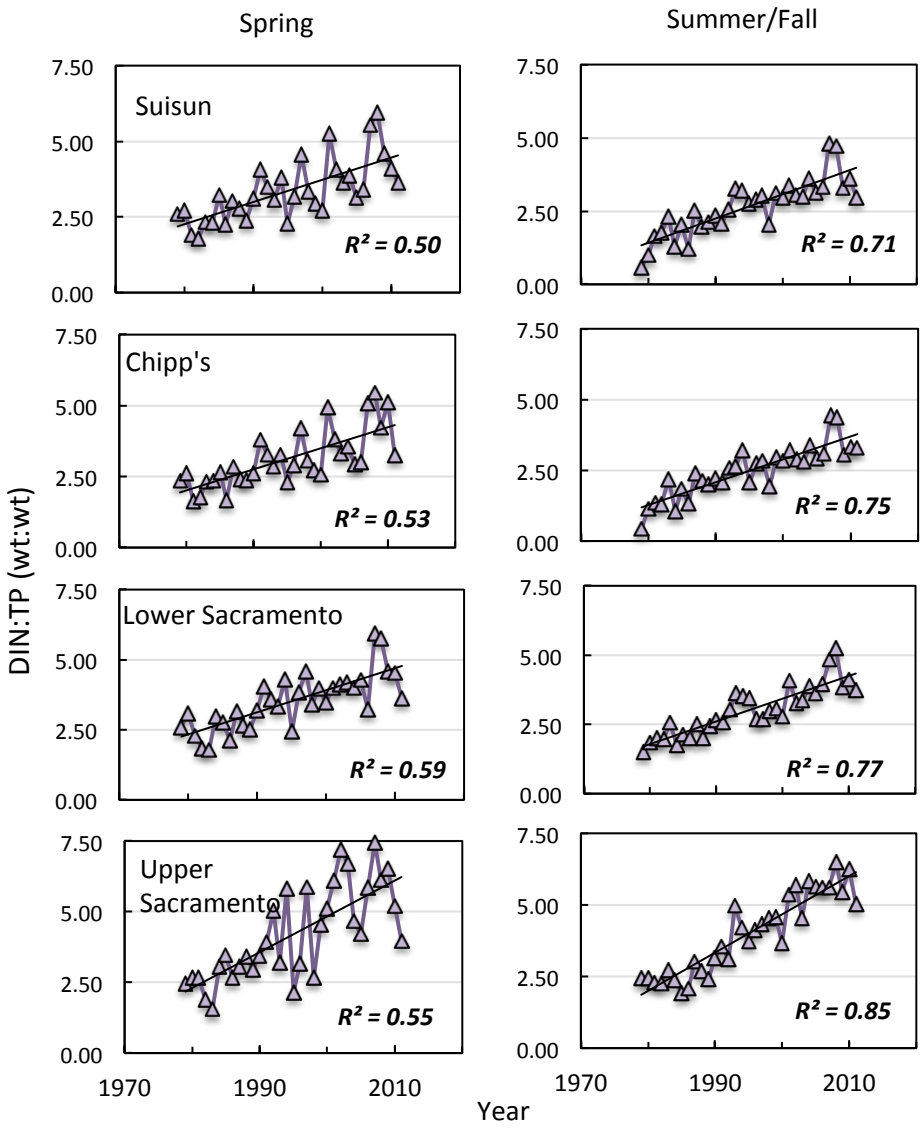


Figure 20. The ratio of inorganic N:total P for spring (March-June; left hand panels) and summer/fall (July-October; right hand panels) for stations sampled in the Bay Delta region indicated. Coefficients of determination of the trends with time are given in the panels. Note that all were significant at $p < 0.01$ (shown in bold italic font), but the coefficients in summer/fall were consistently higher than in the spring.

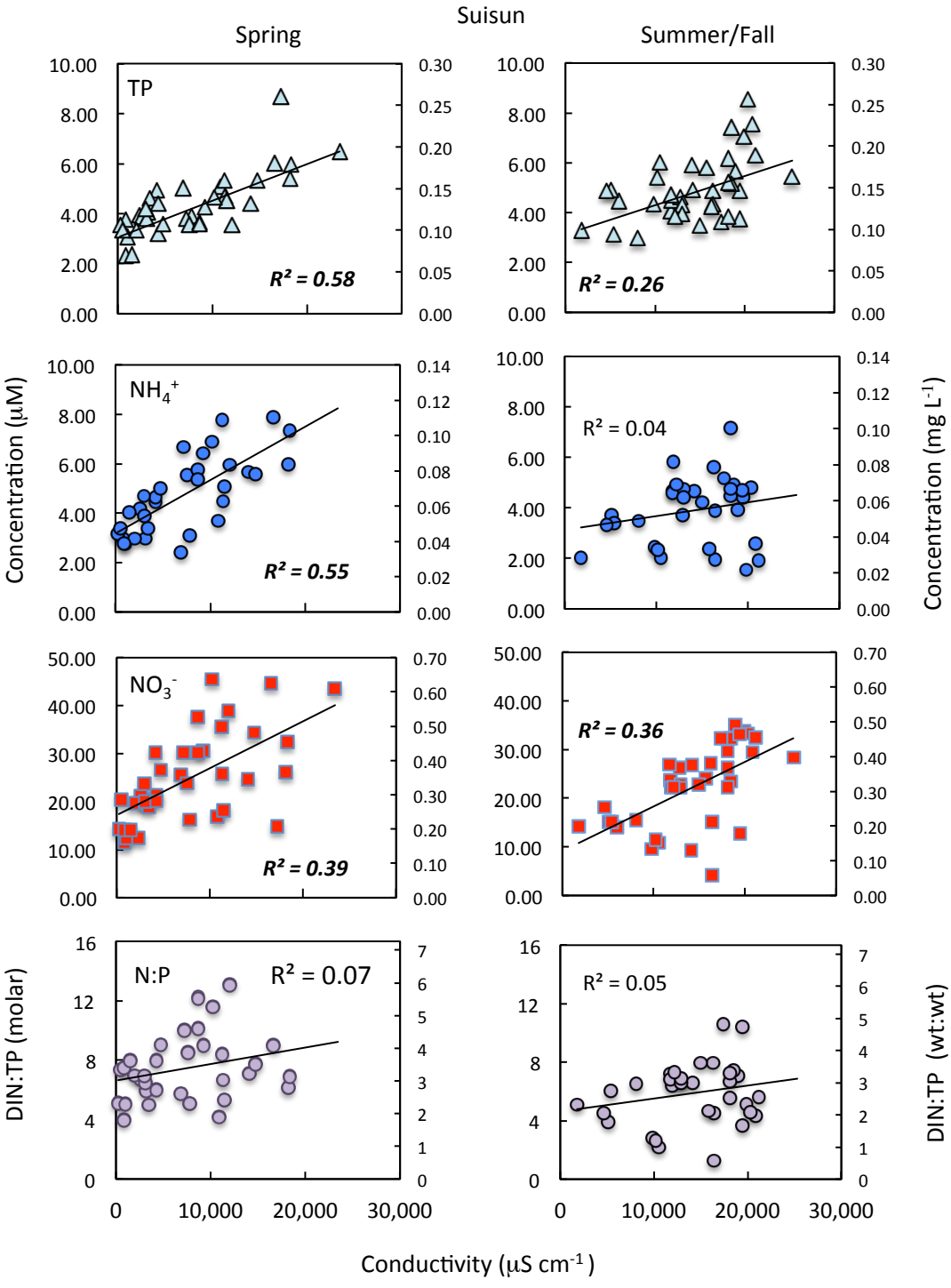


Figure 21. The concentrations of nutrients and their ratio and conductivity for spring (March-June; left hand panels) and summer/fall (July-October; right hand panels) for Suisun Bay. Note that the trend in conductivity over time has been for Suisun Bay to become fresher. Coefficients of determination of the trends with time are given in the panels. Those that were significant at $p<0.05$ are shown in italic font and those significant at $p<0.01$ are shown in bold, italic font. Note that the left hand axis gives the values in molar units and the right hand axis gives the same values in weight units.

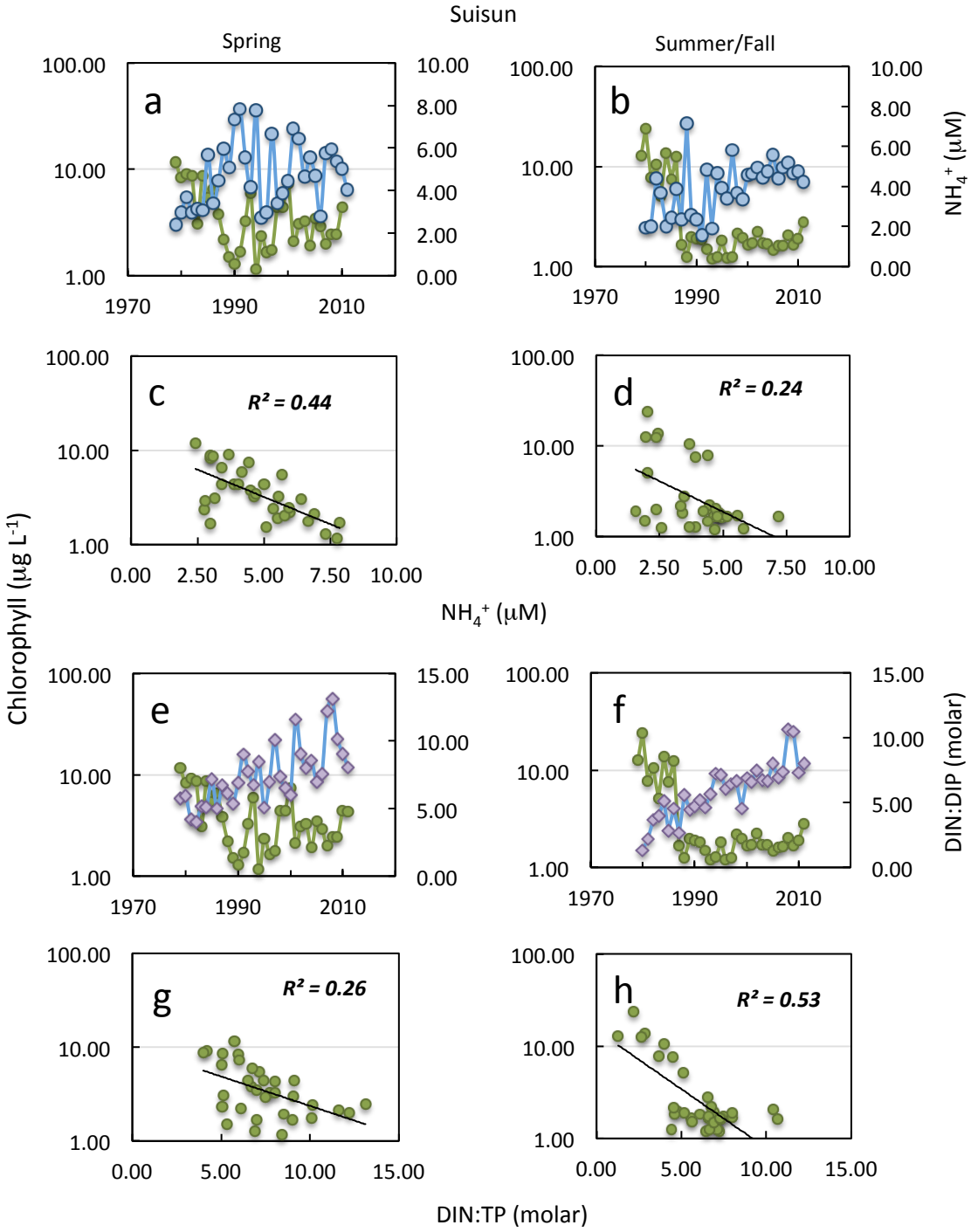


Figure 22. Panels a,b: trends in chlorophyll *a* (green circles) and NH_4^+ (blue circles) over time for data (1979-2011) from Suisun Bay in spring (March-June; left) and summer/fall (July-October; right). Panels c,d: the correlations between chlorophyll *a* and NH_4^+ for the same time period. Panels e-h: the same except that chlorophyll *a* is related to inorganic N:total P ratio (purple diamonds). All coefficients of determination were significant at $p<0.01$ (and shown in bold, italic font)

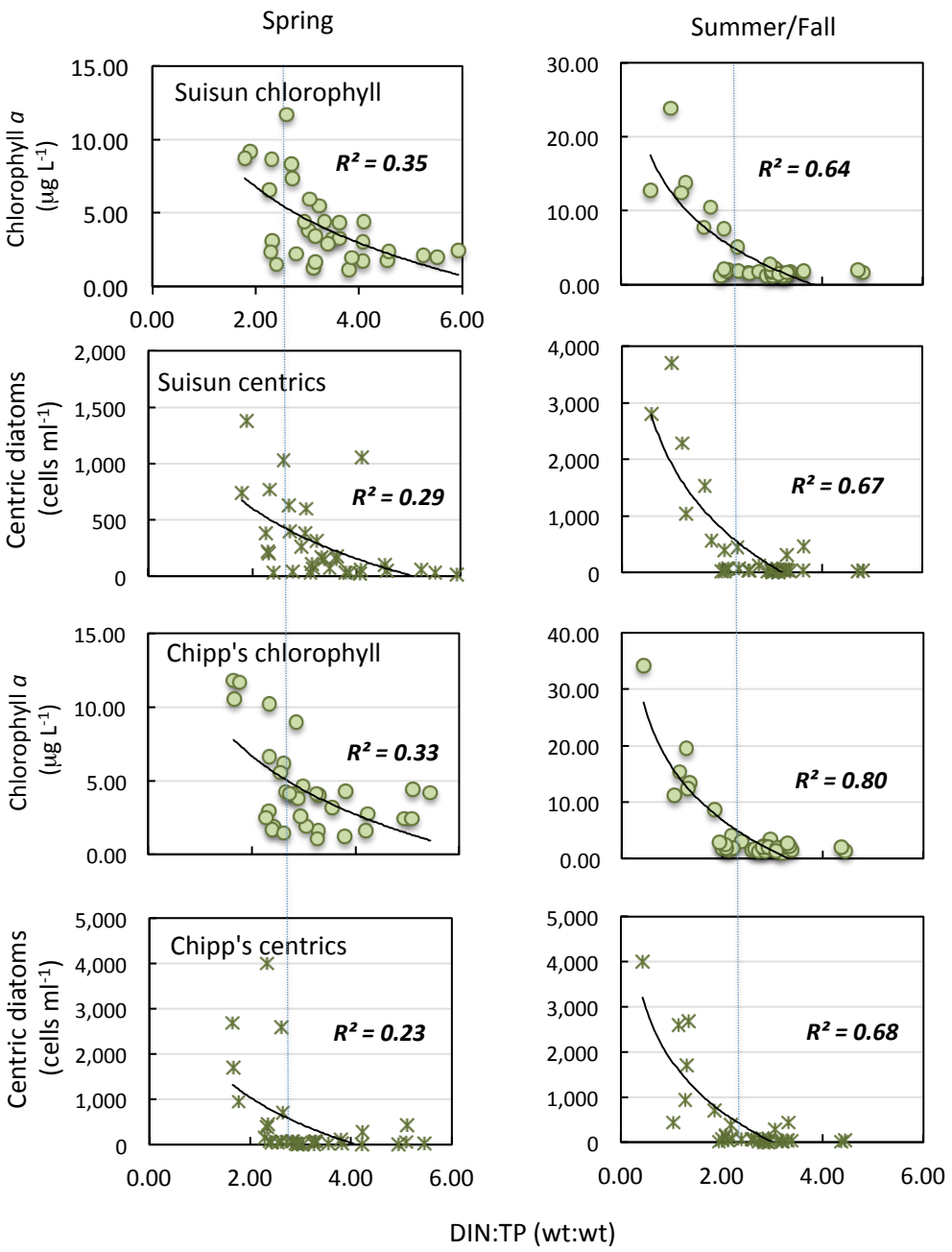


Figure 23. Relationship between chlorophyll *a* and centric diatoms and the inorganic N:total P ratio for two regions of the Bay Delta, Suisun Bay and Chipp's Island. Data encompass the period from 1979-2011. Spring values (left panels) are averages of data from March-June, and summer/fall values (right panels) are averages from July-October. The dotted vertical line approximately delineates values pre-1987 (left) from the post-1987 clam invasion period (right). Logarithmic regressions are given; they were significant at $p<0.01$ (and shown in bold, italic font). Note the much stronger relationships in the fall than the spring.

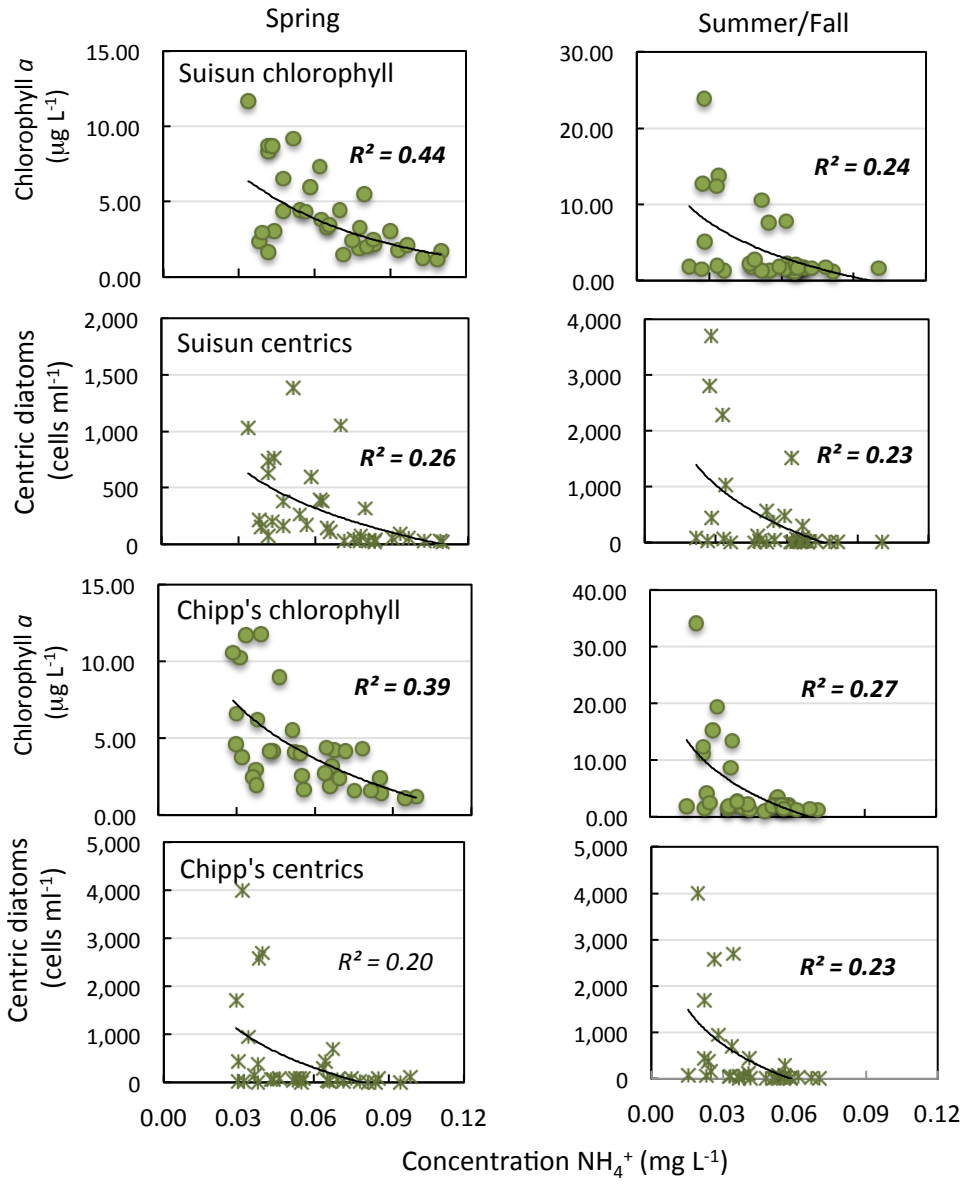


Figure 24. As for Figure 24 except for NH_4^+. All were significant at $p<0.01$ (and shown in bold, italic font) except Chipp's centrics in the spring which was significant at $p<0.05$ (and shown in italic font).

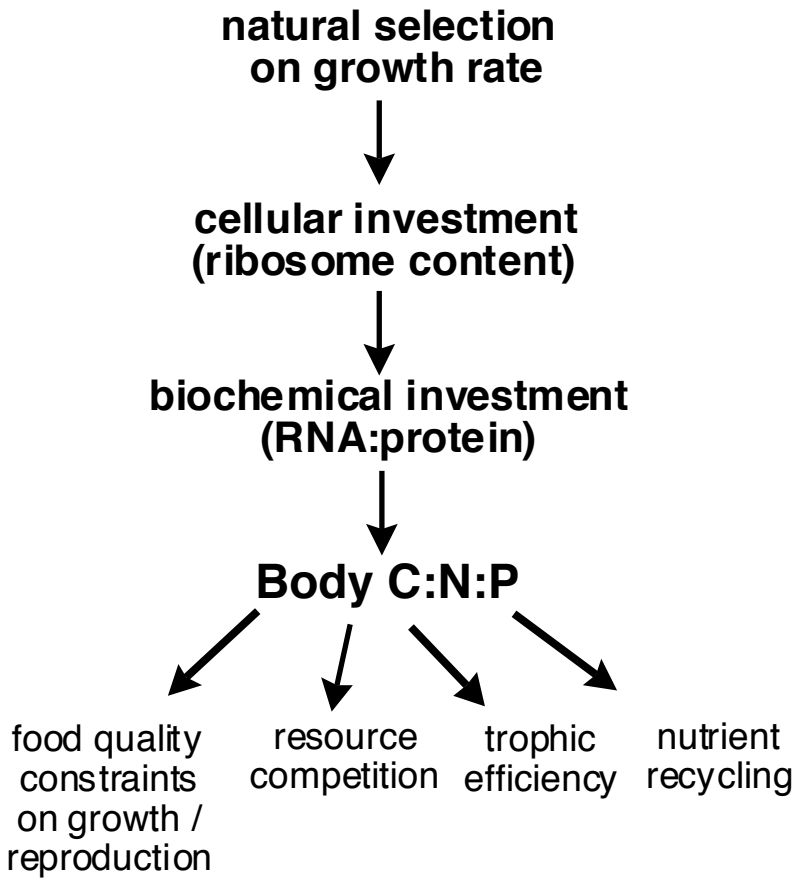


Figure 25. Conceptual illustration of the Growth Rate Hypothesis that has been proposed to explain why different taxa differ in their relative P demands. This biochemical investment affects body stoichiometry and sets constraints on growth, resource competition, and, in the case of animals, trophic efficiency and nutrient recycling.
Figure from Elser et al. (2000).

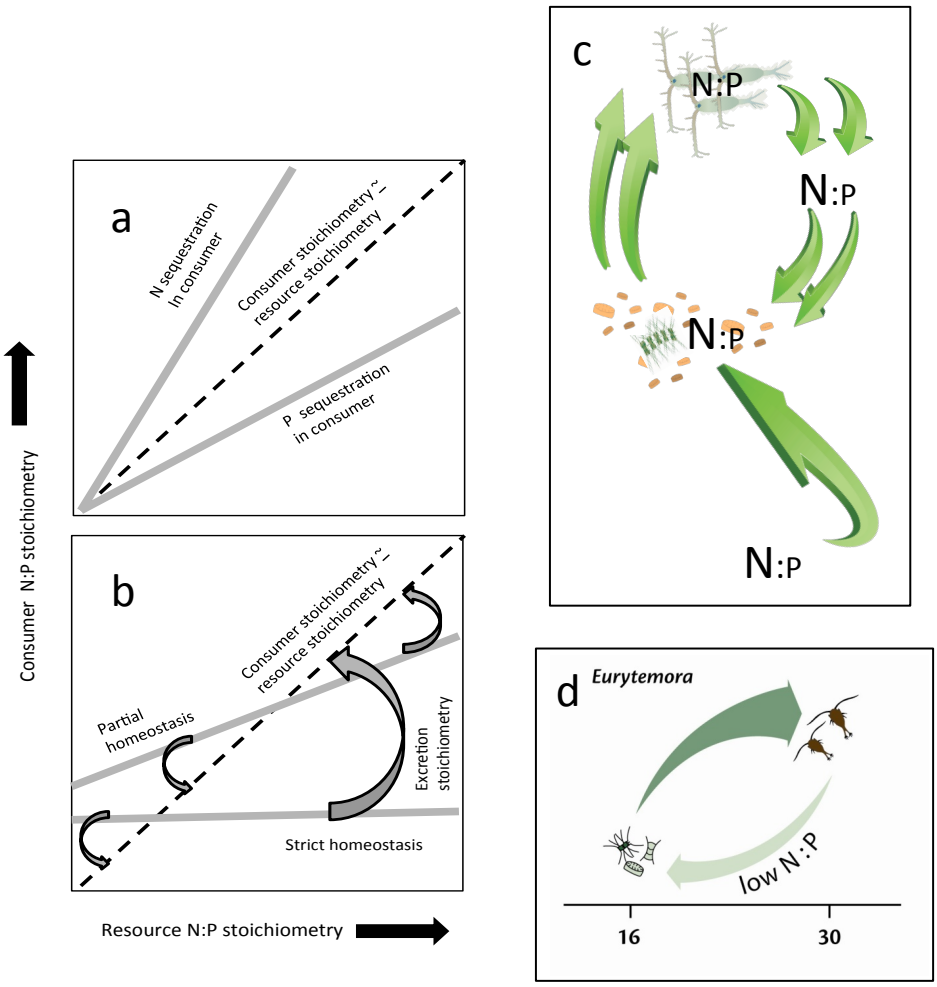


Figure 26. Panels a,b: Schematic relationships between resource N:P (either dissolved nutrients or prey) and consumer N:P. The dashed line in both panels represents the hypothetical situation in which the consumer N:P matches that of its resource. (a) Hypothetical situations in which the consumer is either N or P enriched relative to its resource in a constant proportion. (b) Hypothetical situations where the consumer either partially or strictly regulates its biomass N:P regardless of the N:P of its resource. The arrows depict the extent to which the excreted or released nutrients differ in N:P from that of the consumer biomass N:P. Excretion N:P is expected to be negatively related to substrate N:P when the consumer N:P is constrained. Panel c: Conceptual diagram of the ecological stoichiometric relationship between a change in nutrient input, phytoplankton and zooplankton and their release products. Panel d: Illustration of this phenomenon for *Eurytemora*, which may eat phytoplankton with a lower N:P and sustain their nutrient availability through excretion of low N:P products. The wide arrow represents ingestion of the phototrophs by the grazer; the lighter arrow represents nutrient regeneration in the grazer's excretion.
Left and lower right panels reproduced from Glibert et al. (2011).

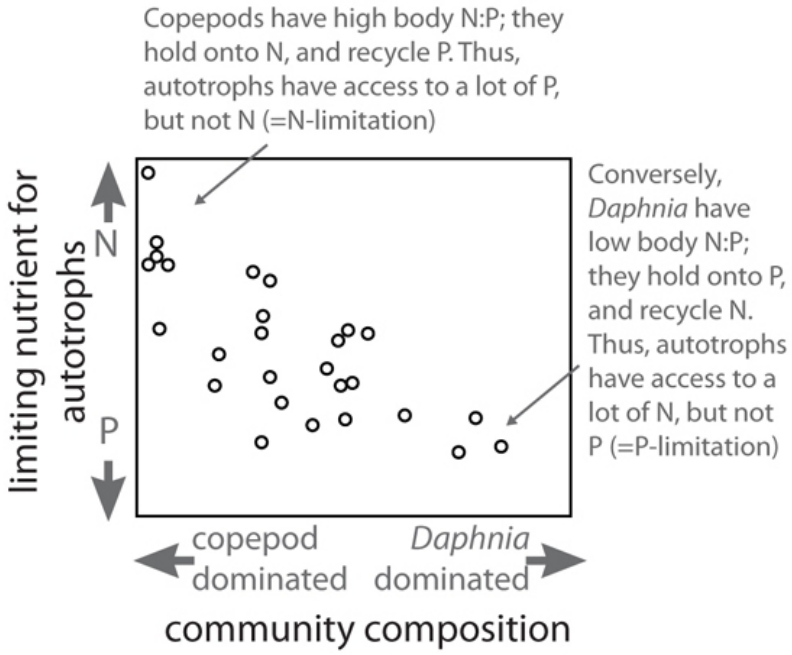


Figure 27. Example of the effect of the differences in elemental composition of different consumer communities on the availability of nutrients for autotrophs.
Figure reproduced from Cease and Elser (2013).

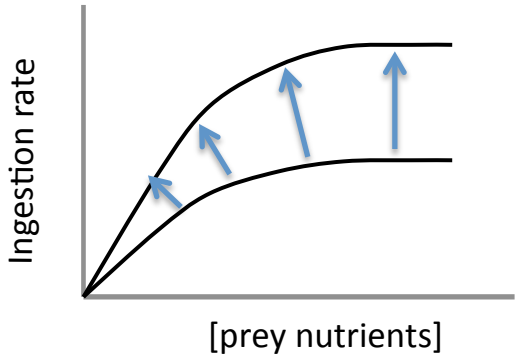


Figure 28. Relationship between ingestion rate and prey nutrients and the directionality of change in this relationship if an organism increases its efficiency of prey acquisition and its maximal rate of ingestion. Note the similarity to Figure 2a.

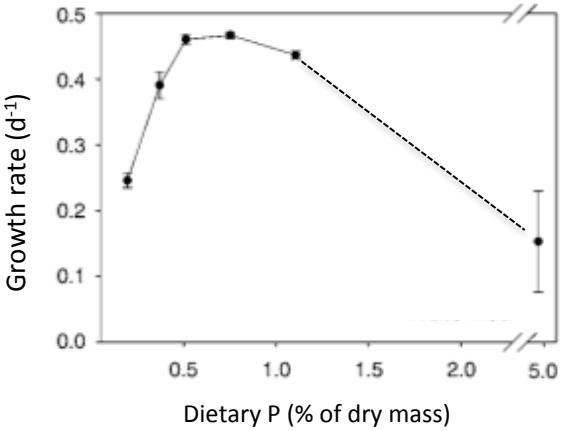


Figure 29. Example of the effect of dietary P on the growth rate (GR) of *Daphnia magna* cultures in the laboratory with algae enriched with varying amounts of P. Note the reduction in growth rate at high P levels (dashed line added for emphasis). Data originally from Plath and Boersma (2001).
Figure reproduced from Boersma and Elser (2006).

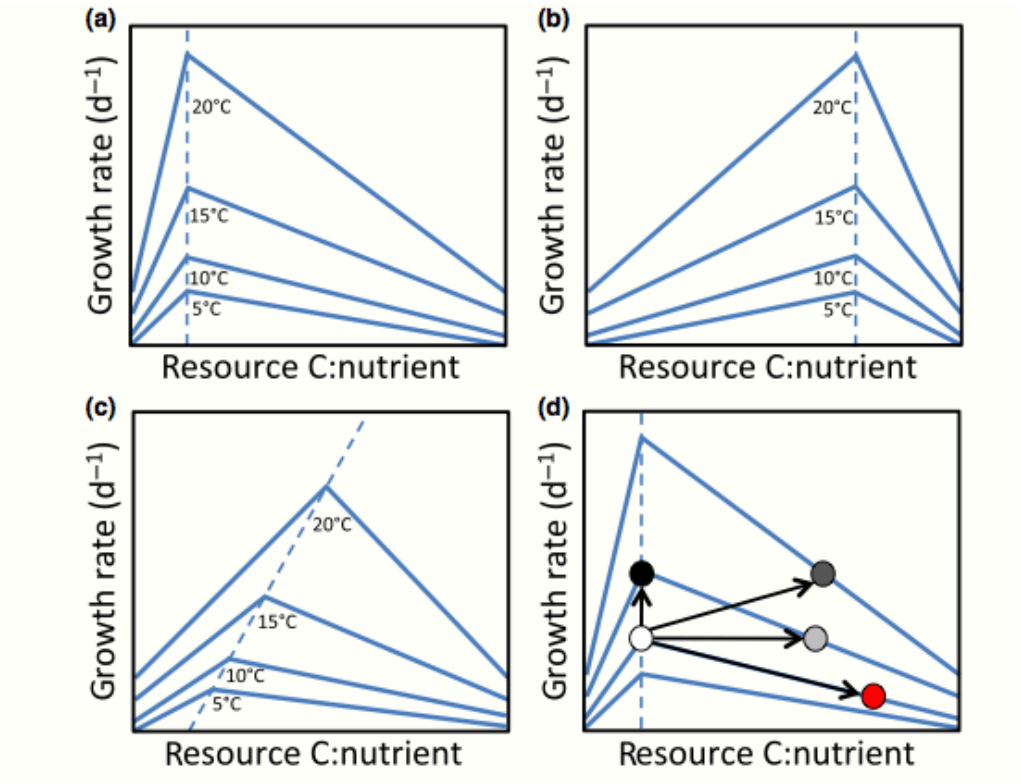


Figure 30. Predicted effects of temperature and food quality (i.e., C:nutrient ratios) on consumer growth rates. Resource C:nutrient ratio is depicted as having a linear effect on growth – up to a maximum (i.e. straight lines in figure), while the effects of temperature are exponential (i.e. increased spacing between lines as temperatures increase). Panels (a) and (b) show predicted response surfaces for consumers with relatively low (a) and high (b) threshold elemental ratio (TER) values (dashed lines). In these panels, TER is assumed be constant across temperatures. Panel (c) depicts an alternative response surface in which the TER increases with temperature. Panel (d) shows predicted changes in growth for a consumer exposed to a change in temperature with no change in food quality (white circle to black circle), a change in food quality with no change in temperature (white circle to red circle), and simultaneous changes in both temperature and food quality (white circle to gray circles). In these scenarios, the initial condition (white circle) is at the consumer's TER at 10 °C, that is, the food quality that produces optimal growth at that temperature. Figure reproduced from Cross et al. (2015).

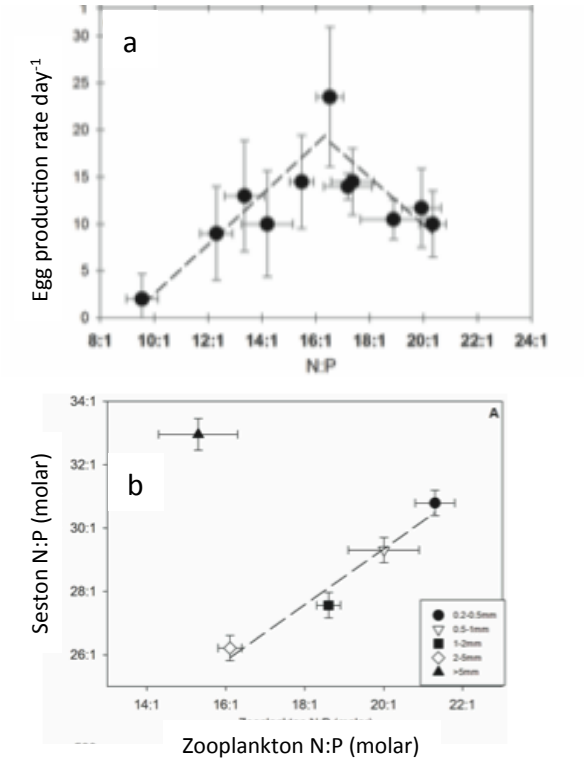


Figure 31. Panel a: Relationship between the molar N:P ratio of *Rhodomonas salina* and the egg production rate of *Temora longicornis.* The dashed lines represent the regression lines. All errors are ± SD.
Panel b: Relationship between the N:P ratio of size-fractionated zooplankton and the corresponding food N:P ratio in the year when the biomass of that zooplankton size-fraction reached a maximum. Data from 1994 to 2010. Errors are ± SE.
Figures reproduced from Nobili et al. (2013).

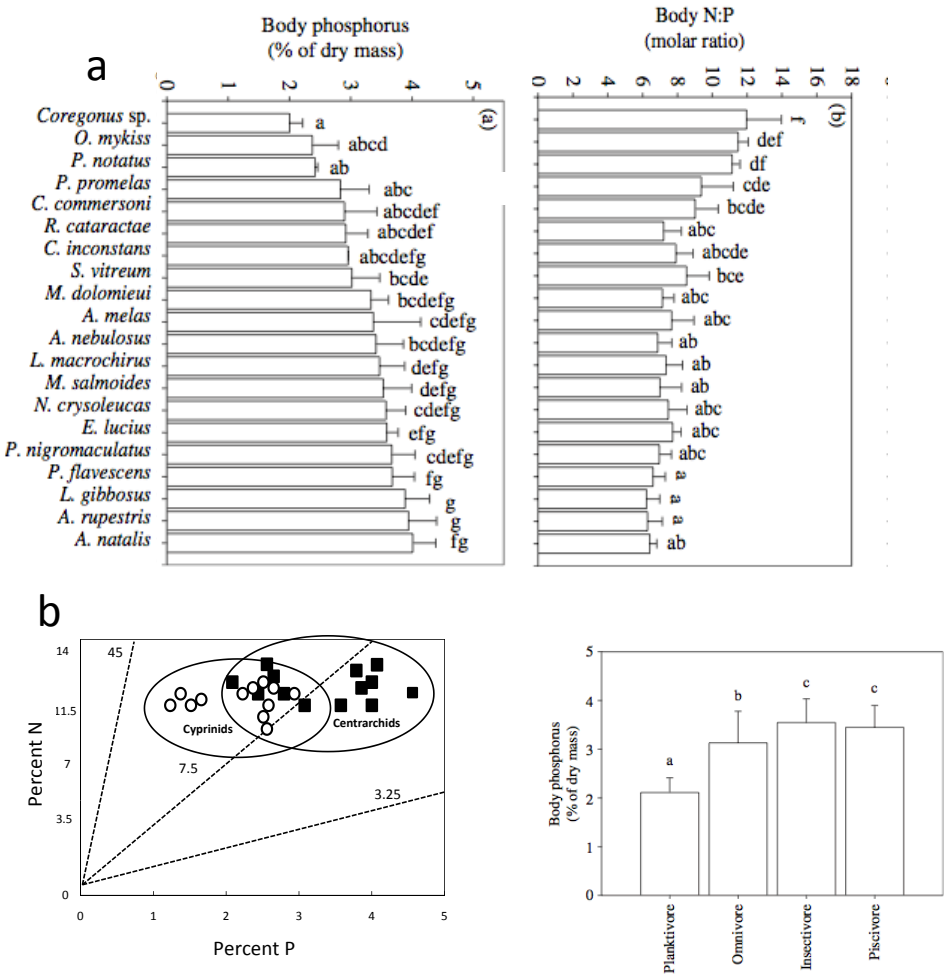


Figure 32. Panel a: Mean whole body P and N:P for various fish species: *Oncorhynchus mykiss, Pimephales notatus, P. promelas, Catosstomus commersoni, Rhinichthys cataractae, Clueae inconstans, Stizostedion vitreum, Micropterus dodomieui, Ameiurus nebulosus, Lepomis macrochirus, Micropterus salmoides, Notemigonus crysoleucas, Esox lucius, Pomoxis nigromaculatus, Perca flavescens, Lepomis gibbosus, Ambloplites ruppertris, Ameriurus natulus.* Similar lowercase letters indicate homogenous groups (Tukey's HSD test). Note that N:P was low in high-P fishes, ranging from ~6 in centrarchids [e.g., *Pomoxis nigromaculatus* (Lesueur), *Lepomis gibbosus* (L.) and *Ambloplites rupestris* (Rafinesque)] to ~12 in salmonids [e.g., *O. mykiss*].
Panel b: Relative N and P content of cyprinid and centrarchid fish. The dashed lines give three N:P ratios for perspective.
Panel c: Body phosphorus (P) as a function of feeding type. In all panels, values are means ± standard deviation and similar lowercase letters indicate homogenous groups (Tukey's HSD test).
Figures a and c reproduced from Hendrixson et al. (2007); Figure b is modified and redrawn from Sterner and George (2000).

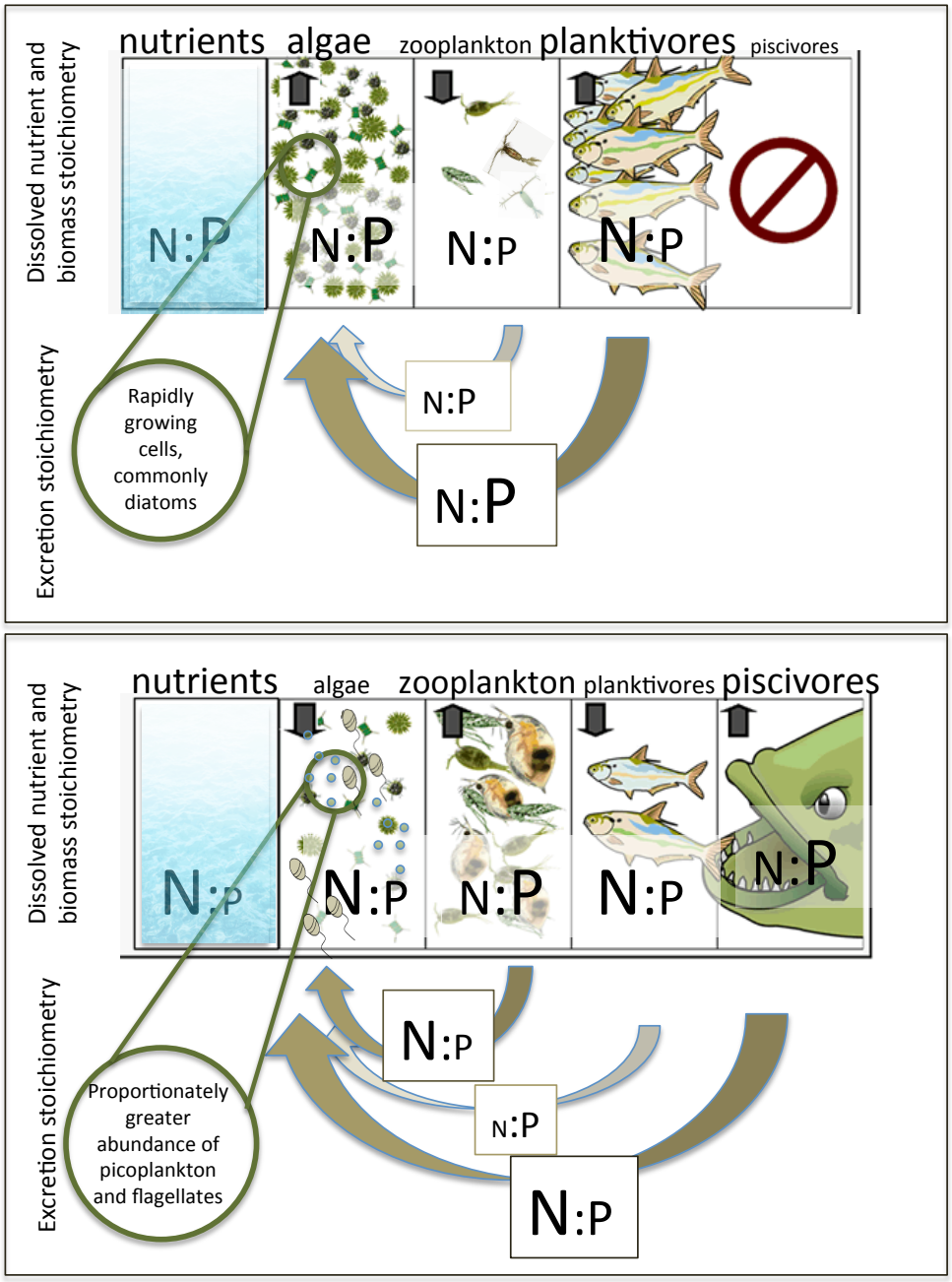


Figure 33. Theoretical trophic cascades and their interactive effects with the proportion of N:P in dissolved nutrients and biomass of different trophic groups. Upper panel depicts a trophic cascade in which there is a virtual absence of piscivores and planktivores dominate. Such a system is maintained under a condition of comparatively low N:P. Lower panel depicts a trophic cascade with high piscivorous grazing pressure (such as largemouth bass). Such a system is maintained under a condition of comparatively high N:P. The variable proportions of N:P in excretion products (consumer-driver stoichiometry) as well as new inputs of N or P are thought to result in these different stable state conditions. Background diagram of trophic cascades modified from http://www.lmvp.org/Waterline/fall2005/topdown.htm.

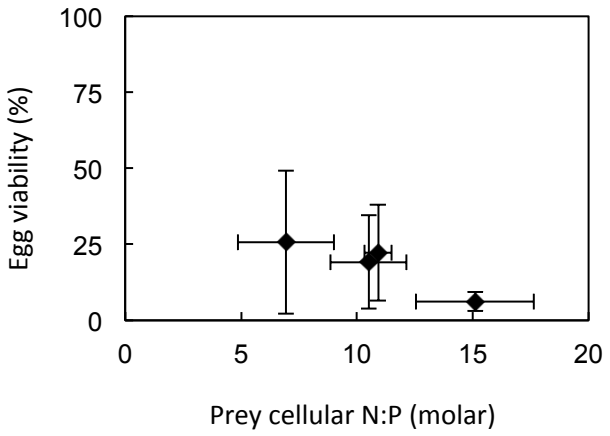


Figure 34. Egg viability of *E. carolleeae* measured over 48 h following 7 days of exposure to prey (the diatom *Thalassiosira pseudonana*) that was grown to have varying cellular N:P ratios. In all conditions prey were provided at the same amount in terms of C. Values are means ± standard deviation.
Reproduced from Bentley et al. (in review).

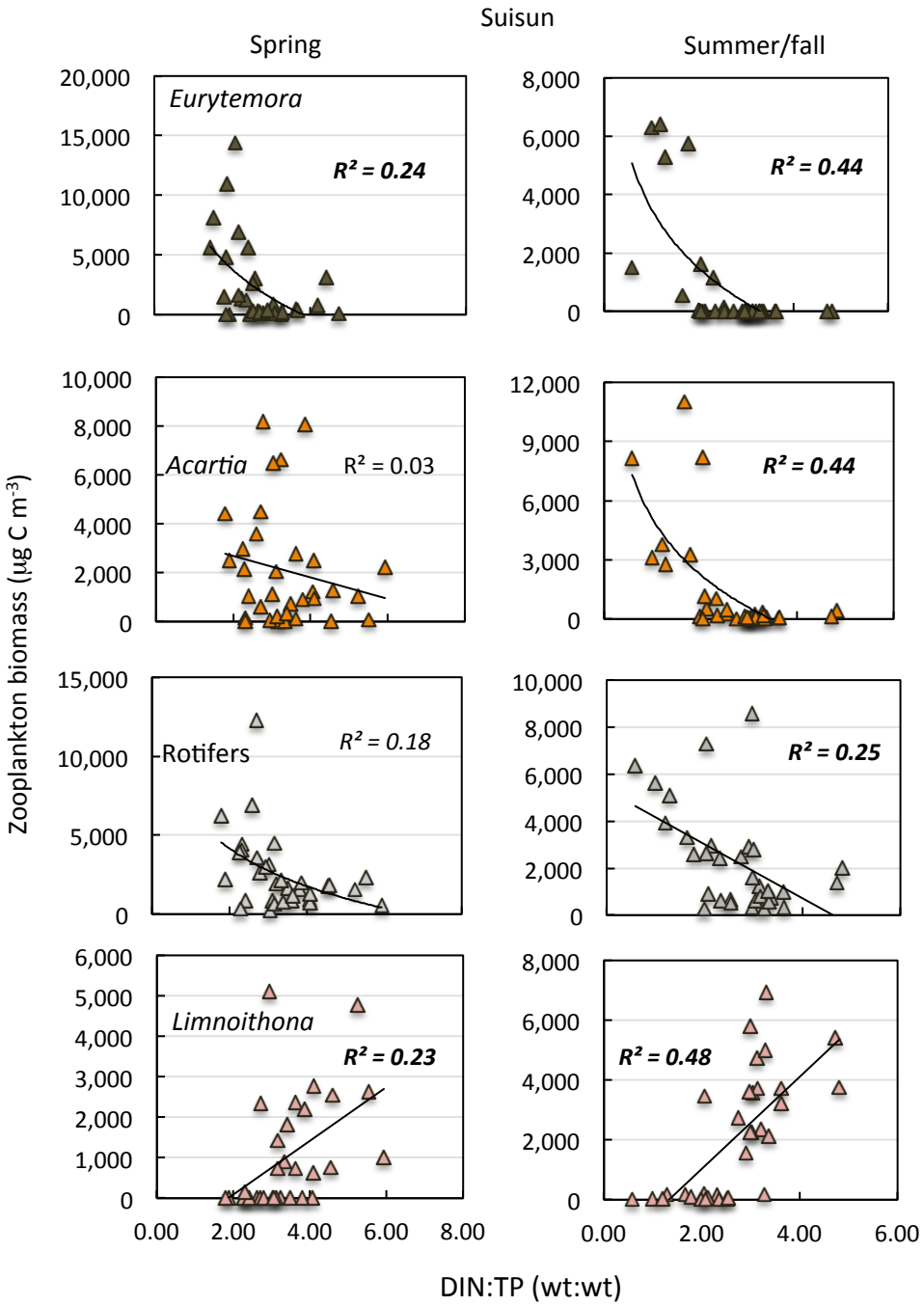


Figure 35. Relationship between zooplankton biomass for the taxa indicated and the inorganic N:total P ratio for Suisun Bay for spring (March-June) and summer/fall (July-October). Data encompass the period from 1979-2011. Correlations that were significant at $p<0.01$ are shown in bold, italic font and those significant at $p<0.05$ are shown in italic font). Note the much stronger relationships in the fall than the spring.

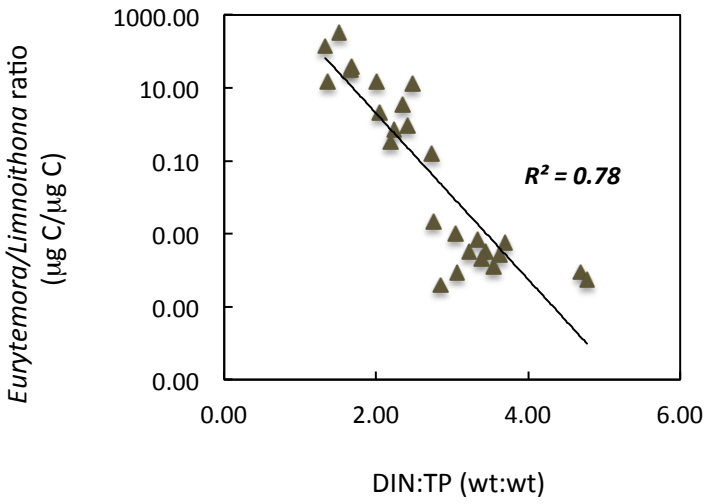


Figure 36. Comparison of the ratio of *Eurytemora/Limn*oithona and the ratio of dissolved inorganic nitrogen:phosphorus (DIN:TP) for the period from 1979-2011. Data are the averages for sites in Suisun Bay, near Chipp's Island, and in lower Sacramento River for the late summer/fall months (July-October). The coefficient of determination was significant ($p<0.01$).

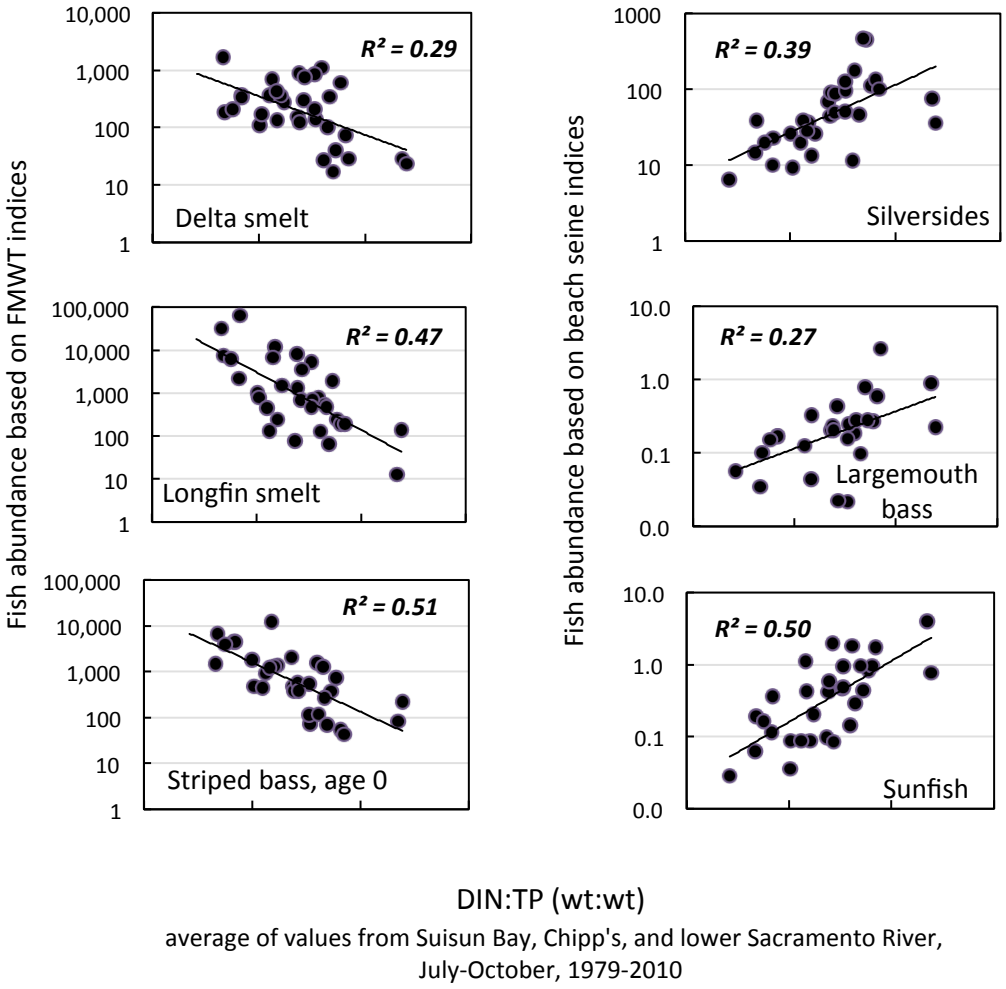


Figure 37. Relationships between abundance of several fish species and the inorganic N:total P ratio of the water column. Fish abundances in the left-hand panels are based on fall midwater trawl (FMWT) indices, and those in the right-hand panels are based on the beach seine index. All nutrient data are the averages of samples collected within Suisun Bay, Chipp's Island, and lower Sacramento River regions of the Bay Delta between the months of July and October. The log coefficients of determination were all significant at $p < 0.01$ (shown in bold, italic font).

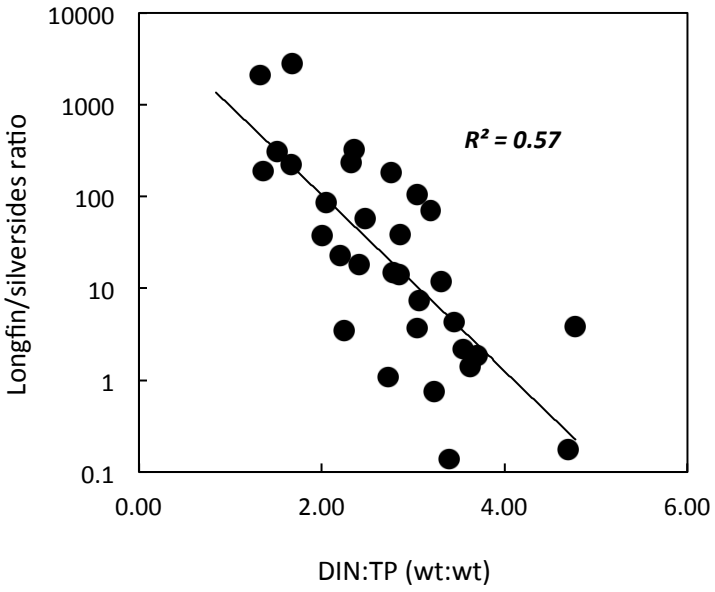


Figure 38. Comparison of the ratio of the abundance of longfin smelt to silversides in relation to the ratio of dissolved inorganic nitrogen:phosphorus (DIN:TP; triangles) for the period from 1979-2011. The coefficient of determination was significant ($p<0.01$).

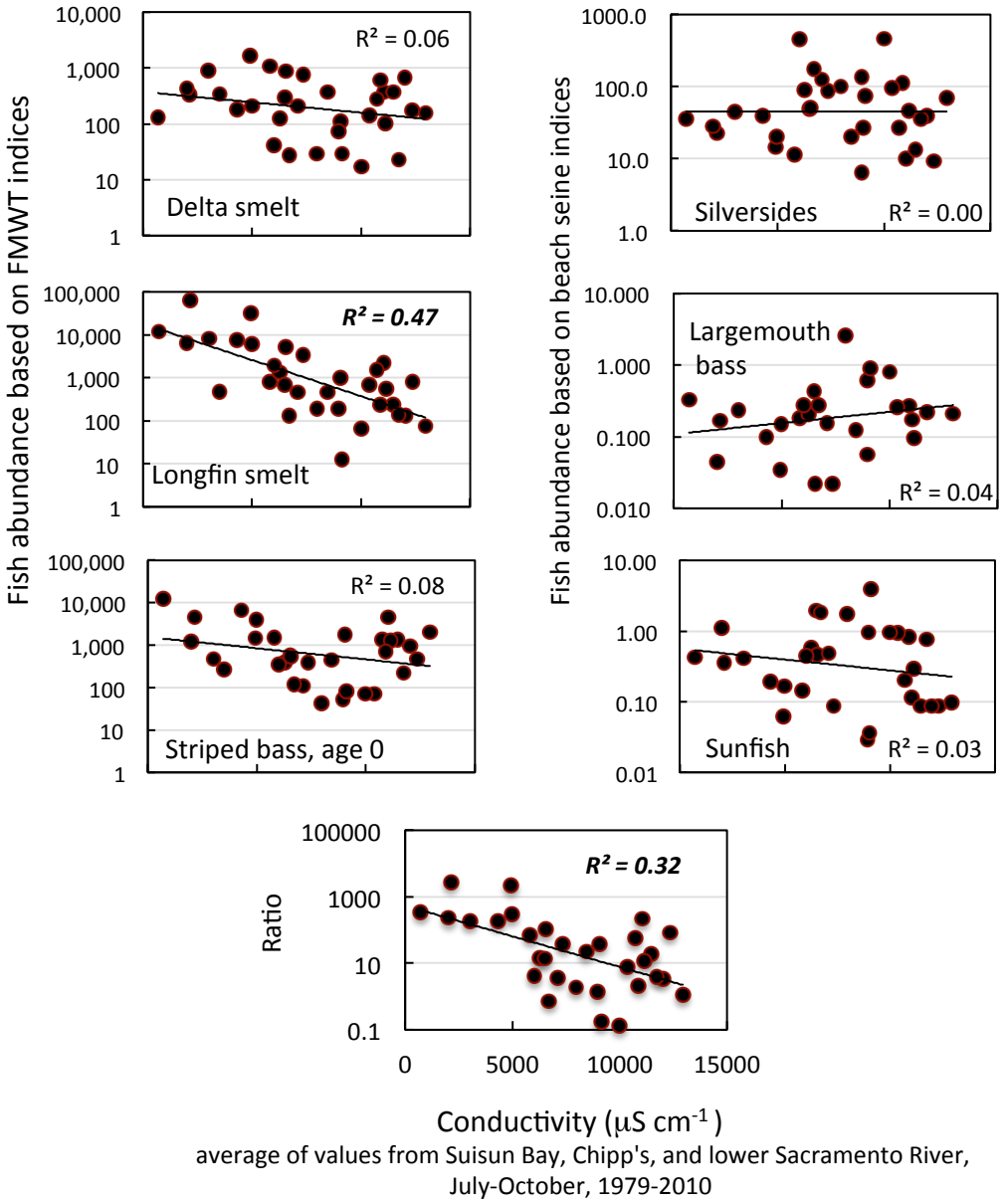


Figure 39. Relationships between abundance of several fish species and conductivity of the water column. Fish abundances in the left-hand panels are based on fall midwater trawl (FMWT) indices, and those in the top three right-hand panels are based on the beach seine index. All conductivity data are the averages of samples collected within Suisun Bay, Chipp's Island, and lower Sacramento River regions of the Bay Delta between the months of July and October. The lower panel gives the ratio of longfin smelt to silversides. Only those log coefficients of determination shown in bold, italic font were significant at $p<0.01$; note that there were fewer significant correlations than with DIN:TP.

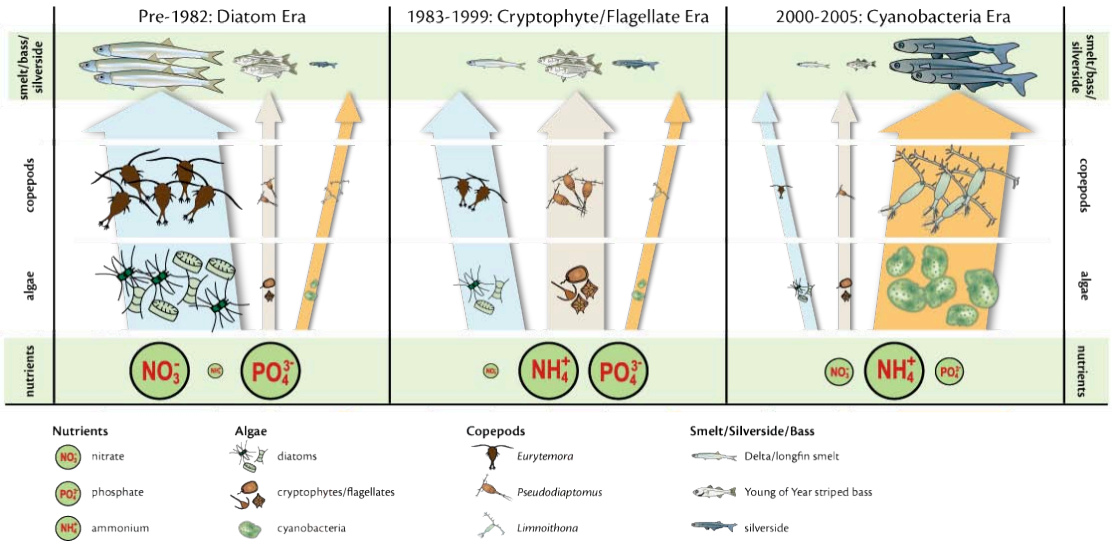


Figure 40. Conceptual diagram of some of the hypothesized changes in the food chain from phytoplankton to fish that have occurred in the Sacramento-San Joaquin Estuary over the past 30 years. Each of these hypothesized food chains has different dominant nitrogen forms or amounts relative to phosphorus. This conceptual model is intended simply to highlight some of the major flows of energy and materials and does not include all organisms, pathways or flows. The size of the symbols is meant to infer relative importance.
Reproduced from Glibert (2010).

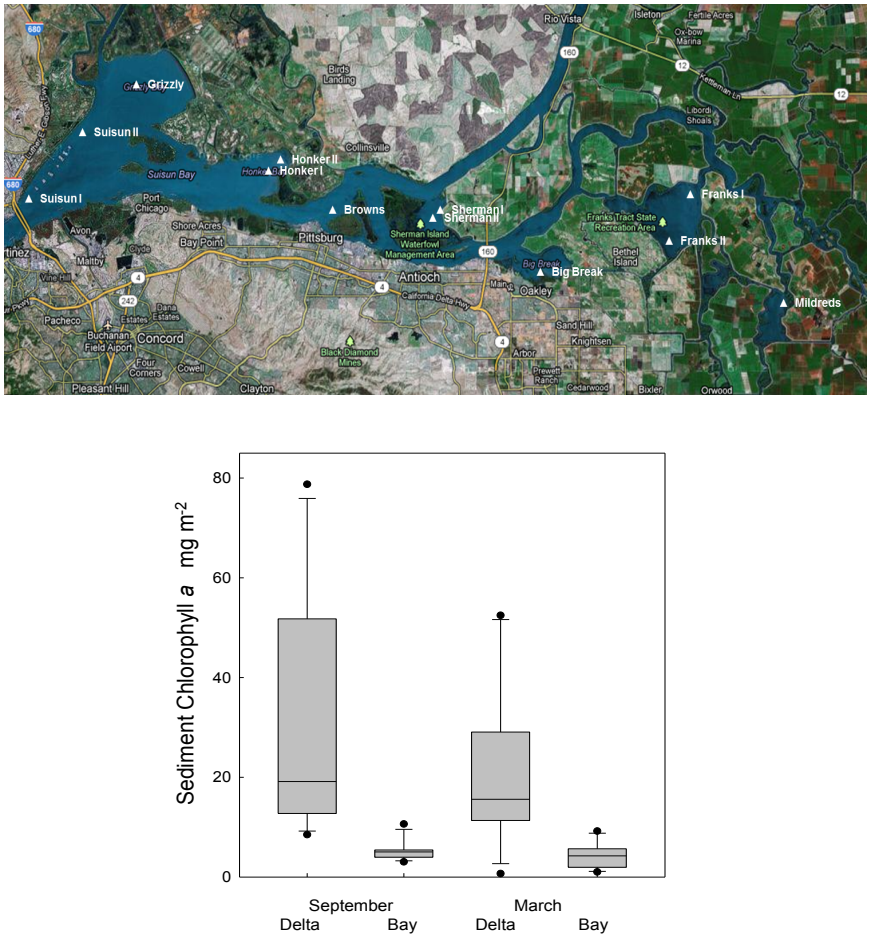


Figure 41. Upper panel: Sampling sites for sediment flux studies conducted along the transects from the Delta to the Bay. The samples from Mildred's Island, Frank's Tract, Big Break, and Sherman were grouped as "Delta" sites and Browns, Honker, Grizzly and Suisun were grouped as "Bay sites.
Lower panel: Sediment chlorophyll *a* concentrations from Delta and Bay environments in September 2011 and March 2012. The box plots show the median as the line within the box, the box represents the 25-75th percentiles, and the error bars are the 0-25 and 75-100 percentiles. A Kruskal-Wallis one way analysis of variance on ranks showed that the Delta and Bay locations were significantly different ($p < 0.01$) and that were no temporal differences.
Figure reproduced from Cornwell et al. (2014).

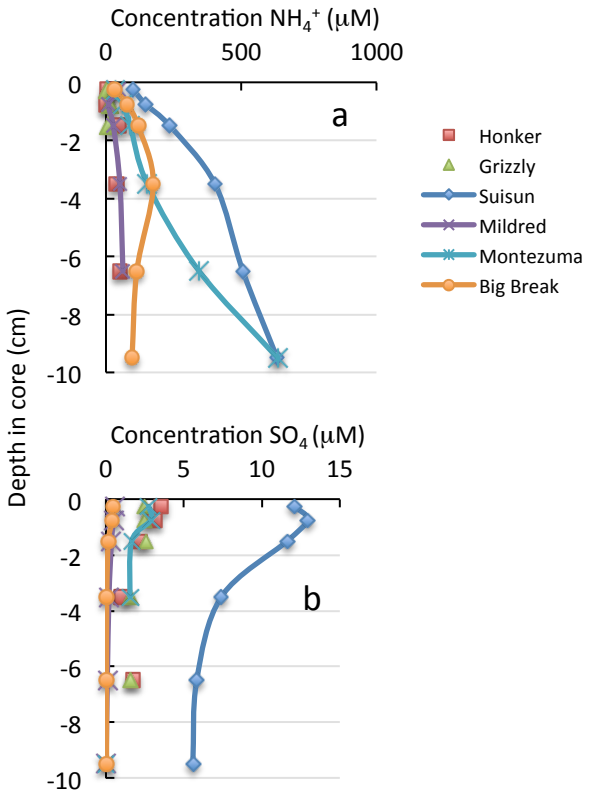


Figure 42. Pore water concentrations of NH_4^+ (panel a) and SO_4 (panel b) for sites indicated for cores collected in March 2013.

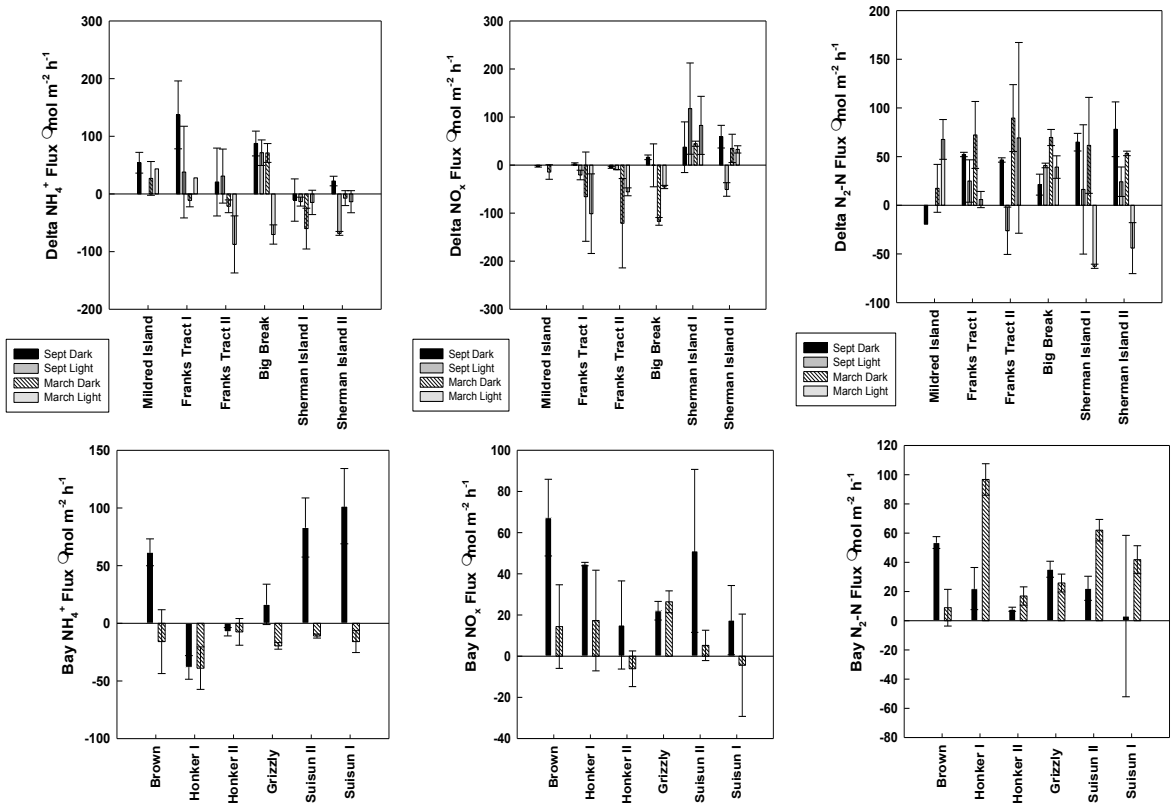


Figure 43. Sediment-water exchange rates of NH_4^+, NO_x (sum of NO_3^- and NO_2^-) and N_2 for Delta and Bay sites. Data include dark fluxes for all sites and light ("illuminated") flux incubations for Delta sites with light at the sediment surface. Each bar is the mean of duplicate cores and error bars show the data range. Positive rates indicate a flux directed from the sediment to the water column. See Cornwell et al. (2014) for methodological details.
Figure reproduced form Cornwell et al (2014).

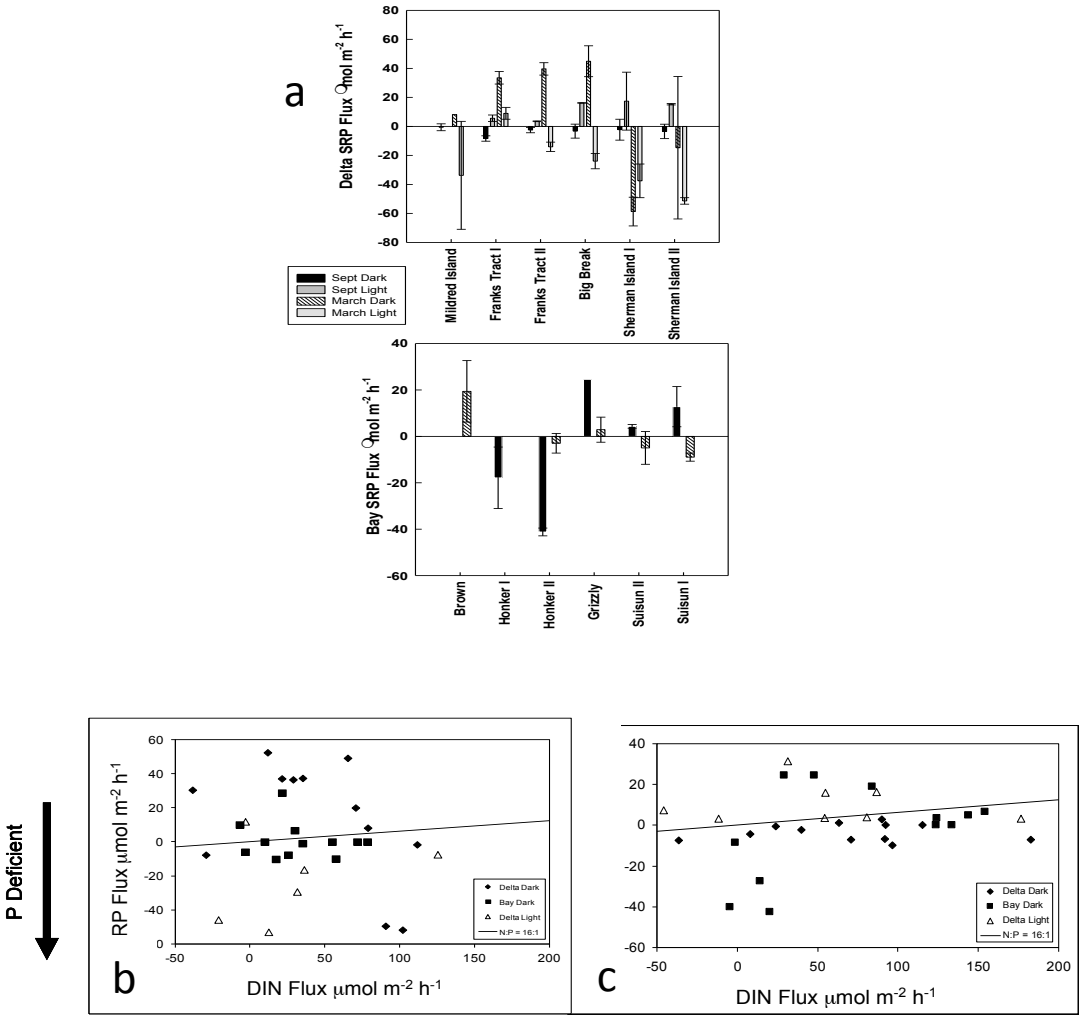


Figure 44. Panel a. As in Figure 39, except for SRP.
Panels b,c: Relationship between SRP and DIN fluxes for (b) March and (C) September. Points above the line indicate a N deficiency relative to P, and those below the line indicate a P deficiency relative to N compared to Redfield stoichiometry. Note that most of the Bay sites for September fall below this line.
Figure reproduced from Cornwell et al. (2014).

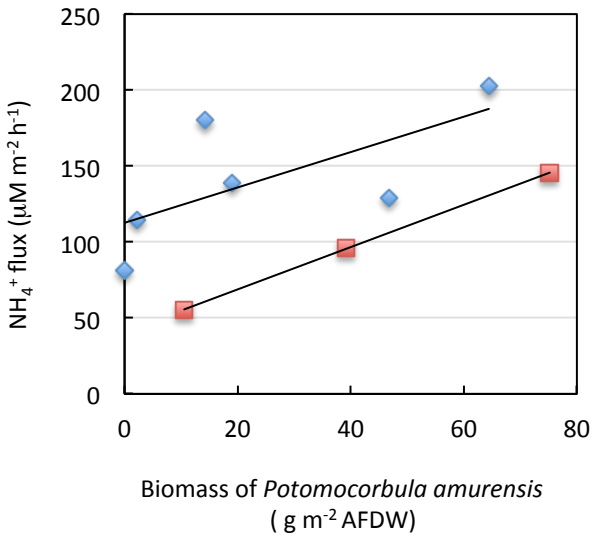


Figure 45. Flux of NH_4^+ measured in cores with variable abundance of clams. Cores were collected from Suisun Bay (squares) and Montezuma Slough (diamonds).

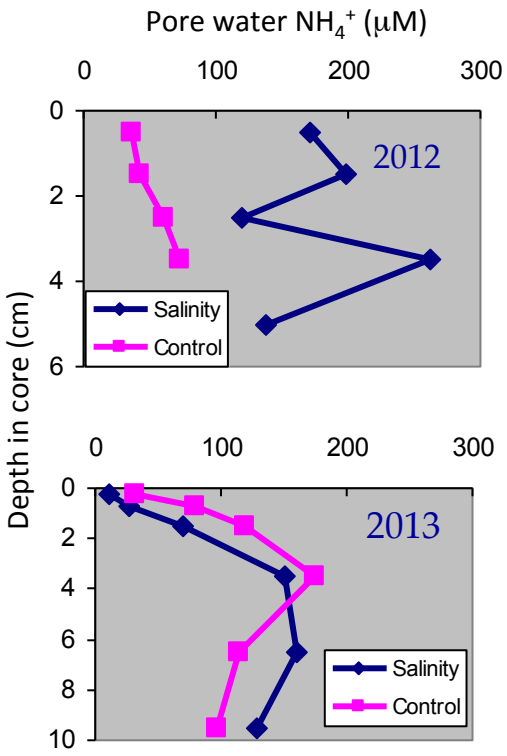


Figure 46. The concentration of NH_4^+ (μM) in cores that were amended with salt to bring the salinity to a value of 2 in comparison to control cores. Measurements were made in 2012 (upper panel) and 2013 (lower panel). Note that the effect was much greater in 2012 when the ambient flow regime was higher and thus the control core was comparatively fresher than in 2013.

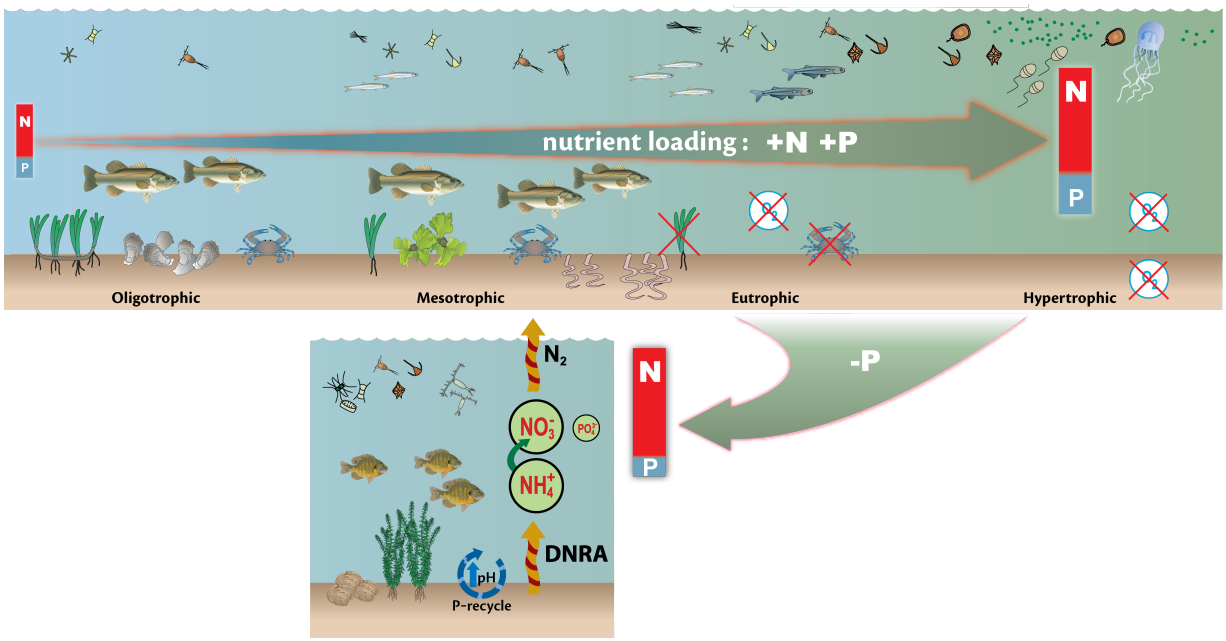


Figure 47. Conceptual schematic showing the progression of a system towards eutrophication with the increased loading of both nitrogen and phosphorus. The panel below illustrates the conceptual change in the system following eutrophication but removal of a single nutrient, phosphorus, without concomitant decrease in nitrogen loading (or even with increases in nitrogen loading), leading to an increase in N:P. Biodiversity changes, but so too does biogeochemistry, in turn altering the environmental suitability for certain types of species.
Figure reproduced from Glibert (2015).

Section 4

Impact of Nutrient Concentrations and Ratios on Phytoplankton Community Composition

With special emphasis on the San Francisco Bay Estuary

Prepared for:
The San Francisco Estuary Institute
In support of
The San Francisco Bay Nutrient Management Strategy

Draft Technical Report
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Mine Berg

APPLIED
marine
SCIENCES

1.0 Introduction

San Francisco Bay (SFB) is considered a high nutrient, low chlorophyll (HNLC) system due to persistent light limitation owing to high water column turbidity (Cole and Cloern 1984, Cloern 1987, Alpine and Cloern 1988, Jassby and Cloern 2002). However, recent decreases in sediment loading and subsequent increases in water clarity (i.e. Schoellhamer 2011, Schoellhamer et al. 2012), have resulted in an increase in summertime phytoplankton biomass. This increase has been distributed unevenly, with a tripling of chlorophyll (Chl *a*) in the southern, but minimal changes in Chl *a* in the northern, regions of SFB (Cloern and Jassby 2012). Why there has not been a stronger recovery of phytoplankton biomass in the north despite significant improvement in water clarity has been the subject of much debate (i.e. Kimmerer 2002, Thompson et al. 2008, Jassby 2008, Cloern and Jassby 2012). Moreover, the low productivity zone in northern SFB has been linked with a decline in the abundances of three pelagic fish species (Sommer et al. 2007).

Two nutrient-related hypotheses for explaining the low phytoplankton productivity zone in northern SFB have been put forward in the past several years. The first is that inhibition of NO_3^- uptake by relatively high ambient NH_4^+ concentrations slows growth of large-sized diatoms (Dugdale et al. 2007, Parker et al. 2012). The second is that dissolved nitrogen:phosphorus (N:P) ratios in excess of Redfield stoichiometry produces phytoplankton with sub-optimal cellular stoichiometry, and/or growth rates, which has a knock-on effect on higher trophic levels (Glibert et al. 2011). The former hypothesis rests on the assumption that diatoms grow slower on NH_4^+ than they do on NO_3^- and are therefore less competitive vis-a-vis other phytoplankton taxa under NH_4^+-dominant conditions. The latter hypothesis rests on the assumption that phytoplankton take up nutrients in the ratio that they occur in the dissolved phase, and that phytoplankton species will be at a disadvantage under conditions of sub-optimal ratios and may possibly be outcompeted.

Motivated by these recent hypotheses, this review examines the broader scientific literature on how phytoplankton physiology is affected by, or responds to, external nutrient concentrations and ratios, specifically with respect to the following questions: 1) How are the cellular N:P ratios of phytoplankton affected by their surrounding nutrient environment when concentrations range from limiting, to sufficient, to excess? 2) Can dissolved N:P ratios be used to predict levels at which nutrients start to inhibit growth of phytoplankton due to toxicity? 3) How do concentrations of nutrients, ratios of dissolved N:P, and phytoplankton community composition in SFB compare with other estuarine systems; and 4) how might these comparisons inform our understanding of the role nutrients play in shaping SFB community composition?

Because these questions span the entire spectrum from cellular physiology to population ecology, this report is divided into two sections. The first section summarizes

the state of knowledge on (a) cellular N and P composition across phytoplankton taxa; (b) conditions under which phytoplankton N:P stoichiometry varies; and (c) how the point at which a phytoplankton cell transitions from N to P limitation is manifested at a under varying resource competition scenarios; and lastly (d) how phytoplankton growth rates differ as a function of N substrate.

The second section examines how nutrient ratios and phytoplankton community composition vary under a range of absolute nutrient concentrations. This section examines four case studies where nutrient concentrations range from strongly limiting, to seasonally in balance with phytoplankton biomass, to in excess of phytoplankton biomass, in order to understand the impact of varying absolute nutrient concentrations as well as dissolved N:P ratios on phytoplankton growth. The lessons learned from this exercise are in turn applied to interpret differences in dissolved concentrations and ratios and phytoplankton community composition along the major (north-south) axis of San Francisco Bay.

2.0 The use of nutrient ratios in marine and estuarine phytoplankton ecology

In 1958 Redfield published his discovery that phytoplankton particulate matter was comprised of N and P in a ratio of 16 (mol:mol), similar to the ratio of dissolved N:P in the water. Redfield (1958) suggested that the ratio of dissolved N:P in the ocean was driven by the remineralization of phytoplankton particulate matter, a theory which has since taken hold (Goldman et al. 1979, Copin-Montegut and Copin-Montegut 1983, Hecky et al. 1993, Falkowski 2000, Geider and LaRoche 2002). Given the average N:P ratio of 16 in phytoplankton, it was deduced that phytoplankton would become limited by N at dissolved N:P less than 16 and limited by P at dissolved N:P ratios greater than 16.

2.1 Taxon-specific differences in phytoplankton cellular stoichiometry

Shortly after Redfield's discovery of the universality of the N:P ratio of 16, investigators turned to phytoplankton cultures to examine how closely individual phytoplankton taxa adhered to this canonical ratio. Parsons et al. (1961) published the first investigation demonstrating variability in cellular N:P ratios of phytoplankton. Subsequent investigations noted that diatoms and dinoflagellates tended to have cellular N:P ratios below 16 whereas chlorophytes and cyanobacteria typically had ratios above 20 (Fig. 1, Geider and LaRoche 2002, Ho et al. 2003, Quigg et al. 2003, Hillebrand et al. 2013). This difference among the taxa stems from slight variations in macromolecular composition of the phytoplankton, principally in their ratio of protein, the largest store of N in the cell, to nucleic acids, the largest store of P in the cell (Fuhs 1969, Terry et al. 1985, Falkowski 2000, Geider and LaRoche 2002, Loladze and Elser 2011). Once it was realized that the N:P ratio of 16 was an average among phytoplankton, and that there were significant departures in this ratio depending on taxa, it also became clear that the stoichiometric demand for N:P differed with respect to taxa (Rhee 1978, Rhee and

Gotham 1980, Terry et al. 1985). That phytoplankton take up N:P in proportion to their cellular composition was subsequently confirmed in culture experiments (e.g. Terry et al. 1985, Leonardis and Geider 2004). In addition to N and P, diatoms, which build silica frustules, are often limited by dissolved silicate (Officer and Ryther 1980, Conley and Malone 1992, Egge and Aksnes 1992). On average, diatoms require silicate in a 1:1 ratio with N in order to grow (Brzezinski 1985, Sarthou et al. 2005), with some variation around this mean depending on the thickness of the frustules and the individual requirements of different species (Harrison and Davis 1979, Tett et al. 2003).

2.2 Adjustments in taxon-specific stoichiometric ratios in response to changes in the environment

2.2.1 Nutrient Limitation

Cellular N:P composition is not a fixed trait and phytoplankton are able to adjust it, within certain limits, in response to changes in environmental conditions. For example, a summary of nearly 50 phytoplankton studies demonstrates that the cellular N:P ratio of P-limited phytoplankton converge around 28 and the cellular N:P ratio of N-limited phytoplankton converges around 16 (Hillebrand et al. 2013). But, individual culture investigations have demonstrated cellular N:P ratios as high as 60 in P-limited species and as low as 5 in N-limited species (Goldman et al. 1979, Terry et al. 1985, Leonardos and Geider 2004). These experiments also demonstrate significant decreases in growth rates associated with the nutrient limitation and changes in ratios of the phytoplankton (Goldman et al. 1979, Terry et al. 1985, Leonardos and Geider 2004).

2.2.2 Irradiance

Irradiance may also change the cellular N:P ratio through its influence on the cellular protein content (LaRoche and Geider 2002). Pigments (Chl *a* and light harvesting antenna pigments) are bound in N rich pigment-protein complexes that increase or decrease, as irradiance decreases or increases, respectively, in order for cells to capture more light or to reduce the size of the light harvesting complex to avoid photodamage and photoinhibition of growth (Falkowski and LaRoche 1991, Suggett et al. 2007). In turn, the cellular pigment, protein, N content, and the cellular N:P ratio of cells increase or decrease, respectively (Wynne and Rhee 1986, Nielsen 1992, Leonardos and Geider 2004). The irradiance-dependent change in cellular N:P ratios is expected to be even more pronounced among cyanobacteria due to a greater association of protein with the light-harvesting phycobilipigments in the phycobilisomes than in the eukaryotic light harvesting complex (Raven 1984, Geider and LaRoche 2002).

2.2.3. Growth Rate

Changes in the growth rates of phytoplankton (as a result of changes in temperature or irradiance) are hypothesized to selectively impact the P content of cells by regulating the synthesis of ribosomes and rRNA, potentially to build new pathways related to growth and reproduction. While both protein and rRNA synthesis increases with increased growth rates, the efficiency of protein synthesis (per ribosomal unit) decreases, therefore cellular requirements for P increase more rapidly than those for N

resulting in a decrease in the cellular N:P ratio. Known as the growth rate hypothesis (Elser et al. 2000, 2003), it has been shown to hold true for a wide range of unicellular organisms including heterotrophic bacteria, fungi and algae (Goldman et al. 1979, Terry et al. 1985, Tett et al. 1985, Leonardos and Geider 2004, Arrigo 2005, Karpinets et al. 2006). However, in a recent review, Flynn et al. (2010) only found experimental support for the growth rate hypothesis in P-limited phytoplankton cultures. N-limited phytoplankton had higher cellular N:P ratios with increased growth rates contradicting the prediction of the growth rate hypothesis with respect to phytoplankton (Flynn 2010).

2.2.4. Elevated nutrient concentrations

Early experiments demonstrated that changing the ratio of dissolved N:P in the growth medium of phytoplankton did not influence their cellular N:P composition when nutrients were in excess and they were growing at maximal rates (Fig. 2A, Goldman et al. 1979). This finding was consistent with culture investigations (mentioned in section 2.1) demonstrating that when the dissolved N:P ratio external to the cell did not match the cellular N:P composition, a residual of the nutrient in excess would build up in the medium (Fig. 2B, C). Subsequent culture investigations and field observation demonstrated that variation in the concentration of the non-limiting nutrient, and therefore in ratios, does not affect cellular N:P ratios or growth rates (Goldman et al. 1979, Tilman et al. 1982, Sunda and Hardison 1997, Roelke et al. 2003). This holds true as long as the concentration of any one nutrient is not so high that it results in toxicity to the cell, which would inhibit growth (Collos and Harrison 2014). In terms of nitrogen substrates, NH_4^+ tends to become toxic to cells at lower concentrations than NO_3^- (Collos and Harrison 2014). For example, a decrease in growth rates by 50% typically occurs around a concentration of 3600 µmoles NH_4^+ L^{-1} for diatoms. This suggests that concentrations typically encountered in natural systems that are in excess of phytoplankton growth demand will not impact diatom growth rates and therefore not result in a decrease in their abundance to impact community composition (Collos and Harrison 2014).

2.3 The critical N:P ratio

An important caveat with respect to the discussion on differences in cellular N:P ratios among phytoplankton is the assumption that phytoplankton growing at or near maximal growth rates do not allocate nutrients into storage, therefore the cell operates at the minimum cell quota for N (q_N) and for P (q_P) (Quigg et al. 2003, Klausmeier et al. 2004). The cellular N:P ratio at the maximal growth rate has been described as the optimal N:P ratio and is widely assumed to be the ratio where phytoplankton cells will transition from N to P limitation (Finkel et al. 2010, Klausmeier et al. 2004, Hillebrand et al. 2013). Contrary to the assumption of minimal cell storage at maximal growth rates, culture investigations demonstrate that phytoplankton cells store increasing amounts of P with increasing external P concentration, or decreasing N:P ratios (Rhee 1978, Terry et al. 1985, Geider and LaRoche 2002, Leonardos and Geider 2004). At decreasing external P concentration, P $cell^{-1}$ will continue to fall until it reaches a minimum. Below this

minimum, P content does not change with further decreases in the external concentrations (Fig. 3A, B). The pivot point that marks the transition point between where P $cell^{-1}$ is stable and where it begins to increase exponentially is termed the critical N:P ratio and is thought to mark the true transition point between N and P limitation (Terry et al. 1985, Geider and LaRoche 2002). Interestingly, a corresponding pivot point does not exist with respect to cellular N concentrations (Fig. 3C) suggesting that phytoplankton N storage does not exist in the same way as it does for P storage (Terry et al. 1985, Leonardis and Geider 2004). Although few culture investigations have determined the critical N:P ratios in phytoplankton cells, those that have converge on N:P ratios varying from 30-40 with respect to the transition point between N and P limitation (Fig. 3A, B, Terry et al. 1985, Geider and LaRoche 2002, Leonardos and Geider 2004). Thus, the critical N:P ratio is shifted higher than the canonical cellular N:P ratio for phytoplankton, providing convincing evidence for why P-limitation is difficult to attain physiologically.

2.4 The influence of the mismatch in cellular and dissolved N:P ratios on competition among phytoplankton and species succession

A variety of factors simultaneously influence phytoplankton growth and succession. These can roughly be divided into "bottom-up" factors such as nutrients and "top down" factors such as grazing. If we imagine a scenario where only bottom-up factors (i.e. resources) matter for the growth of phytoplankton, then the variation among species at the point in which a nutrient becomes limiting is what influences the succession of species over the course of the growing season. The concept of nutrient limitation in this context does not refer to limitation of the population biomass (also known as Liebig limitation) but rather limitation of growth rates (see Beardall et al. 2001 for further details). Thus, the difference at the point where one or another nutrient becomes limiting, and slows the growth of a particular phytoplankter, is the basis of resource-based competition among phytoplankton (Tillman 1977, Rhee 1978, Rhee and Gotham 1980, Sommer 1989). For example, if N is relatively abundant and its rate of uptake by species **A** is at its maximum, but P concentrations have reached a level at which P uptake is less than its maximum, species **A**'s growth rate will be set (or limited) by the sub-maximal rate of P uptake. If species **B** is also present in the same water, and requires less P per unit N (i.e. it has a higher optimal cellular N:P), species **B**'s P uptake rate will still be at its maximum, and may allow species **B** to grow more rapidly than species **A**. As a result, species **A** will be outcompeted and there will be a succession from species **A** to species **B** in the community.

2.5 The ratio of reduced:oxidized nitrogen

If growth rates are directly affected by the type of N used, then the distribution of N between reduced (NH_4^+) or oxidized (NO_3^-, NO_2^-) substrates in the water column can influence phytoplankton growth, and therefore phytoplankton community composition. Whether this is the case has been examined in a variety of species of phytoplankton ranging from diatoms to cyanobacteria (Thompson et al. 1989, Levasseur et al. 1993, Saker and Neilan 2001, Berg et al. 2008, Thessen et al. 2009). Culture investigations

demonstrate that phytoplankton acclimated to growth on either NH_4^+ or NO_3^- have similar rates of growth, typically within ±15% (Fig. 4). The reason for this is that the reductant and energy demands of N assimilation, including assimilation of NO_3^-, are small in comparison to that of C metabolism (Turpin 1991). However, the extra energy cost of assimilating NO_3^- (i.e. the cost of reducing NO_3^- to NH_4^+ before it can be assimilated) can be on the order of 20% (Thompson et al. 1989, Levasseur et al. 1993). The cell may compensate for the extra energy requirement by increasing Chl *a* per cell, decreasing cell size, decreasing the cellular N content, or any combination of these, which may minimize the effect on rates of growth (Paasche 1971, Thompson et al. 1989, Wood and Flynn 1995, Page et al. 1999). The observations summarized in Figure 4 suggest that the ratio of NH_4^+: NO_3^- in the water column should not affect phytoplankton growth or community composition.

Compared with culture data, interpreting observations from the field can be more difficult because the absolute N concentration differs alongside the partitioning of N between NH_4^+ and NO_3^- (Fig. 5A). For example in a system that is not light or trace metal limited, the total N concentration is typically at a maximum when NO_3^- is the dominant N source, and the total N concentration is typically at a minimum when NH_4^+ is the dominant N source (Fig. 5A, Butler et al. 1979, LaRoche et al. 1997, Berg et al. 2001, 2003, Flynn 2010). This occurs primarily because phytoplankton prefer NH_4^+, leaving NO_3^- to accumulate under conditions of N sufficiency. Only when N becomes limiting to phytoplankton growth will NO_3^- be drawn down. Because NH_4^+ is regenerated more rapidly than NO_3^-, NH_4^+ often dominates the inorganic N pool under N limiting conditions (Berman and Bronk 2003). Diatoms typically predominate the phytoplankton community under N sufficient conditions and are almost absent from the community under N limiting conditions (Fig. 5B, Berg et al. 2003, Heil et al. 2007). Therefore it appears that a change in N species is driving a change in community composition but it could just as well be a change in the total N concentration, from replete to limiting (i.e. Flynn 2010, Davidson et al. 2012).

2.6 Summary of cellular N:P stoichiometry

- Phytoplankton take up external N and P in the ratio dictated by their cellular composition; therefore, cellular N:P ratios are indicative of the most favorable dissolved N:P ratios for growth under a situation where nutrient supply balances phytoplankton growth.
- When nutrients are in excess of demand, phytoplankton will continue to take up N and P in the same proportion as their cellular composition. If the external dissolved N:P ratio differs from the cellular ratio, a residual of the nutrient in excess will build up in the dissolved phase (Fig. 2B).
- Cellular N:P composition is plastic and can deviate from the optimal if cells are experiencing nutrient limitation, changing irradiance, or are accelerating or decelerating their growth rates. For example, nutrient limitation can result in

cellular N:P ratios from as low as 5 under N limitation and as high as 60 under P limitation.

- Resource-based competition among phytoplankton results from a mismatch in the dissolved (external) N:P ratio and individual taxa's cellular (internal) N:P, as the nutrient supply becomes limiting to one taxa's growth relative to another.
- The critical N:P ratio describes the cellular N:P ratio where phytoplankton will pivot from being N to being P limited; this ratio is typically greater than the optimal cellular N:P ratio. Therefore, using the optimal cellular N:P ratio of a certain species or taxa to predict competitive outcomes under various scenarios of dissolved N:P ratios may not yield expected results.
- Phytoplankton grow at similar rates using NH_4^+ or NO_3^- as a sole source of N for growth (under otherwise similar conditions); therefore changes in dissolved NH_4^+: NO_3^- are unlikely to substantially influence phytoplankton community composition. In natural systems, NH_4^+ and NO_3^- typically dominate the total N pool under low and high nutrient conditions, respectively, giving the appearance that type of N is driving community succession when it may in fact be a change in total N concentration.

3.0 Variation in dissolved N:P ratios and phytoplankton community composition in open ocean and coastal regions

As described in Section 2.0, cellular N:P ratios are indicative of the most favorable dissolved N:P ratios for growth under a scenario where nutrient supply balances phytoplankton growth. However, nutrient supply is often seasonally or permanently out of balance with phytoplankton demand. In order to understand how phytoplankton community composition is impacted by dissolved N:P ratios under varying scenarios of nutrient supply relative to phytoplankton biomass, the following sections examine four case studies along a nutrient supply continuum from limited to excess. These four case studies include the severely nutrient-limited oligotrophic ocean (Sargasso Sea); the seasonally-limited coastal ocean (North Atlantic Shelf); a seasonally limited but highly eutrophic estuary (Chesapeake Bay); and a high nutrient low chlorophyll system where nutrients generally exceed phytoplankton demand (San Francisco Bay).

Whether nutrients are limiting to, or in excess of, phytoplankton biomass (now referring to Liebig limitation as discussed in Section 2.4) is typically determined by the relationship that it takes 1 µmole N L^{-1} to produce 1 µg Chl *a* L^{-1} (Yentsch and Vaccaro 1958, Gowen et al. 1992). For the purposes of this discussion, a Chl *a*:N ratio of ~ 1 will be used to describe a system where nutrient availability is in balance with phytoplankton biomass, whereas a ratio <1 indicates nutrients in excess and a ratio >1 indicates nutrient limiting conditions. While there is quite a bit of variation in this relationship as a function of light, growth rate, and phytoplankton functional groups (i.e. Manny 1969), it's nevertheless a reasonable approximation over larger regions. Below,

the ratio of phytoplankton biomass to nutrient concentration, represented as Chl *a*:N, will be examined alongside the ratio of dissolved N:P and phytoplankton community composition.

3.1 Open Ocean – nutrient limited system

The North Atlantic Ocean is a system where the influence of dissolved N:P ratios on phytoplankton nutrient limitation and composition have been intensively investigated along a gradient of absolute nutrient concentrations stretching from the impoverished subtropical to the coastally-influenced shelf region (Tyrrell 1999, Cavender-Bares et al. 2001, Ammerman et al. 2003, Mills et al. 2004, Davey et al. 2008, Moore et al. 2008).

In the subtropical Sargasso Sea, ambient nutrient concentrations are close to detection limits and N:P ratios vary from 5-9 (Cavender-Bares et al. 2001, Davey et al. 2008). Nutrient uptake by phytoplankton is very efficient and nutrients are turned over rapidly to support Chl *a* :N > 20 (Fig. 6A-D). In this region, more than 65% (by biomass) of the phytoplankton community is dominated by cyanobacteria such as *Synechococcus* and *Prochlorococcus* (Fig. 6E). The eukaryotic community is comprised of picoeukaryotes, and diatoms are almost non-existent (Cavender-Bares et al. 2001). Bioassay experiments have demonstrated that the entire phytoplankton community (measured as Chl *a*) is most stimulated by the addition of N, and therefore is considered N-limited in the Liebig sense (Graziano et al. 1996, Mills et al. 2004, Davey et al. 2008). Below we compare this N-limited system to a transition zone where nutrients are more plentiful.

3.2 Coastal shelf system – transition zone from nutrient limited to replete

Compared with the Sargasso Sea, both absolute N concentrations and Chl *a* concentrations are greater further north off the coast of Bermuda, in the Gulf Stream and in the North Atlantic Shelf region (Fig. 6A, B). Whereas nutrient concentrations are consistently greater, N:P ratios may vary widely from 50 off the coast of Bermuda to 9 off the Atlantic Shelf (Fig. 6C). Despite a 5-fold variation in N:P ratio, phytoplankton community structure is similar between Bermuda and the North Atlantic Shelf as 50% or more is comprised of eukaryotic phytoplankton (Fig. 6). In contrast, the two communities in the North Atlantic Ocean with the most similar N:P ratios, the Sargasso Sea and the North Atlantic Shelf, have the most divergent phytoplankton communities, comprised of 65% cyanobacteria and 35% picoeukaryotes, in the former region, versus 12% cyanobacteria and 88% eukaryotes, including cryptophytes and diatoms, in the latter region (Fig. 6C, E). What distinguishes these two sectors of the North Atlantic Ocean is 1) the absolute nitrogen concentration, on average 70-100 fold greater on the shelf than in the Sargasso Sea, and 2) Chl *a*:N ratios are 20-fold greater in the Sargasso Sea than they are on the Shelf (Fig. 6D), suggesting that diatoms are absent from the Sargasso Sea community despite what might otherwise be considered favorable dissolved N:P ratios (relative to their optimal or critical N:P) for their growth because of the low nutrient concentrations.

3.3 Estuary – eutrophic

Chesapeake Bay drains a large agricultural watershed and is one of the most eutrophic estuaries on the East Coast of the United States (Boynton et al. 1995, Harding and Perry 1997, Boesch et al. 2001, Hagy et al. 2004, Kemp et al. 2005). It receives 40×10^6 kg of nitrogen on an annual basis (Boynton et al. 1995), largely from the Susquehanna River. Up to 95% of this nitrogen load is taken up by phytoplankton, resulting in mean springtime Chl *a* concentrations of 10-35 µg L^{-1} (Fig. 7A) and annual mean Chl *a* concentrations varying between 5-15 µg L^{-1} (Fisher et al. 1992, 1999, Kemp et al. 2005). Because the source of nutrients to the Susquehanna is largely agricultural, the N:P ratio is high, approaching 600 in spring when water flow is greatest, driving the phytoplankton community closest to the head of the Bay towards P-limitation (Fisher et al. 1992, 1999). At the end of the spring diatom bloom when most of the dissolved silicate used by diatoms to build their frustules has been depleted from the water column (Fig. 7B), the community can also become silicate limited (Conley and Malone 1992, Malone et al. 1996). In summer, when river flow is at its lowest, phytoplankton biomass is principally limited by N (Fisher et al. 1992, Malone et al. 1996, Fisher et al. 1999, Kemp et al. 2005). A spatially and seasonally varying mosaic of N:P ratios supports a succession of different phytoplankton communities. Superimposed on this mosaic of changing N:P ratios is also a sharp change in absolute nutrient levels and in the Chl *a*:N ratios that occur during the spring-summer transition. In spring, when the Chl *a*:N ratio is typically <1, dissolved N:P ratios are >150, and Si:N ratios are <1, diatoms dominate. In summer, when the Chl *a*:N ratio varies from 10-15, dissolved N:P ratios are 4-18, and Si:N ratios are 8-20, the phytoplankton community is dominated by cyanobacteria, cryptophytes and dinoflagellates (Fig. 7B-E). Dinoflagellates also dominate in the lower salinity tributaries of the Bay where N:P ratios are more variable throughout the summer. For example, the ubiquitous dinoflagellate *Prorocentrum minimum* tends to bloom in regions where N:P ratios range from 50-110 while *Karlodinium veneficum* most often dominates in the N:P range of <16 (Li 2011).

Because the change in absolute nutrient concentrations occurs simultaneously with changes in the dissolved N:P (and Si:N) ratios in Chesapeake Bay, it is difficult to attribute the ensuing changes in phytoplankton community composition to either changes in absolute concentration or to changes in ratios. What is clear is that there is also a transition from a system where nutrients are in excess of phytoplankton assimilative capacity in spring, to a system where they are strongly limiting in summer, as evidenced by the changes in the Chl *a*:N ratio from <1 to >10 (Fig. 7)

3.4 San Francisco Bay – high nutrient low chlorophyll

San Francisco Bay (SFB) is distinguished from other estuaries by its high dissolved inorganic nutrient concentrations that tend to remain elevated year-round. Dissolved silicate, transported with the Sacramento River, peaks at greater than 200 µmoles L^{-1} in Suisun Bay decreasing to a low of 50 µmol L^{-1} in Central Bay. Mean annual nitrate (NO_3^-) varies from 19-30 µmoles L^{-1}, ammonium (NH_4^+) from 3-6 µmoles L^{-1}, and phosphate (PO_4^{3-}) from 1.8-5.5 µmoles L^{-1} throughout the estuary and throughout the growing

season (Table 1). Despite these high inorganic nutrient concentrations, Chl *a* is relatively low. Annual mean Chl *a* varies from 2±1µg L^{-1} in Suisun to 6.5±6 µg L^{-1} in South Bay, and springtime Chl *a* varies from 3.1±1.7 µg L^{-1} in Suisun Bay to 10.4±9 µg L^{-1} in South Bay (Fig. 8A). Even the average springtime Chl *a* concentration in South Bay (13.9±11 µg L^{-1}) is only one third of that observed in Chesapeake Bay (Fig. 7A, 8A).

Ratios of dissolved nutrients, or their absolute concentrations, do not vary appreciably over time (i.e. over the growing season) in SFB. The reason for this is that the size of the spring bloom is small relative to the available nutrient pool and nutrients are not drawn down to limiting concentrations over the course of the bloom (exceptions are noted below). Because the spring bloom represents a small increase above the summer-time baseline phytoplankton biomass, the difference in nutrient concentrations and ratios between spring and summer is small (Fig. 8A-C). Consistent with seasonally invariant nutrient and Chl *a* concentrations, there is limited change in the Chl*a*:N ratio between seasons, which remains below 1 at all times of the year (Fig. 8D). South Bay is an occasional exception to the above observations; in some years South Bay nutrients are drawn down to what might be considered limiting levels. However, these low levels of nutrients persist for only short periods of time, as the re-establishment of light-limiting conditions prevents high levels of primary productivity, and limiting levels of nutrients, from being sustained (Cloern and Nichols 1985, Cloern 1991, Cloern 1996). The nutrient dynamics observed in SFB contrast markedly with other coastal systems, such as Chesapeake Bay, that experience a large change in ratios (30-300 fold) following a sharp decrease in nutrient concentrations at the end of an intense spring bloom (Fisher et al. 1999). Moreover, nutrient concentrations remain low for the rest of the growing season in Chesapeake Bay due to high and sustained rates of primary productivity (Malone et al. 1996).

While ratios of dissolved nutrients do not vary appreciably over time, they do vary with embayment in SFB. For example, mean dissolved Si:N ratios vary from 8 in Suisun Bay to 3 in South Bay, both in spring- and summer-time (Fig. 8B). Similarly, dissolved N:P ratios vary from 18 in Suisun Bay to 7 in South Bay in spring, and from 13 in Suisun to 4 in South Bay in summer (Fig. 8C). This is due to mean NO_3^- concentrations being greater in Suisun Bay and PO_4^{3-} concentrations being greater in South Bay (Table 1). It is interesting to note that even at their highest, dissolved N:P ratios are relatively low in SFB, i.e. at Redfield or below, compared with other systems. For example, in spring-time the average dissolved N:P ratio in Chesapeake Bay (N:P=390) is 30-times greater than the average N:P ratio in SFB (N:P=13). This is most likely due to the dominance of agricultural drainage into Chesapeake Bay, which is relatively N-rich, compared with discharge of wastewater effluent into SFB, which is relatively P-rich, as a source of nutrients (i.e. Fisher et al. 1992). Even though dissolved N:P ratios are at or below Redfield throughout most of SFB, N is not considered limiting as the combined concentrations of NO_3^- and NH_4^+ are in the tens of µmoles L^{-1} range in all SFB embayments (Table 1). In fact, if all the unused N was converted into phytoplankton

biomass (using a Chl *a*:N ratio of 1), Chl *a* concentrations would reach 28 µg L^{-1} in South Bay (Cloern and Jassby 2012).

Against this backdrop of nutrient concentrations consistently in excess of phytoplankton demand (as illustrated by a Chl *a*:N < 1), phytoplankton community composition remains remarkably constant between spring and summer, and within each embayment (Fig. 8E). For example, phytoplankton biomass in Suisun Bay is dominated by diatoms in spring and summer. San Pablo Bay is dominated equally by diatoms and cryptophytes both in spring and summer (Fig. 8E). In Central Bay, dinoflagellates comprise the second greatest component of the phytoplankton community after diatoms. The greater fraction of dinoflagellate biomass in this embayment may be due to exchange with the coastal shelf (Cloern et al. 2005, Cloern et al. 2007). Dinoflagellates may also be transported into South Bay where salinities are similar to the shelf and conditions more amenable to their growth. Accordingly, the phytoplankton community in South Bay also has a larger component of dinoflagellates compared with the northern embayments (Fig. 8E), but is still dominated by diatoms (>60%) and cryptophytes.

Unlike the systems described in Sections 3.1-3.3 in which phytoplankton community composition varied in accordance with season and with seasonally changing nutrient levels, SFB's phytoplankton community appears not to vary in composition from spring to summer. The apparent consistency of the phytoplankton community composition with season can been attributed to nutrients almost always exceeding the phytoplankton demand. Given the variation in phytoplankton community composition among embayments, rather than over the course of the growing season, it appears as though other factors - such as salinity, temperature, residence time, average mixing depth, light attenuation, euphotic zone depth and exchange with the ocean (Appendix A) - exert stronger influences on community composition than nutrient concentrations or ratios. For example, greater spring-time light availability, coupled with lower mixing depth and longer residence times, may be a key combination in supporting the spring bloom and greater annual concentrations of Chl *a* in South Bay compared with the other SFB embayments. It is also possible that the specific combinations of salinity, residence time, exchange with the ocean, and light availability play important roles in fashioning the phytoplankton composition unique to each embayment.

3.5 Merging ecosystem scale observations across systems

How variation in dissolved N:P ratios in a range of different systems relate to phytoplankton community composition, with specific attention paid to the fraction of diatoms, and whether there are generalizations that can be made across systems, is examined below.

3.5.1 Predicting the occurrence of diatoms based on specific dissolved N:P ratios

As is evident from Section 2.3, phytoplankton have distinct cellular N:P (and Si:N in the case of diatoms) ratios. If dissolved N:P relative to cellular N:P was a reliable predictor of phytoplankton community or succession, then 'favorable' or 'unfavorable' dissolved N:P

ratios would strongly influence or predict community composition. However, the majority of literature related to both physiology and ecosystem-scale response indicates that the favorable/unfavorable distinction based on dissolved N:P only holds at low absolute nutrient concentrations, where a mismatch of the dissolved and cellular ratios would limit one phytoplankton taxa's uptake of N or P relative to another, and hence decrease its growth rate such that it would become outcompeted. Even at low nutrient concentrations, making a prediction about phytoplankton community composition based on ratios is complicated by the fact that cellular ratios change in response to nutrient limitation and we generally do not know at what exact cellular ratio phytoplankton growth starts to slow down. The cellular ratio that marks the point at which growth rates slow down, is at the true transition between N and P limitation is known as the critical N:P ratio. This ratio is generally not known, but tends to occur at a greater N:P ratio than the cellular N:P ratio (Section 2.0).

Predictions of phytoplankton competitive fitness under various environmental nutrient ratios are complicated even further by additional factors that change cellular ratios. For example, increases in growth rates could potentially increasing the P demand of the cell and variations in irradiance could change the cellular protein content (thereby N content) of phytoplankton. Together, these factors make it almost impossible to predict phytoplankton composition based on the occurrence of certain ratios of dissolved nutrients in the water column. To illustrate this fact, we can examine the variation in dissolved N:P ratios that encompass communities dominated by diatoms. Using all the data presented in this review, from the North Atlantic, Chesapeake Bay, and San Francisco Estuary, it is clear that diatoms dominate communities where the dissolved N:P ratio varies from 7 to >600 (Fig. 9A). The fact that dissolved N:P ratios vary by two orders of magnitude across disparate systems where diatoms consistently comprise >70% of the community, suggests that the former cannot be used to predict the latter. In other words, when nutrients are not limiting, ratios of dissolved N:P cannot be used to predict phytoplankton community composition.

3.5.2 Predicting phytoplankton succession based on changes in dissolved N:P ratios

While a specific dissolved nutrient ratio cannot be used to predict a certain phytoplankton community composition, could a substantial change in the ratio be used to predict phytoplankton succession? For example, going back to the optimal cellular N:P ratios presented in Figure 1, could a change in ratio from 25 to 7, at low absolute nutrient concentrations, drive a change from cyanobacteria to diatoms, by changing conditions from P-limiting in the former case to N-limiting in the latter? Making predictions regarding the successional pattern of phytoplankton is complicated by a different set of factors than those discussed Section 3.5.1. As noted earlier, it is often difficult to separate the effect of the change in the absolute nutrient concentration (which typically drives the change in dissolved nutrient ratios) from the effect of a change in the ratio. For example, in Chesapeake Bay, the transition from diatom-dominated in spring to flagellate and cyanobacteria-dominated communities in summer follows a transition in the dissolved N:P ratio from high to low, and a transition in

absolute N concentration from high to low (Table 2). Therefore, while it may seem that the change in the ratio is what leads to succession it could also be the decrease in the absolute N concentration, from replete to limiting, that leads to succession. Separating the effect of a change in the ratio from change in concentration is generally not possible in a natural system unless additional factors are taken into consideration (Flynn 2010, Davidson et al. 2012). Culture studies however, demonstrate that changes in the concentration of the non-limiting nutrient does not matter to growth (Tilman et al. 1982, Sunda and Hardison 1997, Roelke et al. 2003) which argues that the change in ratio argument is really a change in concentration phenomenon.

If we examine the Chl *a*:N ratio, a pattern emerges among all four systems (subtropical North Atlantic, North Atlantic Shelf region, Chesapeake Bay and San Francisco Bay) in that diatoms comprise >40% of phytoplankton community biomass when the Chl *a*:N ratio is equal to or below unity (Fig. 9B). A compelling physiological reason for this observation is that diatoms need nutrients to be in excess of their biomass (i.e. Chla:N<1) in order to remain competitive for nutrients vis-à-vis smaller phytoplankton with larger surface area:volume ratios (Pasciak and Gavis 1974, Riebesell et al. 1993, Sunda and Hardison 1997, Beardall et al. 2009). In a study of diatoms and small eukaryotes, Sunda and Hardison (1997) demonstrated that diatoms become diffusion-limited for uptake of N, slowing their growth rates, at concentrations that were higher than those impacting smaller-sized eukaryotes. These types of studies, combined with the dominance of diatoms at Chl *a*:N ratios below 1, suggests that it's a change in concentration rather than change in ratio that drives succession, of diatoms, at least (see also Flynn 2010, Davidson et al. 2012). Conversely, small species such as cyanobacteria become progressively more important in the phytoplankton community as the fraction of Chl *a*:N increases and N becomes more limiting (Fig. 9C). The take-home message from these data is that the ratio of total phytoplankton biomass to available nitrogen (giving an indication of whether or not nutrients are in excess of phytoplankton demand) may be a better predictor of phytoplankton succession and community composition than changes in nutrient ratios.

4.0 Summary and answers to questions

A number of the key findings from this review can be applied to answer the questions posed at the beginning of this review as follows:

1) *How are the cellular N:P ratios of phytoplankton affected by their surrounding nutrient environment (from limitation to sufficiency and even excess)?* The cellular N:P composition of phytoplankton is determined by the relative abundances of macromolecules (protein, nucleic acids, lipid bilayers) comprising the cell. At nutrient sufficient concentrations, phytoplankton can be characterized by a specific cellular N:P ratio (also called the optimal ratio); this ratio will remain the same no matter what the external ratio of nutrients is as long as absolute nutrient concentrations remain in excess of phytoplankton

requirements. At limiting concentrations of nutrients, cellular N:P ratios can change dramatically to accommodate a minimum cellular quota for the limiting nutrient in question.

2) *Can dissolved N:P ratios be used to predict levels at which nutrients start to inhibit growth of phytoplankton due to toxicity*? Each phytoplankton species has a toxicity threshold for nutrients, be it NH_4^+, NO_3^- or PO_4^{3-}. These levels differ by orders of magnitude from species to species (i.e. Collos and Harrison 2014) and are for the most part not known. Because toxicity and subsequent growth inhibition is determined based on an absolute concentration, this concentration can be achieved at an infinite number of different ratios by varying the non-inhibiting nutrient. Therefore, ratios of dissolved N:P cannot be used to predict levels at which nutrients negatively affect growth of phytoplankton due to toxicity and/or growth inhibition. With respect to toxicity of NH_4^+, it is most likely not due to NH_4^+ itself but to unionized NH_3 (see Drath et al. 2008 for details).

3) *How do concentrations of nutrients and ratios of dissolved N:P in San Francisco Bay compare with other estuarine systems?* While concentrations of nutrients, including Si, PO_4^{3-}, NH_4^+ and NO_3^-, in SFB are greater than typical for estuarine systems, they are well below concentrations that may result in toxicity or inhibition of phytoplankton growth. San Francisco Bay is a classical high nutrient, low chlorophyll (HNLC) system where a factor other than N or P limits the biomass accumulation of phytoplankton. This factor is most commonly the trace metal iron (i.e. De Baar et al. 1995, Cole et al. 2004, LaRoche et al. 1996) or irradiance (i.e. O'Donohue and Dennison 1997). In SFB, the limiting factor is irradiance (Cole and Cloern 1984, 1987, Alpine and Cloern 1988, Jassby et al. 2002). The hallmark of a HNLC system is that phytoplankton cannot grow according to their potential, and therefore cannot deplete all the nutrients available. As a result, a residual of nutrients is left over in the water column. This is in stark contrast with estuarine systems such as Chesapeake Bay where there is no limitation and phytoplankton grow until they deplete all the available nutrients from the water column (Malone et al. 1996).

4) *How do nutrient ratios observed in SFB affect phytoplankton community composition along the major north-south axis*? Phytoplankton cellular N:P ratios are most likely not affected by variations in dissolved N:P ratios along the north-south axis of SFB because total N concentrations are in excess of phytoplankton requirements. This allows phytoplankton to take up N and P in the proportions that each individual species requires without becoming limited, therefore there is no competition for resources. As a result, factors other than nutrient ratios become more important in determining phytoplankton community composition. In SFB major factors are turbulent, fast-moving water, relatively constant but low water temperatures, and absolute nutrient concentrations in excess of phytoplankton requirements. These conditions appear to function as diatom-producing factories, not only in SFB but also in 85 other coastal regions world-wide (Cloern and Dufford 2005, Carstensen et al. 2015).

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Table 1. Mean dissolved inorganic nutrient concentrations (µmol L^{-1}) in San Francisco Bay by embayment and season. Means and standard deviations based on data from 2006-2013 (http://sfbay.wr.usgs.gov/access/wqdata/). Suisun Bay = Station S6; San Pablo Bay = Station S15, Central Bay = Station S18, South Bay = Station S27. Stations in lower South Bay with higher nutrient concentrations have been omitted from this analysis. Data from the USGS sampling program (http://sfbay.wr.usgs.gov/access/wqdata/).

	Suisun Bay	**San Pablo Bay**	**Central Bay**	**South Bay**
DSi spring	246±43	139±41	72±37	72±33
DSi summer	211±22	96±24	49±10	90±29
NO_3^- spring	29±11	25±7	19±5	21±13
NO_3^- summer	26±9	24±5	19±4	22±6
NH_4^+ spring	5.7±2.4	4.4±1.3	3.5±1.5	4.4±2.9
NH_4^+ summer	3.3±1.9	4.5±1.5	5.0±1.7	4.4±1.9
PO_4^{3-} spring	1.9±0.5	2.2±0.6	1.8±0.4	3.0±1.0
PO_4^{3-} summer	2.2±0.5	2.9±0.5	2.3±0.3	5.5±1.0

Table 2. Changes in ratios and concentrations of nutrients in Chesapeake Bay from spring to summer. Data from Fisher et al. 1992, 1999

Bay Segment	**Dissolved N:P ratio (mol:mol)**		**DIN Concentration (µmol L^{-1})**	
	Spring	Summer	Spring	Summer
Oligohaline	>600	<20	>40	<5
Mesohaline	>300	<5	>20	<0.5
Polyhaline	>150	<5	>15	<0.5

Figure Legends

Figure 1. Cellular N:P ratios of various phytoplankton taxa compiled from culture studies. Horizontal red line indicates the Redfield Ratio (N:P=16 mol:mol). Adapted from Hillebrand et al. (2013).

Figure 2. A) Cellular N:P content of three species of phytoplankton as a function of external (medium) N:P ratio at nutrient concentrations in excess of phytoplankton demand. Blue=*Thalassiosira pseudonana*, green=*Monochrysis lutheri*, orange=*Dunaliella tertiolecta*. Data from Goldman et al. 1979. Note that species with higher cellular N:P ratios (such as cyanobacteria) were not included in the experiment. B) Schematic drawing of a phytoplankton cell illustrating the process of N and P uptake into the cell via dedicated transporters (ovals in diagram), and incorporation into macromolecules and organelles. Most of the N transported into the cell becomes associated with pigment-protein complexes in the chloroplast, the greatest store of N in the cell, while most of the P becomes associated with nucleic acids (RNA/DNA) in the nucleus and with ribosomes outside the nucleus, the greatest stores of P in the cell. See also Arrigo (2005) for further details. C) Build-up of N molecules outside the cell, exposed to a relatively high dissolved N:P ratio, as a result of the cell's uptake of N and P in a ratio dictated by its internal macromolecular composition (diagram of cell structure by Peter Westbrook). This build-up occurs because nutrient molecules are charged and cannot freely diffuse into the cell; transport into the cell occurs principally via active transport. However, facilitated diffusion, via aquaporins and channel proteins, documented to occur in vascular plants, is also hypothesized to occur in phytoplankton (Loque et al. 2009 and references therein). In root hairs, these channel proteins allows an influx of nutrients down its concentration gradient at external concentrations above thousand µmoles L^{-1} (Bligny et al. 1997). Whether this type of influx occurs in marine phytoplankton is not known.

Figure 3. A) Changes in cellular phosphorus content of the prymnesiophyte *Pavlova lutheri* content as a function of dissolved N:P ratio of the medium during faster growth (green) and slower growth (red). The green vertical line (N:P=30) denotes the critical ratio at faster growth, and the red vertical line (N:P=40) denotes the critical ratio at slower growth. Adapted from Terry et al. 1985. B) Changes in cellular phosphorus content of the diatom *Chaetoceros muelleri* as a function of dissolved N:P ratio of the medium at high irradiance (700 µmol photons m^{-2} s^{-1}) and low irradiance (50 µmol photons m^{-2} s^{-1}). Green vertical line (N:P=45) denotes the critical ratio at low light, and the red vertical line (N:P=30) denotes the critical ratio at high light C) Changes in cellular nitrogen content of *C. muelleri* as a function of dissolved N:P ratio of the medium at high irradiance and low irradiance. Adapted from Leonardos and Geider 2004.

Figure 4. Growth rates (GR, d^{-1}) of various phytoplankton taxa using NH_4^+ as their sole source of N for growth plotted as a function of growth rates of phytoplankton using NO_3^- as the sole source of N for growth (GR_{NH4}=0.9536(GR_{NO3})+0.028, R2=0.976). Data compiled from Ferguson et al. 1976, Dortch and Conway 1984, Levasseur et al. 1993, Saker and Neilan 2001, 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 5. A) Typical changes in NO_3^- (red line) and NH_4^+ (blue line) concentration with season in a temperate coastal system that is not light or trace-metal limited (data based on Butler et al. 1979). Period 1 (dark grey shading): the total N concentration (and also silicate, not shown here) varies from 8 to 34 µmol L^{-1} and NO_3^- comprises on average 92% and NH_4^+ comprises 8% of total N concentration. Period 2 (dark grey shading): the total N concentration varies around 1 µmol L^{-1}; NO_3^- comprises on average 12% and NH_4^+ comprises 87% of the total N concentration. B) Typical changes in phytoplankton community composition associated with the seasonal progression and changes in N concentrations above in A).

Figure 6. Nutrient and phytoplankton biomass variables from different regions of the North Atlantic Ocean, including the Sargasso Sea (red), Bermuda (green), Gulf Stream (cyan) and the New York Shelf Region (purple). A) Chl *a* (µg L^{-1}). B) Total nitrate (NO_3^- + NO_2^-, µmol L^{-1}), N. C) Ratio of dissolved N:P (mol:mol). Grey horizontal line indicates Redfield Ratio. D) Ratio of Chl *a*:N (µg:µmol). E) Phytoplankton community composition, measured with flow cytometer, converted to biomass (fraction 0-1). Ultraplankton denotes eukaryotic phytoplankton and includes diatoms for the Shelf region. Data from Cavender-Bares et al. 2001.

Figure 7. Nutrient and phytoplankton biomass variables from different portions of Chesapeake Bay, including the Oligohaline (freshwater endmember), Mesohaline (middle of the Bay), and Polyhaline (saltwater endmember). A) Chl *a* (µg L^{-1}). B) Ratio of dissolved silicate:nitrogen (Si:N, mol:mol). C) Ratio of dissolved N:P (mol:mol). Horizontal line indicating Redfield Ratio not distinguishable from baseline. D) Ratio of Chl *a*:N (µg:µmol). E) Phytoplankton community composition (fraction 0-1) based on biomass of various taxa. Data from Fisher et al. 1992, Adolf et al. 2006.

Figure 8. Mean dissolved nutrient and phytoplankton biomass variables (averaged across years 2006-2013) from different embayments in San Francisco Bay, including Suisun Bay (Station S6; freshwater endmember), San Pablo Bay (Station S15), Central Bay (Station S18; saltwater endmember), and South Bay (Station S27; marine lagoon). A) Chl *a* (µg L^{-1}). B) Ratio of dissolved Si:N (mol:mol). C) Ratio of dissolved N:P (mol:mol). Grey horizontal line indicates Redfield Ratio. D) Ratio of Chl *a*:N (µg:µmol), data from the USGS sampling program (http://sfbay.wr.usgs.gov/access/wqdata/). E) Phytoplankton community composition based on biomass of various taxa. Data from

Berg and Kudela (Interagency Ecological Program Project 2012) and from the USGS sampling program with permission from J. Cloern.

Figure 9. A) Diatoms as a fraction of total phytoplankton community biomass as a function of dissolved N:P ratio (mol:mol). B) Diatoms as a fraction of total phytoplankton community biomass as a function of the Chl *a*:N ratio (µg:mol). C) Cyanobacteria as a fraction of total phytoplankton community biomass as a function of the Chl *a*:N ratio (µg:µmol), in three different marine systems including Chesapeake Bay, the North Atlantic Ocean, and San Francisco Bay. Data from Fisher et al. 1992, Cavender-Bares et al. 2001, Adolf et al. 2006, and the USGS sampling program.
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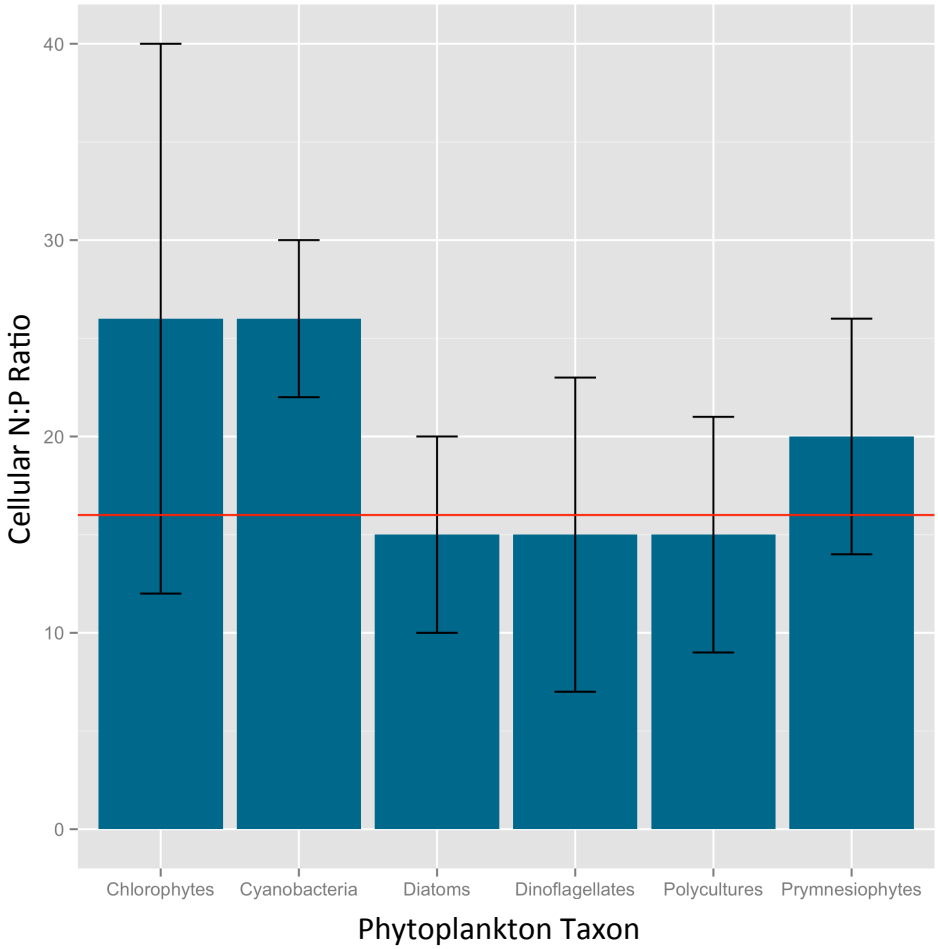
Cellular N:P Ratio
40
30
20
10
0
Chlorophytes
Cyanobacteria
Diatoms
Dinoflagellates
Polycultures
Prymnesiophytes
Phytoplankton Taxon

Figure 1

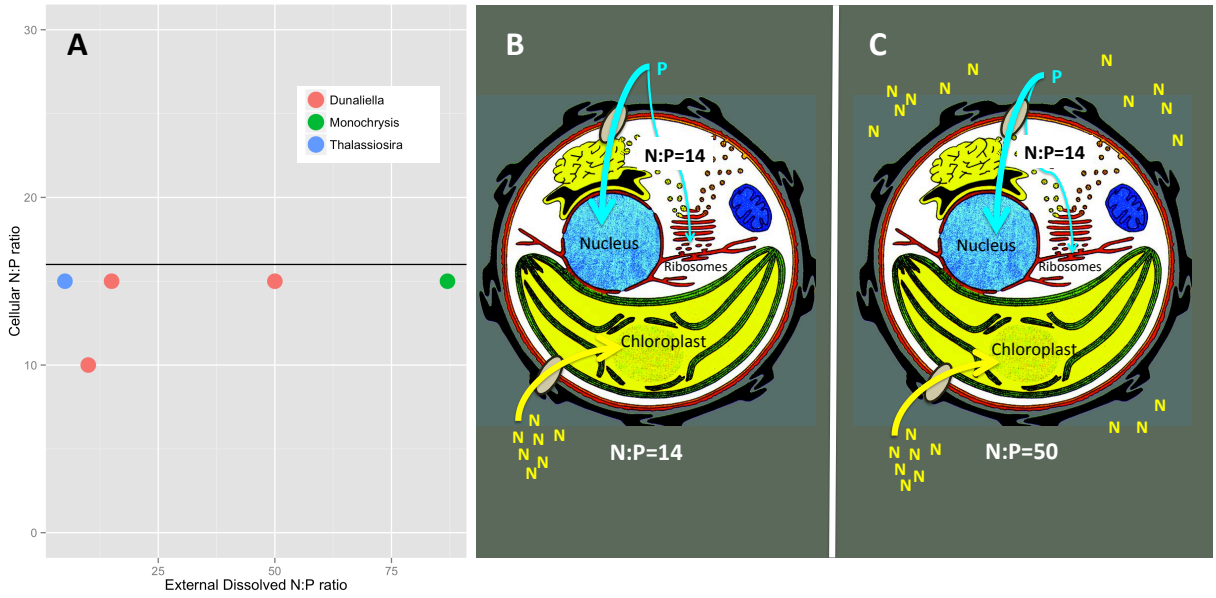
A
B
C
Dunaliella
Monochrysis
Thalassiosira
Cellular N:P ratio
External Dissolved N:P ratio
30
20
10
0
25
50
75
N:P=14
Nucleus
Ribosomes
Chloroplast
N:P=14
N:P=14
Nucleus
Ribosomes
Chloroplast
N:P=50
P
N

Figure 2

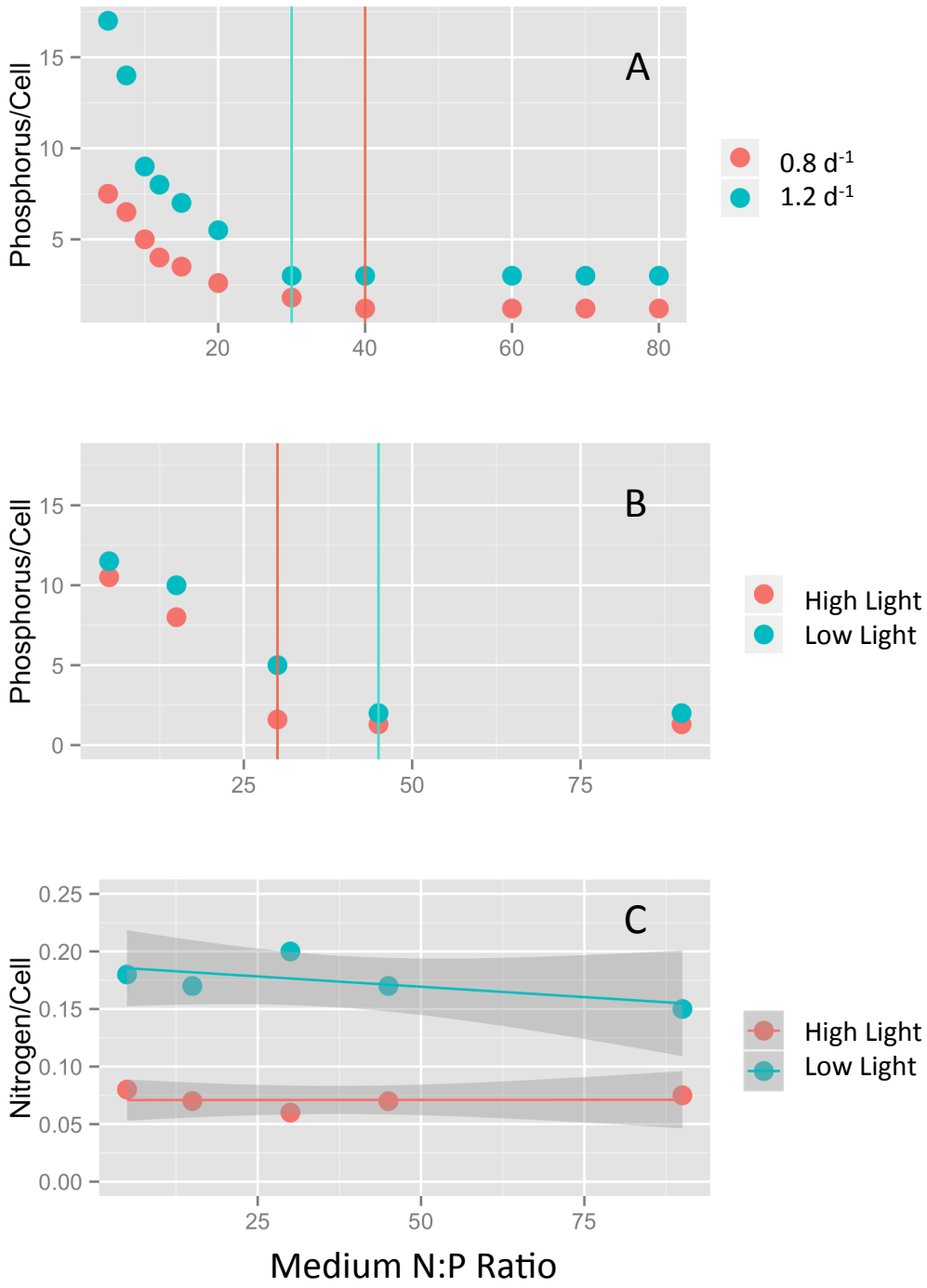
A
Phosphorus/Cell
15
10
5
20
40
60
80
0.8 d-1
1.2 d-1
B
Phosphorus/Cell
15
10
5
0
25
50
75
High Light
Low Light
C
Nitrogen/Cell
0.25
0.20
0.15
0.10
0.05
0.00
25
50
75
High Light
Low Light
Medium N:P Ratio

Figure 3

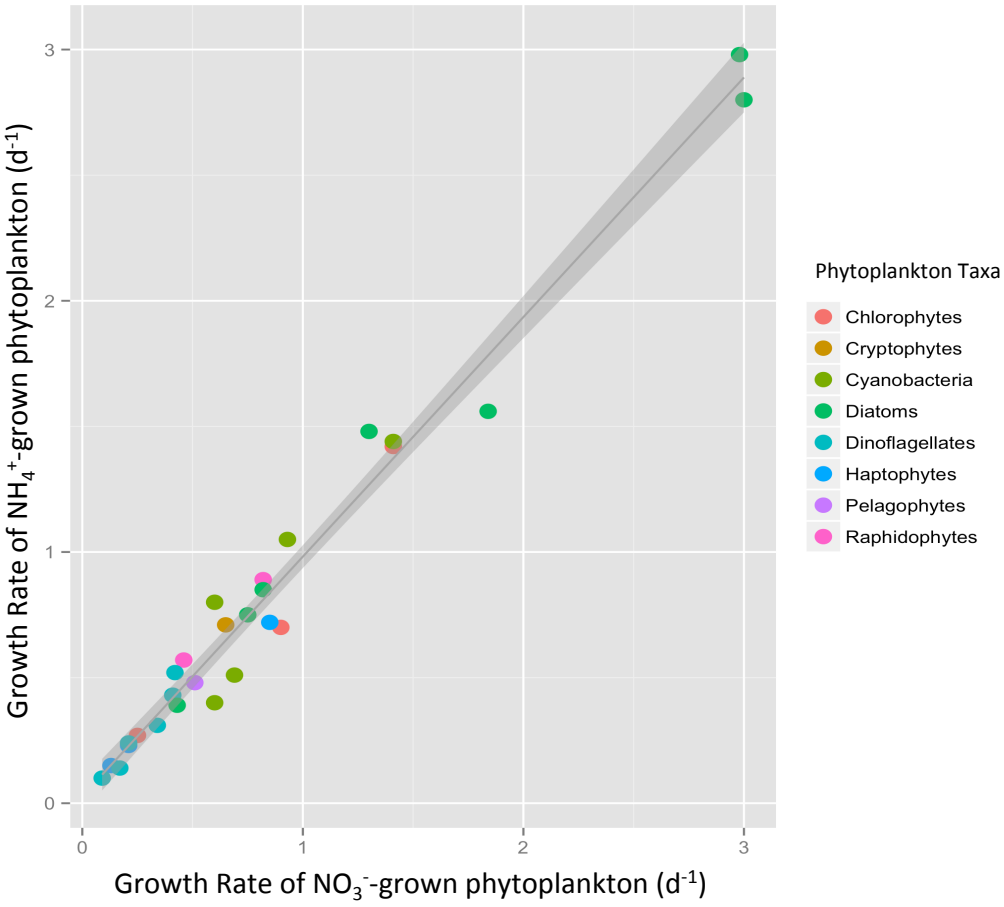
Growth Rate of NH_4^+-grown phytoplankton (d^{-1})
Growth Rate of NO_3^--grown phytoplankton (d^{-1})
0
1
2
3
Phytoplankton Taxa
Chlorophytes
Cryptophytes
Cyanobacteria
Diatoms
Dinoflagellates
Haptophytes
Pelagophytes
Raphidophytes

Figure 4

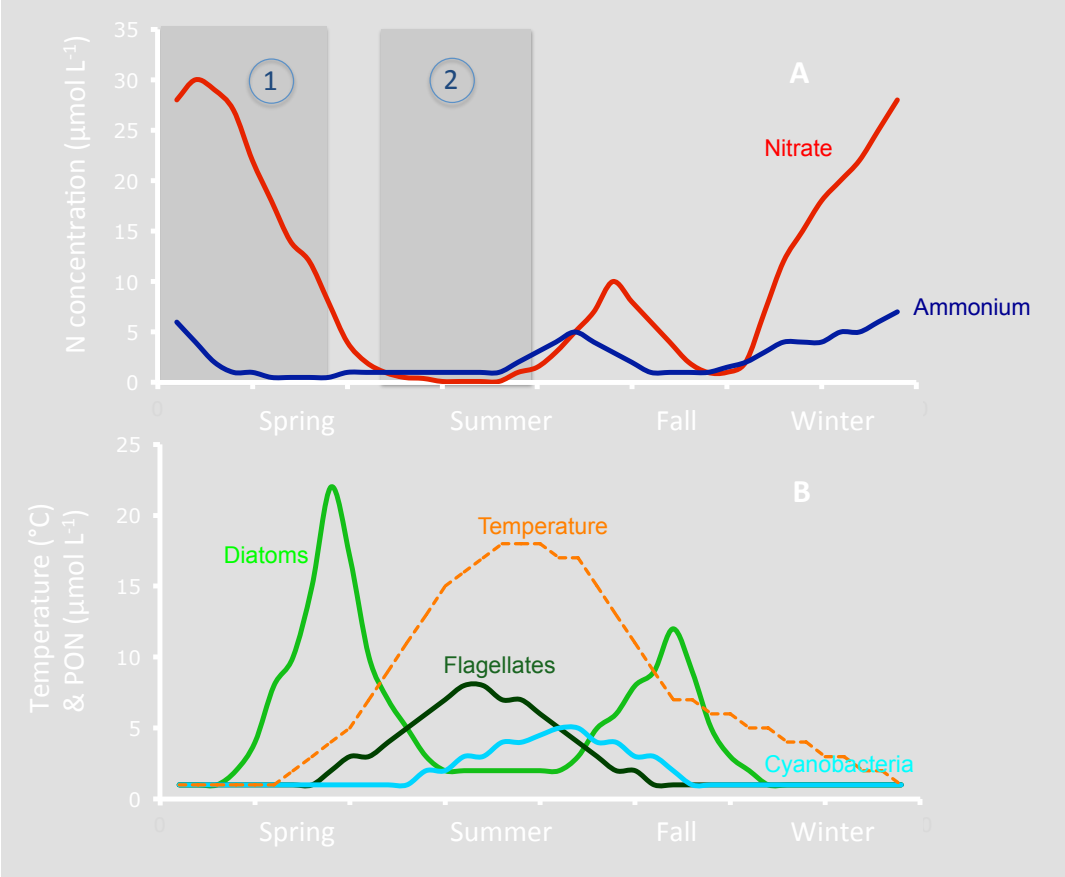
N concentration (µmol L⁻¹)
35
30
25
20
15
10
5
0
1
2
A
Nitrate
Ammonium
Spring
Summer
Fall
Winter
Temperature (°C)
& PON (µmol L⁻¹)
25
20
15
10
5
0
B
Diatoms
Temperature
Flagellates
Cyanobacteria
Spring
Summer
Fall
Winter

Figure 5

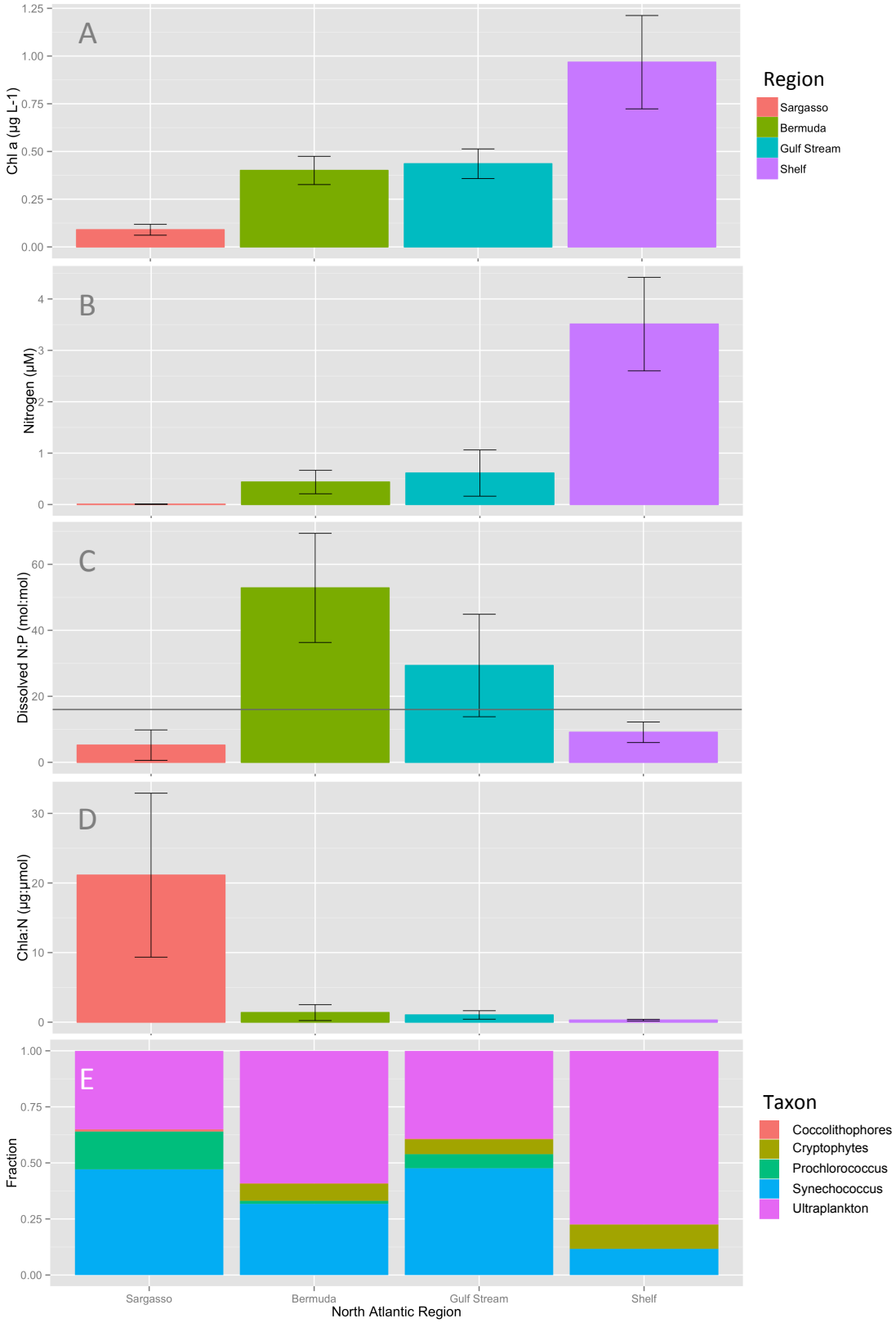
A
B
C
D
E
Chl a (µg L-1)
Nitrogen (µM)
Dissolved N:P (mol:mol)
Chla:N (µg:µmol)
Fraction
Region
Sargasso
Bermuda
Gulf Stream
Shelf
Taxon
Coccolithophores
Cryptophytes
Prochlorococcus
Synechococcus
Ultraplankton
North Atlantic Region

Figure 6

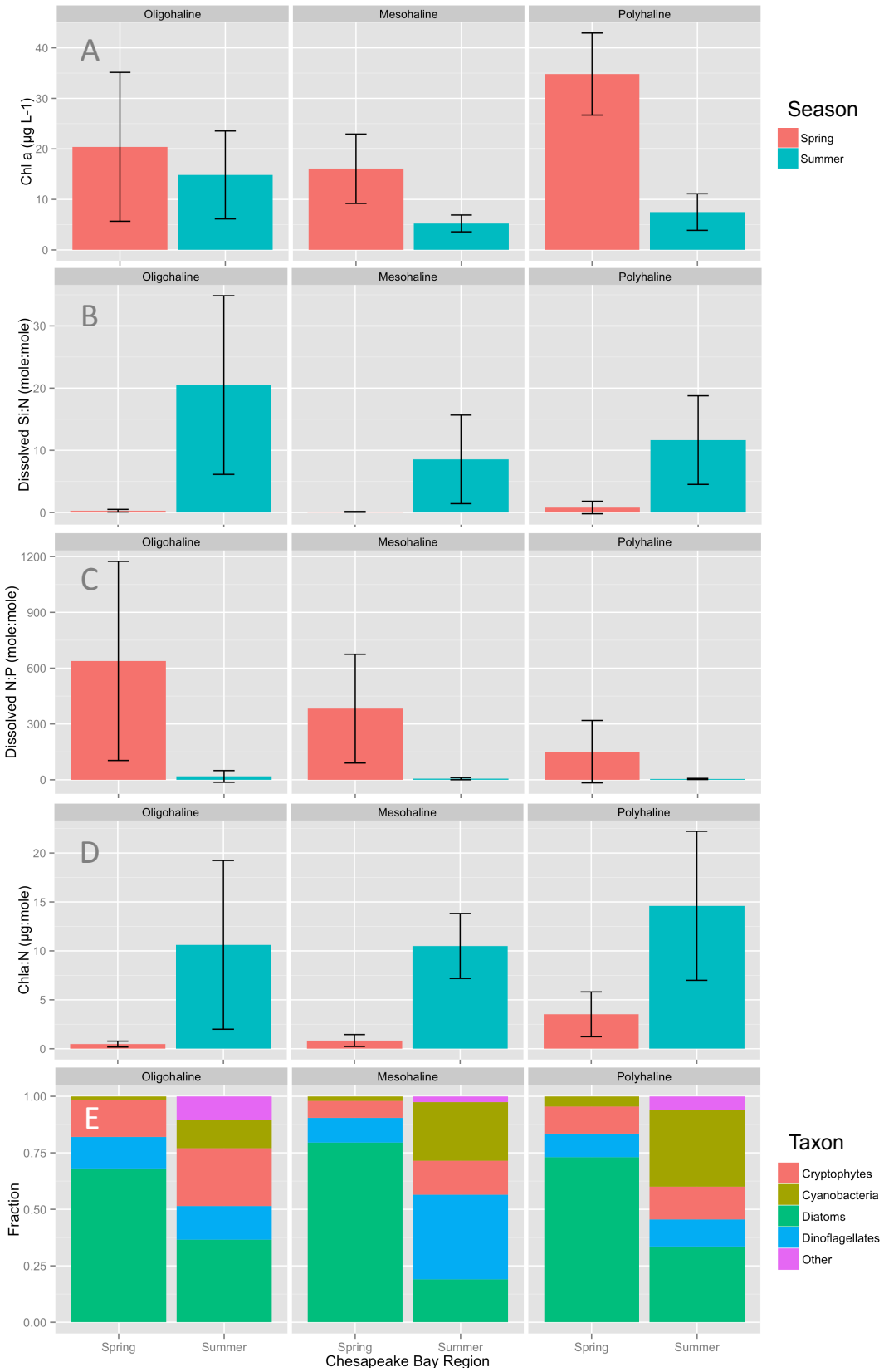
Oligohaline
Mesohaline
Polyhaline
A
Chl a (µg L-1)
B
Dissolved Si:N (mole:mole)
C
Dissolved N:P (mole:mole)
D
Chla:N (µg:mole)
E
Fraction
Spring
Summer
Chesapeake Bay Region
Season
Spring
Summer
Taxon
Cryptophytes
Cyanobacteria
Diatoms
Dinoflagellates
Other

Figure 7

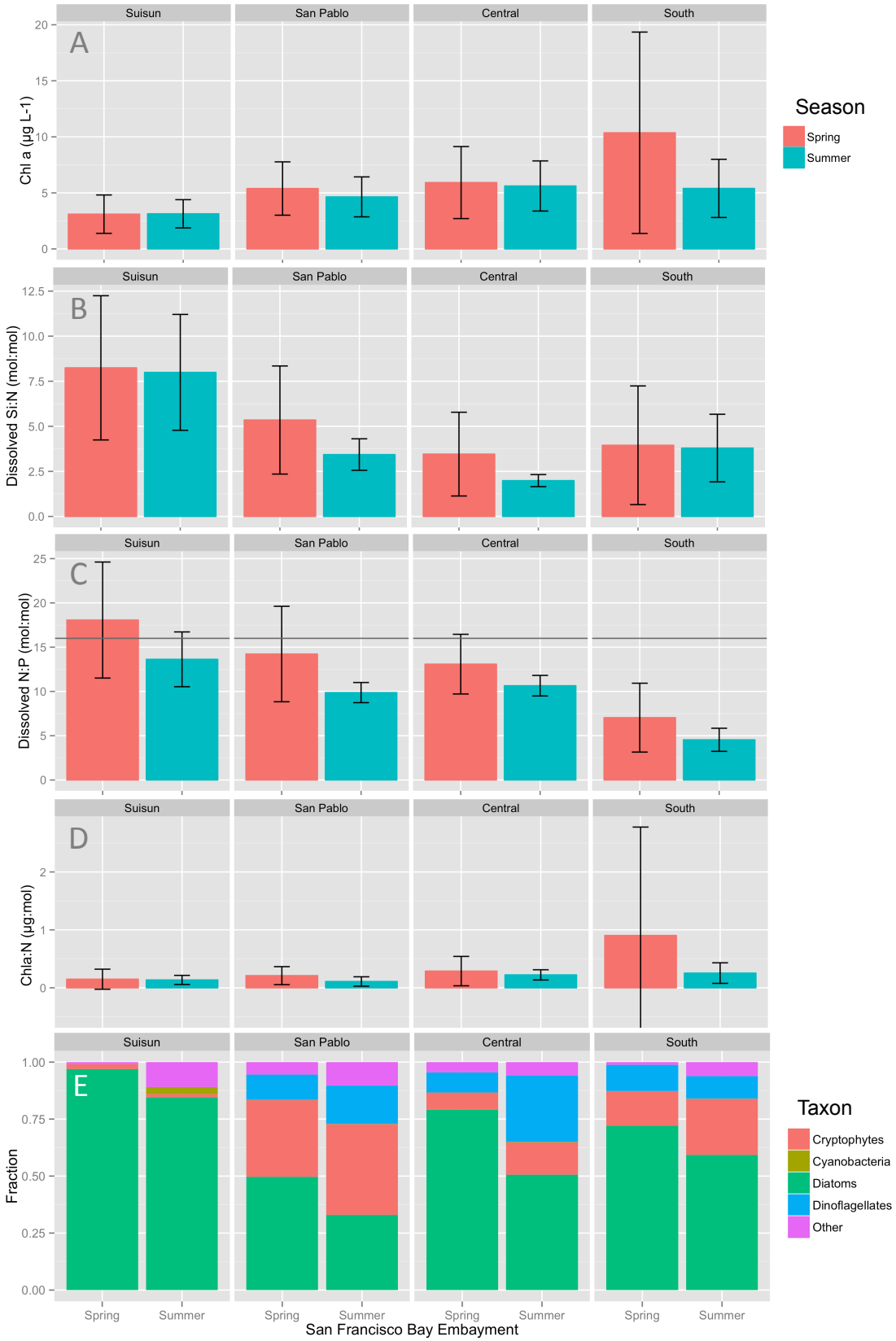
Suisun
San Pablo
Central
South
A
Chl a (µg L-1)
B
Dissolved Si:N (mol:mol)
C
Dissolved N:P (mol:mol)
D
Chla:N (µg:mol)
E
Fraction
Spring
Summer
San Francisco Bay Embayment
Season
Spring
Summer
Taxon
Cryptophytes
Cyanobacteria
Diatoms
Dinoflagellates
Other

Figure 8

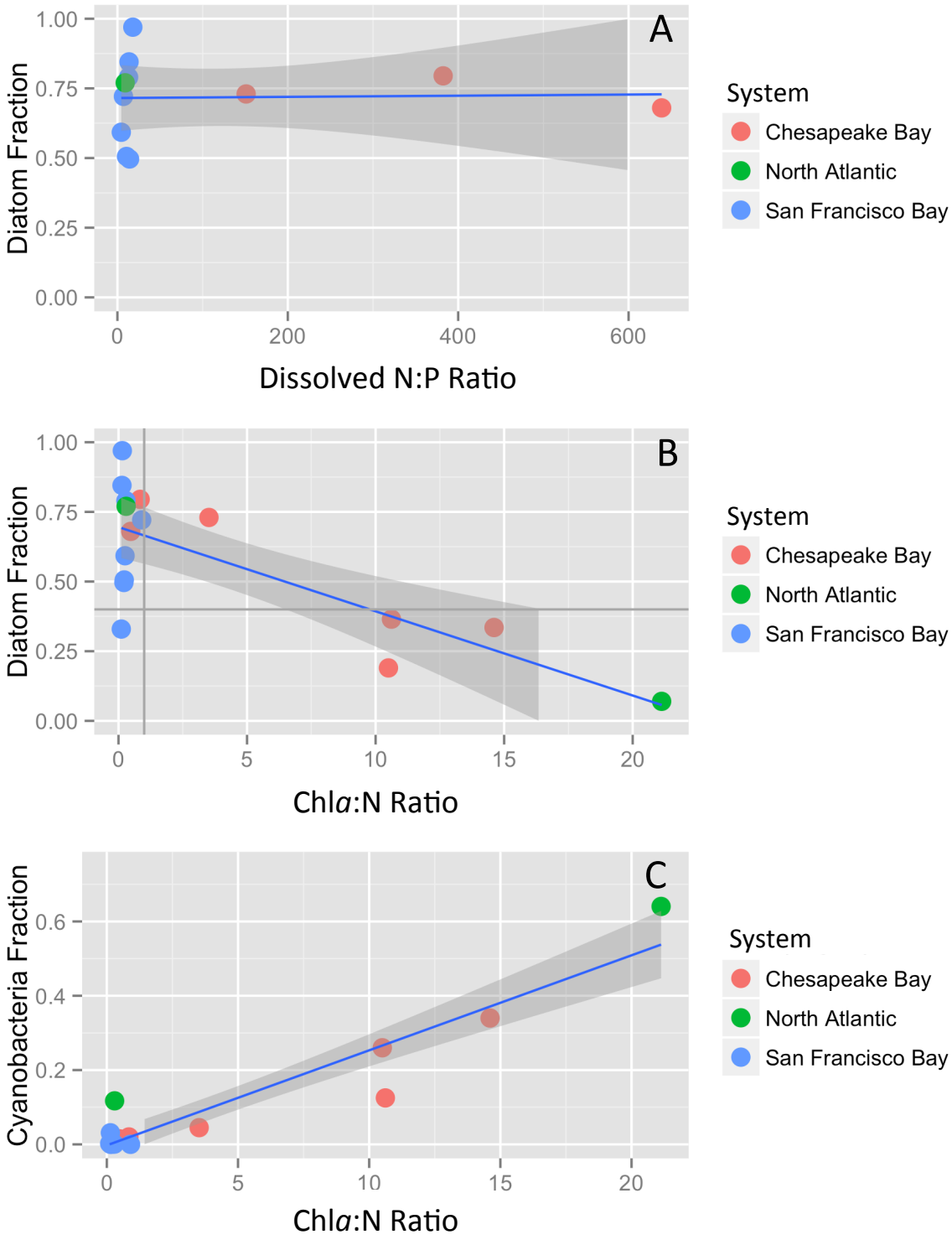
A
Diatom Fraction
1.00
0.75
0.50
0.25
0.00
0
200
400
600
Dissolved N:P Ratio
System
Chesapeake Bay
North Atlantic
San Francisco Bay
B
Diatom Fraction
1.00
0.75
0.50
0.25
0.00
0
5
10
15
20
Chl*a*:N Ratio
System
Chesapeake Bay
North Atlantic
San Francisco Bay
C
Cyanobacteria Fraction
0.6
0.4
0.2
0.0
0
5
10
15
20
Chl*a*:N Ratio
System
Chesapeake Bay
North Atlantic
San Francisco Bay

Figure 9

Appendix A.

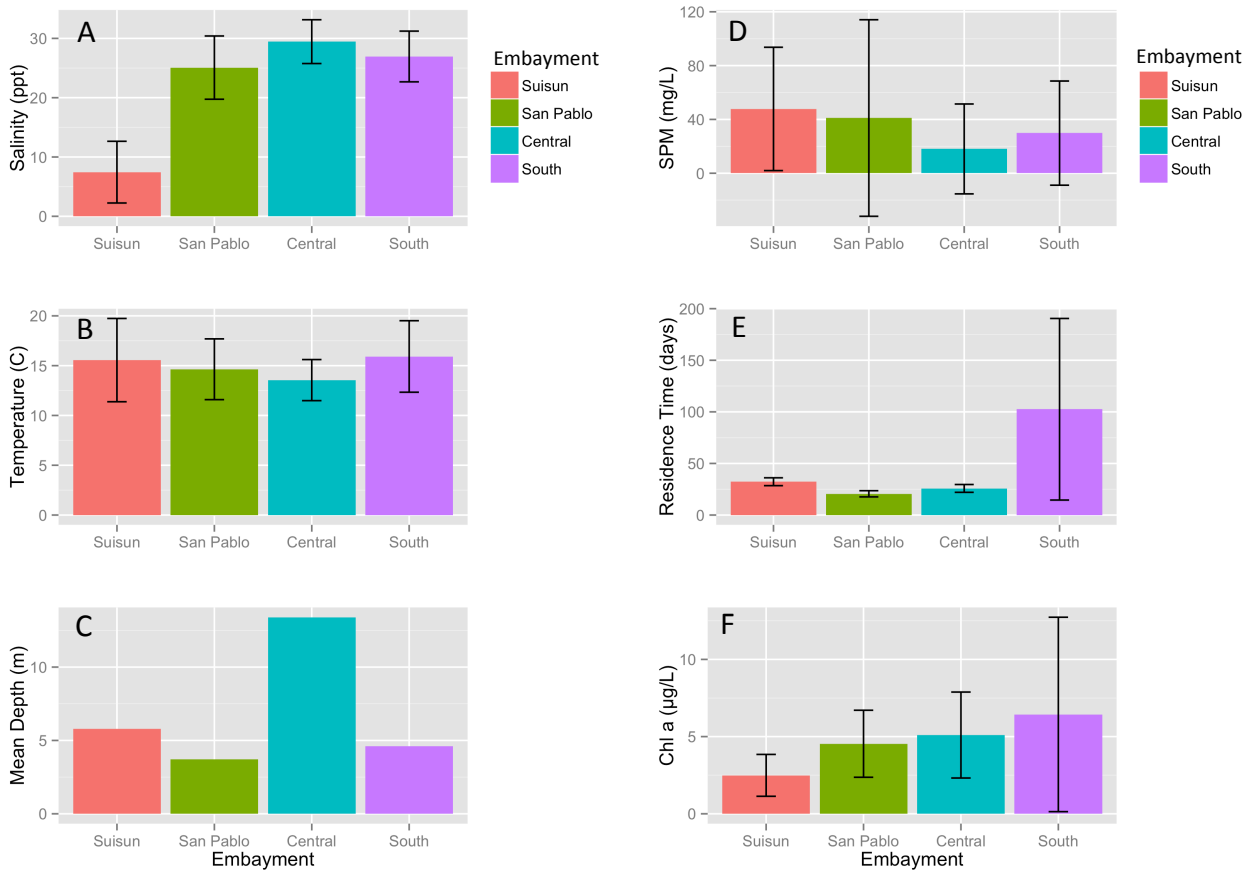

Figure A-1. Annual mean and standard deviation of environmental and biological variables (averaged across years 2006-2013) from different embayments in San Francisco Bay. A) Salinity, B) Temperature, C) Mean basin depth, D) Suspended particulate matter, E) Residence time (Data from Smith 1987), F) Chl *a*. Data from the USGS sampling program (http://sfbay.wr.usgs.gov/access/wqdata/).

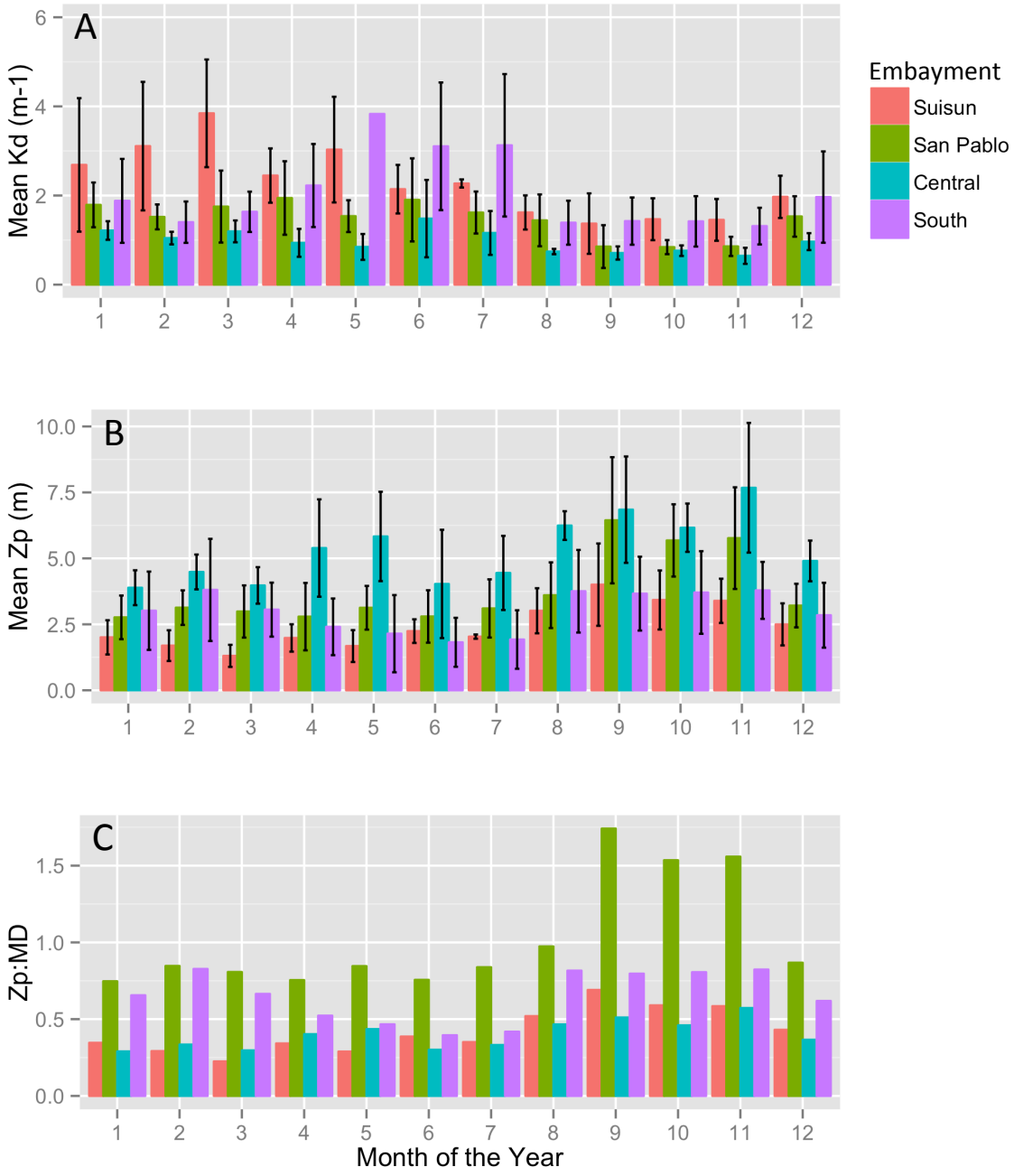

Figure A-2. Mean and standard deviation of irradiance variables (averaged across years 2006-2013) from different embayments in San Francisco Bay. A) Light attenuation coefficient, (K_d, m^{-1}) reflects how SPM in the water column attenuates incoming surface irradiance. B) Euphotic zone depth, Z_p., the depth that light penetrates in the water. C) Ratio of euphotic zone depth to mixed layer depth, Z_p:MD, used to calculate the time phytoplankton spend in the light versus the dark. Light availability combined with residence time may be critical for phytoplankton growth in SFB. Data from the USGS sampling program (http://sfbay.wr.usgs.gov/access/wqdata/).

“Shifts” in Suisun Bay and Delta phytoplankton communities? Addressing issues with data quality.

David B. Senn
San Francisco Estuary Institute

1. Introduction: northern San Francisco Bay-Delta

The San Francisco Bay-Delta, or San Francisco Estuary (SFE), is California’s largest estuary (Figure 1). Flows from Sacramento and San Joaquin Rivers move through and merge within the Delta, carrying drainage from approximately 40% of California, including the agriculturally-rich Central Valley. The Delta has undergone physical alterations over the past 150 years that have dramatically altered the system’s hydrology, terrestrial and aquatic habitats, and the ecosystem services these habitats provide (e.g., Whipple et al., 2012). The Delta also receives considerable inputs of treated wastewater effluent and agricultural runoff, both of which contribute nutrients and other contaminants. In addition, a substantial portion of water entering the Delta (≥25% annually) is exported for irrigated agriculture and domestic water use (Jassby 2008).

Long-term monitoring shows that the northern SFE (nSFE; Suisun Bay and the Delta) is in a state of severe ecological decline, with population collapses of several pelagic fish species. A multi-agency applied science program (Interagency Ecological Program, IEP) has been exploring the causes of those declines, with results pointing to the combined effect of multiple anthropogenic stressors (Sommer et al, 2007; Baxter et al. 2010; Hanak et al. 2013, Meyer et al. 2009 and NRC 2012), including: landscape alterations, species invasions, water withdrawals, and agriculturally- and wastewater-derived contaminants including nutrients.

An alternative conceptual model was recently proposed that identifies excess nutrient inputs as a primary driver of ecosystem decline in the nSFE (‘ecological stoichiometry’; Glibert 2010; Glibert et al. 2011). The ecological stoichiometry conceptual model is based on the hypothesis that elevated ammonium (NH_4^+) concentrations, or altered ratios of N to P (N:P), caused shifts in the phytoplankton community composition toward assemblages that poorly support the food web, which in turn exerted bottom-up adverse impacts on primary and secondary consumers. Excess anthropogenic nitrogen (N) and phosphorous (P) inputs have indeed profoundly impacts on many estuarine and coastal ecosystems worldwide (Diaz and Rosenberg, 2008; Cloern, 2001; Nixon, 1995; NRC coastal hypoxia report), causing hypoxia, loss of submerged aquatic vegetation (SAV) habitat, and harmful algal blooms (HABs; Anderson et al., 2002). Nutrient concentrations are indeed elevated in areas of the nSFE, due to both wastewater inputs and agricultural runoff (e.g., Cloern and Jassby, 2012; SFEI 2014, 2015), and excess nutrients are recognized to be among the nSFE’s multiple stressors (NRC 2011, Baxter

2010). The ecological stoichiometry conceptual model is unique, though, in that it assigns nutrients a pivotal role.[1]

Since publication of the initial studies (Glibert 2010, Glibert et al 2011), the notion of a nSFE phytoplankton community "shift" has become a common baseline assumption about the Delta's ecological condition in subsequent literature (e.g., Dugdale et al., 2012; Parker et al., 2012a,b; Glibert et al 2012; Glibert et al 2014). In addition, the hypothesized mechanistic link between the phytoplankton community shift and increased anthropogenic ammonium loads has influenced management decisions (e.g., CA State Water Board, 2012) as well as policy decisions related to science and monitoring priorities to support restoration and management of the Delta (DSC 2013).[2]

The empirical evidence for the proposed phytoplankton shift is the analysis of long-term phytoplankton monitoring data from Suisun Bay and the Delta. As part of a separate study, we began a project that continued the lines of inquiry initiated by Glibert and colleagues (2010, 2011), with the goal of exploring associations between phytoplankton community and environmental factors that could shape community (e.g., nutrients, salinity, light, residence time, grazing). After delving into initial data analysis, we began noticing repeated peculiarities within the dataset, especially post-1987, which led us to more closely examine data quality. This closer examination revealed that data quality is compromised by major departures from standard phytoplankton enumeration methods which resulted in extremely small cell counts and large uncertainties.

In this paper, we explore the same phytoplankton community dataset used by Glibert and colleagues (2010, 2011), and present:

1. Descriptive and quantitative overviews of the phytoplankton community data quality issues and underlying causes.
2. Reanalysis of the data with statistical models that address the uncertainty and methodological issues, and resulting interpretations

2. Study Sites and Data

The Department of Water Resources (DWR) has managed water quality and biological monitoring programs in the nSFE since ~1975 (DWR Environmental Monitoring Program, EMP; Figure 1). Samples for phytoplankton characterization have been collected once or twice per month over the period of 1975-present at a total of 32

[1] *"...management strategies to date have not reversed fish declines because they have not addressed the ultimate cause of the change at the base of the food web and the complex role of nutrient form and quantity. The present study supports the premise that reduction of the NH_4^+ effluent into the Bay Delta is essential to restoring historic pelagic fish populations and that until such reductions occur, other measures, including regulation of water pumping or manipulations of salinity, as has been the current strategy, will likely show little beneficial effect."* [Glibert et al 2011]

[2] For example, the recent "Delta Plan" (DSC, 2013), which lays out high-priority management challenges and science needs for the Delta and Suisun Bay, states: "Changes in the types of algae that form the base of the aquatic food web, including growth of toxic algae, have been linked to excessive amounts or altered ratios of plant nutrients...Ratios of nutrients in Delta waters are thought to be a primary driver in the composition of aquatic food webs in the Bay-Delta (Glibert et al. 2011)."

stations. Of these stations, 8 have complete phytoplankton over the entire period and others for shorter periods (Figure 1).

Phytoplankton data was downloaded from the DWR website as the file 'flatfile.xls' (http://www.water.ca.gov/iep/products/data.cfm; last accessed 9/1/2015) which contained both density estimates (cells/ml) and number of cells counted at the species level (total of 592 phytoplankton species) for the period 1975-2012. As discussed in more detail below, DWR changed its methodology for microscopy enumeration in 2008. The analysis presented below focuses on the ~30 year record of 1975-2006, similar to prior studies (Glibert 2010; Glibert et al., 2011). We aggregated the data by binning densities into seven phytoplankton classes (diatoms, dinoflagellates, euglenoids, green algae, cyanobacteria, cryptomonads, and flagellates), using classifications at this level included as a field in the dataset, consistent with the data aggregation methods in prior studies (Lehman 1992, 2000, 2004; Glibert 2010; Glibert et al. 2011). The results and interpretations are similar for all the Suisun stations, and, for the sake of brevity, the discussion below focuses primarily on data from station D7 in Suisun Bay (Figure 1). Results from other stations are presented in the Appendix. DWR EMP data is also available for nutrients, chl-a, suspended sediment concentrations (relaed to light levels or turbidity), and other parameters, but was not used in this paper.

3. Results and Discussion

3.1 First-glance observations

Prior to 1987, Suisun Bay experienced substantial phytoplankton blooms, with elevated phytoplankton biomass (chl-a > 10 µg/L) occurring for multiple months each year (Figure 2). Considerable interannual variability in the blooms magnitde was also evident through the mid-1980s, some of which resulted from large variability in freshwater flow entering Suisun including years with among the highest (1983) and lowest (1977) flows on record (Alpine and Cloern 1992). Beginning in 1987, phytoplankton biomass dropped precipitously, and remained at low levels with only rare and short-lived blooms over the subsequent 25 years. The large decrease in phytoplankton biomass in Suisun Bay and the western Delta (Jassby 2008) is considered to be among the factors contributing to fish population declines due to decreased food availability (Sommer et a.l 2007; Baxter al. 2010). Because of its major ecological impact on higher trophic levels, the overall phytoplankton biomass decrease has been studied extensively, with most investigations attributing the large decrease to the establishment of the invasive clam *Potamocorbula amurensis* in Suisun Bay, San Pablo and the far-western Delta around 1987 (e.g., Alpine and Cloern 1992; Nichols et al, 1990; Jassby, 2008; Kimmerer and Thompson, 2013). More recently, several studies have argued that high NH4 inputs have contributed to the biomass decrease in Suisun Bay through different mechanisms, including: limiting phytoplankton growth rates (e.g., Dugdale et al., 2007; Parker et al., 2012a,b; Dugdale et al, 201X).

Our analysis of the nSFE phytoplankton community composition began by using the density values (cells mL^{-1}) provided as a pre-calculated field in the DWR dataset (Figure

3A-D). We initially used this data “as is”, and deemed it to be an acceptable starting point in terms of data QA/QC since several peer-reviewed studies had already used those same values reported changes in phytoplankton community composition (Lehman, 1992, 2000, 2004; Glibert 2010, Glibert et al. 2011). Prior to 1986, diatom abundance prior to 1986 suggest there was a strong seasonality in diatom densities prior to 1986, and that a dramatic decrease in diatom densities occurred after 1986 (Figure 3a). In general, cell densities are an imprecise metric for describing phytoplankton community biomass or biovolume (e.g., μg C L^{-1} or μm^3 L^{-1}), because the size of individual cells can differ by several orders of magnitude, especially between classes or genera (ref). In San Francisco Bay, however, diatoms have generally dominated phytoplankton biovolume during blooms (e.g., Cloern and Dufford, 2005). The timing of the apparent diatom density decrease in Figure 3A coincides with the dramatic decrease in chl-a (Figure 2), consistent with diatoms having comprised a large portion of the phytoplankton biomass, and observation consistent with other studies (e.g., Kimmerer 2004).

Monthly density time series for four other phytoplankton classes are also presented in Figure 3A-D: flagellates, cryptophytes, greens, and cyanobacteria. While their densities fluctuated over time, clear temporal trends are not as readily discernible by visual inspection for these classes.

3.2 Interpretations made for ecological stoichiometry conceptual model

The data presented in Figure 3A-D are the observational data upon which the ecological stoichiometry conceptual model is based, specifically its premise that phytoplankton community composition has undergone a shift toward assemblages that poorly support the foodweb (Glibert 2010; Glibert et al., 2011). Glibert (2010), after a statistical transformation (CUSUM) of monthly class-level data at a Suisun Bay station (D8), argued that diatom densities decreased over time, and that the densities of several other classes increased (flagellates, cryptophytes, green algae, and cyanobacteria). The paper describes 3 eras of phytoplankton community in which classes of organisms play roles of greater relative importance in terms of major flows of energy and material: diatom era (pre-1982); cryptophyte and flagellate era (1983-1999); and cyanobacteria era (2000-2005). Glibert 2010 explains the cause of the community shift as resulting from a shift in the competitive advantage “to phytoplankton taxa that can that can more efficiently use reduced forms of N. Among the phytoplankton groups that replaced diatoms in this system, cyanobacteria and many flagellates have a preference for chemically reduced forms of N…” The underlying statistical analysis used to support this NH4:community-shift linkage has been challenged (Cloern et al., 2010).[3] Since the current paper remains focused on phytoplankton data quality, the statistical approaches from other studies are not explored further here..

[3]The proposed causal relationship between increasing NH4 concentration and changes in phytoplankton densities was based on regressions of CUSUM-transformed densities vs. CUSUM-transformed NH4 concentration. Cloern et al. 2010 subsequently argued that, when doing regression analysis of CUSUM-transformed variables, “high correlations may appear where none are present in the untransformed data”, and that “[r]egression analysis on CUSUM-transformed variables is, therefore, not a sound basis for making inferences about the drivers of ecological variability measured in monitoring programs.”

In their subsequent study, Glibert et al. (2011) used the same EMP phytoplankton dataset, but combined all Suisun Bay stations (D7, D8, and D4) and computed annual average densities (March-November). Their analysis pointed to statistically significant changes in the diatom (decrease) and dinoflagellate (increase) densities. In contrast to Glibert (2010), Glibert et al (2011) note that increasing trends were not detected for green algae, cryptophyte, or cyanobacteria.

3.3 Examining data quality and uncertainty

The motivation for our initial foray into exploring the EMP phytoplankton data was the opportunity it presented for systematically analyzing a large, diverse, multi-station, and multi-decade dataset (Figure 1), and quantitatively exploring potential causal links between variations in phytoplankton community composition and chemical and physical drivers (nutrients, temperature, light, etc.). We expected that answers to the following questions would shed light on some of the major drivers influencing phytoplankton community:

1. Which best describes the changes in phytoplankton community (Figure 4)?
    a. H0: No change in composition.
    b. H1: Primarily a loss a loss of diatoms, with little change in other classes
    c. H2: All classes decreased proportionally
    d. H3: Decreased diatoms, substantially increased non-diatoms
2. When did shifts occur, were they gradual or sharp, and in which areas of Suisun Bay and Delta were they most pronounced?
3. What combination of chemical and physical drivers (data that is also available for those sites) best explain the spatial and temporal variability in the phytoplankton community and biomass, and what are the relative importance of those drivers?
4. If nutrient concentrations played an influential role in shaping phytoplankton community, can ‘protective’ nutrient concentrations be inferred from analyzing historic data?

After delving into initial data analysis, we began to repeatedly notice peculiarities with the dataset, especially post-1987. For example, between 1987 and 2007, entire classes were frequently absent during monthly observations (no diatoms: 41%; no flagellates: 57%; no cryptophytes: 44%; no green algae: 74%). Flagellates and cryptophytes were often absent for multi-month stretches, followed by windows when flagellates or cryptophytes were detected, before dropping back to zero. If taken literally, the data also suggested that more than a third of the time conditions at D7 were selecting for mono-class phytoplankton assemblages (all flagellates: 10%; all cryptophytes: 12%; all diatoms: 15%). These observations are at odds with basic principles of phytoplankton ecology, and provided early hints of data quality issues, prompting us to more closely examine the raw data and laboratory methods.

Figure 5 illustrates a standard enumeration method for counting and estimating densities (cells/mL) of phytoplankton taxon (species, class, etc.) in which phytoplankton are allowed to settle from the sample and are then counted by microscopy (Karlson et

al, 2010). Three variables determine the density estimate: the area of the counting chamber that is analyzed by the microscopist (A_{count}); the volume of water that settles on the chamber (V_{settle}); and the actual number of enumerated cells (C). In order to obtain a statistically robust enumeration, a minimum number of counts is necessary. The uncertainty associated with a density estimate decreases as the number of cells counted increases, with sharply increasing uncertainty at low counts (Figure 5). A standard practice in phytoplankton enumeration is to count a minimum of 400 phytoplankton units per sample, which yields an expected error, or uncertainty, of ±10%. Since counting is labor-intensive and therefore expensive, many studies trade less effort for greater uncertainty and count 50 units of the dominant species, yielding an expected uncertainty of 28% (Karlson et al. 2010). While the microscopist counts randomly selected grid cells, eventually reaching their target for the most abundant organism taxa (e.g., 50 or 400 cells), they also count all the other organisms in those grid cells. If a similar level of uncertainty is desired for less abundant taxa, 50 or 400 of those organisms would also need to be counted.

A closer examination of the EMP dataset, focusing on the actual cell counts (C) instead of densities, found that the cell enumeration protocol deviated considerably from standard practices. Low cell counts were common at D7, with a mean of ~6 total cells counted each month over the period 1987-2006 (Figure 5). Over the entire period of 1975-2006, counts never exceeded the 400-cell threshold, and the 50-cell threshold for the dominant species was met or exceeded in only 12% of samples, with most of these (52 out of 53) occurring prior to 1987. The units in Figure 5 are total cells counted; therefore, the number counted for each class of organisms was less than or equal to the total counts. The low cell count issue observed at D7 was common to all phytoplankton stations (Figure A.1), indicating that this was standard practice. After noting this low-count issue, we looked closer at the description of EMP's enumeration method. It states that a modified version of the standard protocol was followed[4]: 20 microscope fields (F) were counted for each sample, not a minimum number of organisms.

The method modification and low cell counts substantially impacted the confidence intervals. In Figure 7, the densities are replotted, this time including confidence intervals based on the number of enumerated cells. During bloom periods prior to the mid-1980s, >50 total cells were commonly counted in each sample (Figure 5). While the number of counts falls short of recommended practices and results in large confidence intervals, the confidence intervals are sufficiently small relative to seasonal diatom variations that a seasonal cycle can still be discerned. After the mid-1980s, however, enumerated diatoms dropped dramatically. On many dates post-1987, no diatoms were reported, and, for the majority of dates from 1987-2006 on which diatoms were detected, only a single diatom was counted.

The other phytoplankton classes are more severely impacted by data quality issues. The number of flagellates enumerated was almost always quite low, especially

[4] http://www.water.ca.gov/bdma/meta/phytoplankton.cfm

post-1987 (Figure 7B). In addition, flagellate densities were reported as 0 cells/mL for a large portion of observations, both before and after 1987. However, total counts for these dates were often low, and, as a result, the flagellate density estimates have large confidence intervals (Figure 7B). Even prior to 1987, flagellate counts were low enough that the confidence intervals commonly spanned a range of ±50% fo the estimated value. Cryptophytes, green algae, and cyanobacteria densities suffer similar data quality issues, and also have large confidence intervals.

These data quality issues have not been described in past studies that have proposed temporal shifts in Bay-Delta phytoplankton community composition (Lehman, 1992, 2000, 2004; Glibert 2010, Glibert et al. 2011). The uncertainties were not taken into account in statistical tests used by Glibert and colleagues (2010, 2011). In addition, upon closer examination, in at least some of those studies, dates when densities were zero appear to have been excluded when computing annual means (Glibert et al., 2011) and when plotting and analyzing monthly data (Glibert 2010).

After presenting initial findings on the data quality problems (Malkassian et al., 2014; Cloern et al, 2015), we learned of additional methodological issues. DWR provided us with another dataset that contained relevant information for each sample: the V_{settle}, magnification used, and the number of grids (G) counted (T Brown, DWR, pers. comm., Sep 3, 2015). Prior to receiving this data, our working assumption about the sharp change in counts around 1988 (Figure 6) was that it was related to the *Potamocorbula* invasion, and generally lower phytoplankton biomass in samples. The additional data, however, indicate that a substantial method change occurred in 1988: the magnification changed from 350x to 700x for station D7 (Figure 8) and all other stations (Figure A.1). As a result of the increased magnification, the area captured within the microscope's view decreased approximately 4-fold (Figure 8). Although DWR had also adjusted magnification in prior years, those adjustments were generally offset by changes in the number of fields counted (G) or in $V_{settled}$, thus maintaining the volume counted (V_{count}) at a fairly constant value. When magnification changed in 1988, however, neither G nor $V_{settled}$ was consistently increased, resulting in V_{count} decreasing by a factor of ~4. After this 1988 method change, reported densities were based on counting only 0.1-0.2% (typically 0.01-0.02 mL) of the sample (Figure 8).

Beginning in 2008 DWR changed its method to a protocol that is aligned with best practices, now counting a minimum of 400 total cells, and a minimum of 100 cells of the dominant taxon.[5]

3.4 What can still be inferred from the EMP phytoplankton data?

The data quality issues with the EMP phytoplankton dataset create a challenging situation for scientists and managers who have used this data to make inferences about ecological health, and to inform nSFE management decisions. The original ecological

[5]http://www.water.ca.gov/bdma/meta/phytoplankton.cfm

stoichiometry investigations (Glibert 2010, Glibert et al. 2011) that idereported shifts in phytoplankton community composition based on analysis of the EMP data did so unaware of its severely-compromised quality. Subsequent investigations have cited those studies as background on ecological condition in the nSFE, (e.g., Dugdale et al., 2012; Parker et al., 2012a,b; Glibert et al 2012; Glibert et al 2014). The proposed phytoplankton community shift and hypothesized link to NH_4^+ has also diffused into management contexts, shaping the prioritization of applied science and monitoring (DSC 2013) and informing major regulatory decisions.[6] The large uncertainties accompanying the density estimates (Figure 8), however, raise important questions about whether a shift occurred as proposed (i.e., Figure 4 H3), whether it could be detected, and the confidence that can be placed in any associations between a community shift and proposed causal factors. With this issue in mind, we reanalyzed the EMP data, guided by the following questions:

1. *What changes in diatom densities can be detected?*
2. *What changes in non-diatom densities can be detected Suisun Bay?*
3. *What can be inferred about the type of phytoplankton community shift (Figure 4)?*

We used general additive models (GAMs) to explore these questions (see Box A for additional description). Separate models were developed for diatoms and non-diatoms (combined flagellates, cryptophytes and chlorophytes). Models with and without controlling for magnification and using different station combinations were used to test for and quantify the effect of the 1988 method change.

Q.1 Detectable change in diatom densities in Suisun Bay?

Several studies have documented the seasonal and long-term variability in diatom production in the nSFE (e.g., Jassby 2008, Kimmerer 2004), and these observations can serve as one way of assessing the EMP diatom data quality -- a 'positive control' against which trends from GAMs results can be compared. The dramatic drop in peak-annual chl-a concentrations, and change in the seasonal chl-a cycle, indicate a major loss of phytoplankton biomass and production around 1987 (Figure 2, and e.g., Jassby 2008). Kimmerer (2004), using trends in dissolved silicate, demonstrated that diatoms accounted for most of the production (and chl-a) in Suisun Bay prior to 1987 and that the 1987 precipitous decrease in chl-a was due to the loss of diatoms. By extension, it is reasonable to argue that the strong pre-1987 seasonal variability in chl-a levels, and occasionally-large interannual variations (1977, 1983) resulted from variations in diatom densities

[6] http://www.waterboards.ca.gov/board_info/agendas/2012/dec/120412_11.pdf CA State Water Board statement on requirements to upgrade the Sacramento regional wastewater treatment plant: "The consequences of excessive nutrients, including changes in phytoplankton and zooplankton communities, negatively impact the survival and success of these threatened and endangered species [ref: Glibert 2010]...The Northern San Francisco Bay, specifically Suisun Bay, has undergone significant changes in ecosystem structure. These changes are presently being attributed to ecosystem perturbations over the past several decades resulting from changes in nutrient ecosystem stoichiometry [ref: Glibert 2010]. Historically, Suisun Bay was a diatom-based food web. In 1982, [Sac Regional] began operations and began discharging secondarily treated effluent, discharging up to 14 tons of ammonium-nitrogen into the Sacramento River daily. This discharge of ammonium-nitrogen coincided with the Sacramento River and Suisun Marsh shifting from a nitrate-based diatom phytoplankton system, to an ammonium-based small phytoplankton system [ref: Glibert 2010; Dugdale et al., 2007]"

The model does capture a long-term trend of decreasing diatom densities at D7 over the 40 year record (Figure 9 A and B). The fitted seasonal cycle at D7 also compares favorably with pre-1987 seasonal differences in chl-a (Figure 10A and B). The model also captures substantial interannual variability in diatoms, again exhibiting similar patterns as chl-a (Figure 3). The year 1987 marks a sharp change in diatom densities in the both the long-term (Figure 9) and seasonal (Figure 10) model predictions. At the other Suisun stations (D4, D8), the model captures the same seasonal patterns and pronounced post-1987 decreased diatom densities (Figure 10, Figure A.3). At the Delta stations, the model captured large interannual differences in seasonal diatom blooms (Figure A.3). However, abrupt drops like those in Suisun were not detected at non-Suisun sites.

A non-trivial magnification effect was detected in the diatom model (Figure 9B): i.e., higher magnification was associated with higher reported densities. This suggests that changes in magnification introduced a bias in the reported data. Adjusting the densities over time for magnification and comparing model predictions provides a means for estimating the magnitude of the magnification bias w (Figure 9B): ~50% higher model predictions for diatoms when comparing 700x and 280x (Figure 9B, and Figure 10A and B). While the magnification effect is nontrivial, for diatoms it did not alter or obscure the inferred long-term trends at D7, the interannual differences in seasonal trends, or seasonality. An important caveat regarding the magnification effect is that the sustained change in magnification occurred in 1988, within 1 year of the *Potamocorbula* invasion and the sharp drop in chl-a (Figure 2). It is therefore possible that some of what the model attributed to the magnification effect was actually real change in ecosystem condition. In order to isolate and more narrowly test the magnification effect, we ran a second GAM for diatoms, using the same structure as in *Eq. B.1* but using only Delta stations (i.e., only including C3, MD10, P8, and D26). *Potamocorbula* is only found in areas with elevated salinity; thus, the perturbation caused by its introduction would not have been felt at these Delta stations. The magnification effect was again detected in the Delta-only model (Figure A.5), and its magnitude was similar to that in the Delta+Suisun model (i.e., Figure 9B). Based on this evidence, it is reasonable to argue that the magnification effect is 'real' and independent of the *Potamocorbula* invasion.

A.1: Despite the EMP datasets major data quality limitations, it does appear possible to detect the dramatic changes in diatom densities to be identified, and to identify some pronounced spatial differences in diatom seasonal cycles among stations in the Suisun-Delta complex.

Q.2 Detectable change in non-diatom densities in Suisun Bay?

Unlike with diatoms, there is no positive control, against which GAMs predictions can be compared. We therefore evaluated the meaningfulness of any potential change over time in non-diatom densities relative to the variability or 'noise' in the data, and the magnitude of any magnification effect. The necessity of modeling non-diatoms as a

combined group is a limitation of this analysis, but allows us to test, in an aggregated way, the shift in non-diatoms proposed by Glibert and colleagues (2010, 2011).

The no-magnification model detected an increase in non-diatom densities in the mid-1980s at D7 (Figure 11A). The no-magnification seasonal predictions provide additional perspective (Figure 12.A): at Suisun stations (D4, D7, D8) annual maximum densities initially increased in the mid-1980s and then decreased to an intermediate level in the late-1990s through early-2000s.

However, the non-diatom magnification effect was relatively large, and adjusting for magnification substantially influences the interpretation of the non-diatom data (Figure 11B). The magnification effect translates into a >4-fold difference between predicted densities if the sample was counted on low and high magnificaiton (Figure 11B). Magnification-adjusted densities exhibit no upward trend in non-diatom densities over time (i.e., Figure 11B). If anything, the magnification model suggests that non-diatom densities decreased over time at D7 and the other Suisun stations (Figure 12B). Similar to the case for diatoms, we ran a Delta-only non-diatoms GAM, and found again that magnification was an important factor, and its effect-magnitude was similar between the Suisun+Delta and Delta-only models (Figure A.6).

The magnification-adjusted predictions did not identify monotonic increases in non-diatom densities at the Delta stations, but did capture large interannual differences at some sites (Figure A.6). Since only Suisun stations were used in proposing the phytoplankton community “shift” (Glibert 2010; Glibert et al., 2011), we have not yet further explored the non-diatom densities at Delta stations. However, there may be some merit to revisiting those stations and time periods.

A.2 The EMP data do not support the assertion that non-diatom densities have increased over time in Suisun Bay. While a modest (and sharp) increase in the mid-1980s might be gleaned from the no-magnification model, that change was not evident once densities were adjusted for magnification. This observation is consistent with the increase captured by the no-magnification model actually being an artifact of the change in counting method (magnification). Because of the low counts and the large confidence intervals for non-diatom classes (Figure 7), we cannot strictly rule out that some changes occurred, either smaller than the current ‘noise’ level or otherwise not well-captured because of data quality issues. However, the non-diatom increase proposed by Glibert and colleagues (2010, 2011) is inconsistent with our analysis.

Q.3 What can be inferred about changes in the Suisun phytoplankton community over time based on the EMP data?

The characteristics of any Suisun Bay phytoplankton community shift (i.e., Figure 4) are important because those characteristics can help differentiate between competing mechanistic explanations for the shift. The answer to Q.3 is organized below around the hypotheses presented in Figure 4.

- *H0: No change in composition.* A dramatic drop in diatoms in Suisun Bay around 1987 can be detected with the EMP data (Figure 9B). Results from the analysis of the EMP data reject the null hypothesis that no change occurred.
- *H1: Substantially decreased diatoms, with little change in other classes*. H1 can not be rejected based on the data. After adjusting for magnification, the predicted non-diatom densities in Suisun Bay exhibited some interannual variability but no prolonged trends (Figure 11B). It is possible that uncertainties introduced by the data quality issues, plus any additional unaccounted for bias in the data, are preventing the detection of changes that did occur, and, therefore, changes in non-diatom densities cannot be ruled out. However, of the three categories of potential shifts in Suisun Bay, H1 appears to be the most plausible when the EMP data are reanalyzed using a model structure that controls for some of the major data quality issues.
- *H2: All classes decreased proportionally.* The data do not support H2. A modest decrease in non-diatom density may have occurred (Figure 11B), but any change appears to have been relatively much smaller than the diatom decrease.
- *H3: Substantially decreased diatoms, substantially increased non-diatoms.* The data do not support H3. While there was clearly a substantial loss of diatoms in Suisun Bay, the data do not suggest that non-diatom densities increased substantially.

Using the same EMP data, Glibert and colleagues (2010, 2011) reached substantively different conclusions about the Suisun Bay phytoplankton community shift. Glibert (2010) describes 3 eras of phytoplankton community and shifts in which cryptophytes and flagellates (1983-1999) and cyanobacteria (2000-2005) "replaced" the diatoms that dominated early in the record (pre-1982), and depicts this shift as leading to major changes in the flows of energy and material (see Glibert 2010, Figure 23).

Our reanalysis of the EMP density data for Suisun Bay, does not support this description. In addition, it is important to point out that while our analysis here has focused entirely on cell densities (cells mL^{-1}), phytoplankton abundance in terms of biomass (μg C L^{-1}), or biovolume (μm^3 L^{-1}), are is more ecologically-meaningful metrics of community composition, especially when considering issues of energy and material flow. Diatom biovolume, on a per cell basis, is generally orders of magnitudes larger than other classes (e.g., chlorophytes, cryptophytes). Therefore a community shift that meaningfully shifted the flow of energy and material flowed away from diatoms and toward other classes that are much smaller in size would require a larger increase in non-diatom densities relative to the diatom density decrease.

A.3 The EMP data do not support the notion that phytoplankton community in Suisun Bay has undergone a shift similar to that described by Glibert and colleagues (2010, 2011), which forms the basis for the ecological stoichiometry hypothesis.

4. Summary

1. The reported shift in the phytoplankton community composition in Suisun Bay, based on analysis of EMP data, has been a central component of the ecological stoichiometry conceptual model for ecosystem decline in the northern Bay-Delta (Glibert 2010; Glibert et al., 2011).
2. During our analysis of the EMP data, we identified severe data quality issues due to low cell counts. At Suisun sites over the period 1988-2005, <5 cells were counted in the majority of samples at Suisun sites. At one station (D7), for >15% of the samples, densities were determined based on a total count of only 1 cell. The low cell counts result in extremely large confidence intervals for the data from this period. A major methodological change made in 1988 (doubling the magnification) appears to have contributed to the low cell counts post-1988.
3. The data quality issues and large uncertainties were not considered in the earlier papers that proposed the phytoplankton community shift.
4. From a qualitative perspective, the data quality issues raise major questions about the empirical evidence at the foundation of conceptual model proposed by Glibert and colleagues (2010, 2011): what can confidently be inferred about changes in the phytoplankton community over time using this dataset? if a shift did occur, what were the causal factors? what were and the bottom-up effects on higher trophic levels?
5. A reanalysis of the EMP phytoplankton data determined the following:
    a. Despite the datasets major data quality limitations, it does appear possible to detect the dramatic changes in diatom densities that occurred in 1987, coinciding with the *Potamocorbula* invasion. The sharp loss of diatom production has also been identified in other studies with independent data.
    b. The EMP data do not support the assertion that non-diatoms have increased over time in Suisun Bay. While a modest (and abrupt) increase in non-diatoms in the mid-1980s might be gleaned from the data, that change was not evident once densities were adjusted for magnification effects. This observation is consistent with the method change (magnification), and any related bias in detection, introducing an artifact that, if not controlled for, could otherwise be construed as a modest increase in non-diatoms. Because of the low counts and the large confidence intervals for non-diatom classes (Figure 7), we cannot strictly rule out that some true changes occurred, either smaller than the current ‘noise’ level or otherwise not well-captured because of data quality issues. However, it does not seem possible to conclude that a substantial non-diatom increase occurred using the current data, at least not with the methods used here or the techniques used in past studies.
    c. The poor data quality severely limits what conclusions can be confidently reached about phytoplankton community shifts over time This reanalysis of the EMP data does not support the notion that phytoplankton community in Suisun Bay has undergone a shift similar to that described by Glibert and colleagues (2010, 2011).

d. The statistical model did capture some perhaps meaningful interannual variability at sites in the Delta, which may be worthy of further exploration.

Box A

General Additive Models (GAMs) are flexible and powerful statistical models that are well-suited for exploring the EMP data for several reasons:

1. GAMs allow for fitting relationships when the trend over time is not necessarily expected to be linear: e.g., if there is seasonal patterns on top of long term trends; or if there are both increases and decreases over time.
2. GAMs can cope with the issue of autocorrelation in time series data (i.e., data from one time point are autocorrelated with data from previous time periods).
3. Both long-term trend terms and seasonal terms can be incorporated into the same model, and then those individual terms separately inspected in the model output (see Figures 9-10 and 11-12). In other words, it is possible to see not just a long term trend, but also how the seasonal cycle has changed over time.
4. Other factors, other than time, can also be included and their importance evaluated. (e.g., the magnification effect discussed below and in the text).

Separate models were developed for diatoms and non-diatoms, using the package *mgcv* in R (Wood 2015; R Core Team, 2016). In the case of non-diatoms, individual models were originally run but failed to solve, presumably because of small counts or numerous zeros. We therefore combined (flagellates, cryptophytes, chlorophytes), which, although perhaps not as satisfying as if individual classes could have been run, nonetheless remains consistent with our key question of whether non-diatom classes changed over time (Figure 4). GAMs also allows the user to define the data's error distribution, which we suspected would be important to test because of dataset's low counts and the large number of zeros. Three different data distributions were tested (normal, Poisson, quasi-Poisson, negative binomial). The importance of error distribution and the individual independent variables were tested through a formal model selection procedure (below).

Although the initial focus of the analysis was to examine trends at D7, we developed 'global' models that included all stations having complete data records (Figure 1), and included the following terms:

Eq. B.1 $\text{Density} = f_1(\text{time: station}) + f_2(\text{season: station, time}) + f_3(\text{magnification})$

f_1 : Long-term trend in density, varies by station
f_2 : Seasonal pattern (day of year), varies by station and time, and
f_3 : Magnification effect, same effect across all stations

Long-term trends and seasonal patterns were allowed to vary among the stations in the models. However, for magnification, if there was a magnification effect, it is reasonable to argue that its effect would be the same across sites. Thus, using all sites actually allowed us to test for the magnification effect, since magnification was the only factor that was common to all stations, and the times when changes occurred were known. Having model predictions for time and season at other stations also proved useful for comparing magnitudes of changes

Model selection involved the use of standard metrics for GAMs (e.g., primarily Aikeke's information criteria, AIC; also generalized cross validation scorce, GCV). AIC provides a relative measure of the 'goodness' of a model, and scores each model based on a balance of how well it fits the data and how simple (better) vs. complex (worse) it is -- i.e., a simpler model that fits the data reasonably well is considered better than a model that fits the data similarly well but is more complex (i.e., e.g., more parameters). . The model with the lowest AIC is generally considered the best. In our case the error distribution was the most important distinguishing factor among models. The negative binomial treatment proved best, indicating that taking into account the large number of low-count and zero data is important for appropriately analyzing the data. For both the diatom and non-diatom models, the best model also included the magnification effect. Results and interpretations for both the magnification and no-magnification models are discussed below.

Table B.1 Results from model selection for Suisun+Delta models

	#	Model	Error distribution	GCV/UBRE	Rsq	Deviance explained (%)	AIC
Diatoms							
	1	Magnification + Season + Date	normal	2564.4	0.343	36.8	62,317
	2	Magnification + Season + Date	poisson	27.851	0.491	63.1	125,057
	3	Magnification + Season + Date	quasi-poisson	29.369	0.488	62.9	NA
	4	Magnification + Season + Date	negative binomial	1.0435	0.311	59.9	39,964
	5	Season + Date	negative binomial	1.0775	0.333	59.6	40,021
Non-Diatoms							
	1	Magnification + Season + Date	normal	274.48	0.321	33.3	49,340
	2	Magnification + Season + Date	poisson	10.317	0.397	59.8	65,579
	3	Magnification + Season + Date	quasi-poisson	12.822	0.382	58.2	NA
	4	Magnification + Season + Date	negative binomial	1.0442	0.351	53.9	36,319
	5	Season + Date	negative binomial	1.0796	0.343	52.4	36,498

As noted in the main text, Delta-only models (excluding Suisun stations D7, D8, and D4) were also run for both diatoms and non-diatoms. These models were used to test whether a magnification effect was evident at stations uninfluenced by the *Potamocorbula* invasion. The same model selection process was applied to those models, and inclusion of the magnification term substantially improved the models (much lower AIC values).

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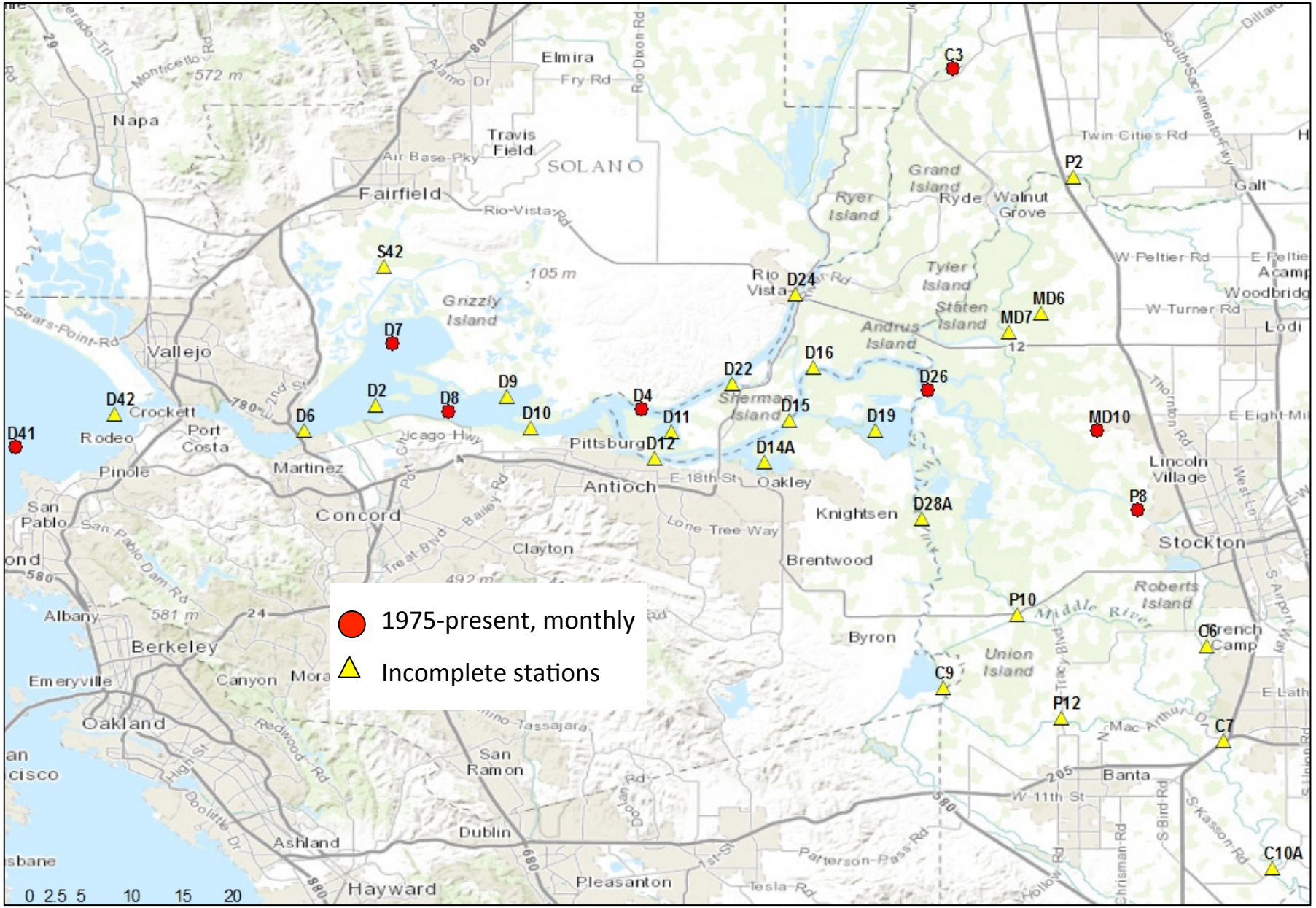


Figure 1 Location of EMP phytoplankton stations, with symbol indicating complete (1975-present) and incomplete records. Some incomplete records are actually 20+ years, and have been included in some analyses.

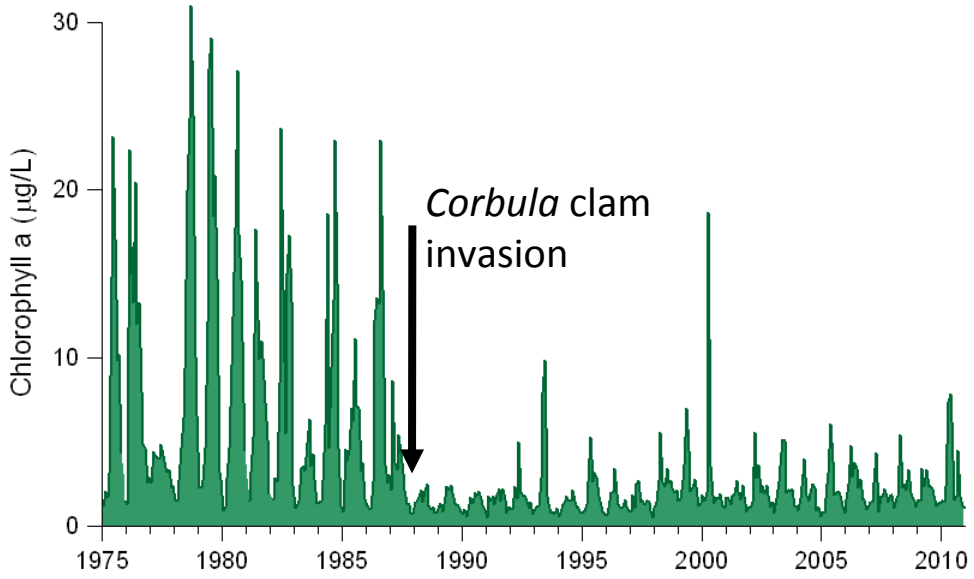


Figure 3 Chl-a concentration as a surrogate for phytoplankton biomass in Suisun Bay. This is based on a combination of USGS and EMP data from multiple sites, and binned monthly.

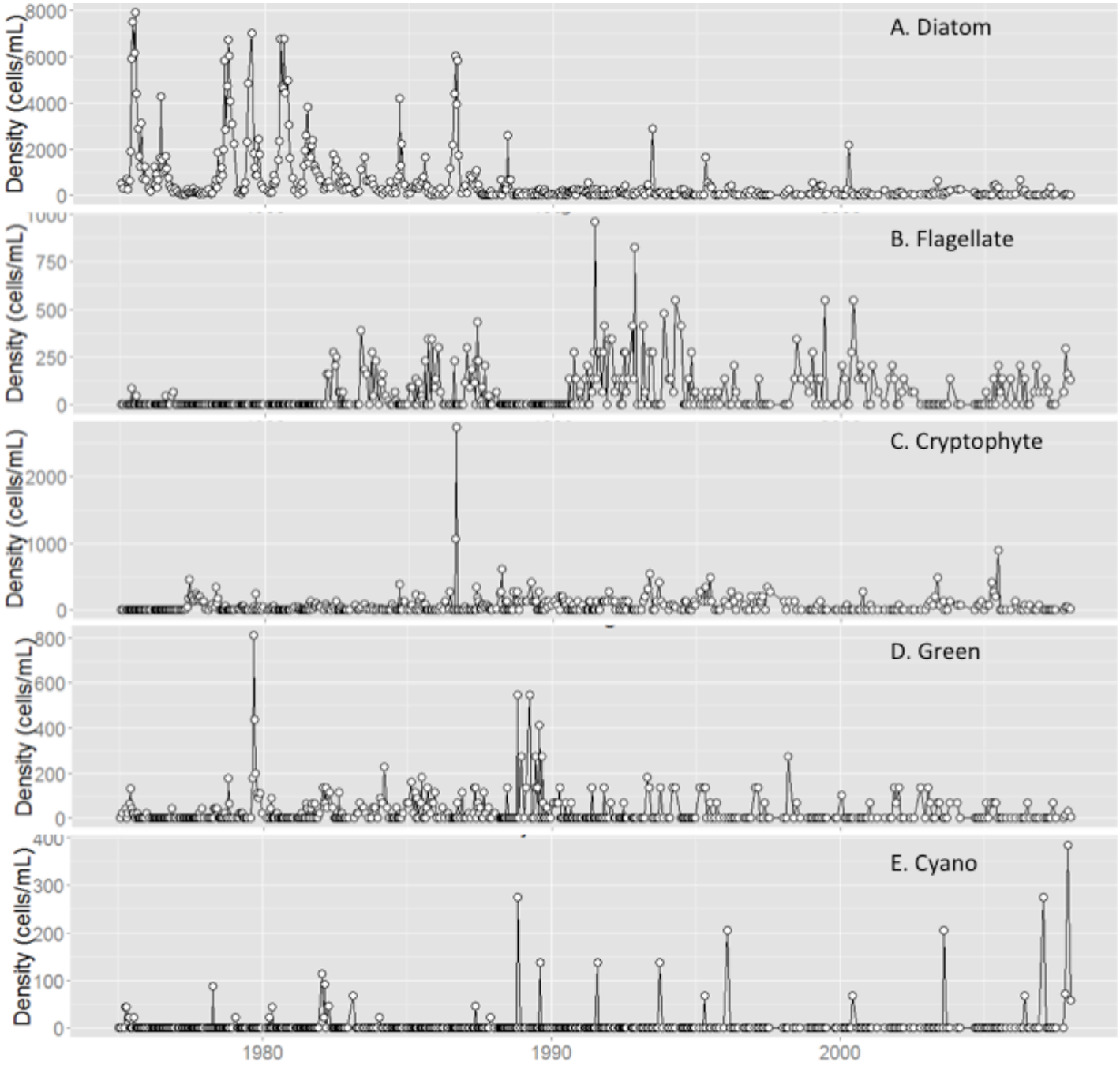


Figure 4 Reported phytoplankton densities at D7 (Suisun Bay, Grizzly Bay) for five phytoplankton classes. Densities were provided as a pre-calculated field in the DWR downloadable dataset, and are the same values used in prior studies that have suggested community composition shifts.

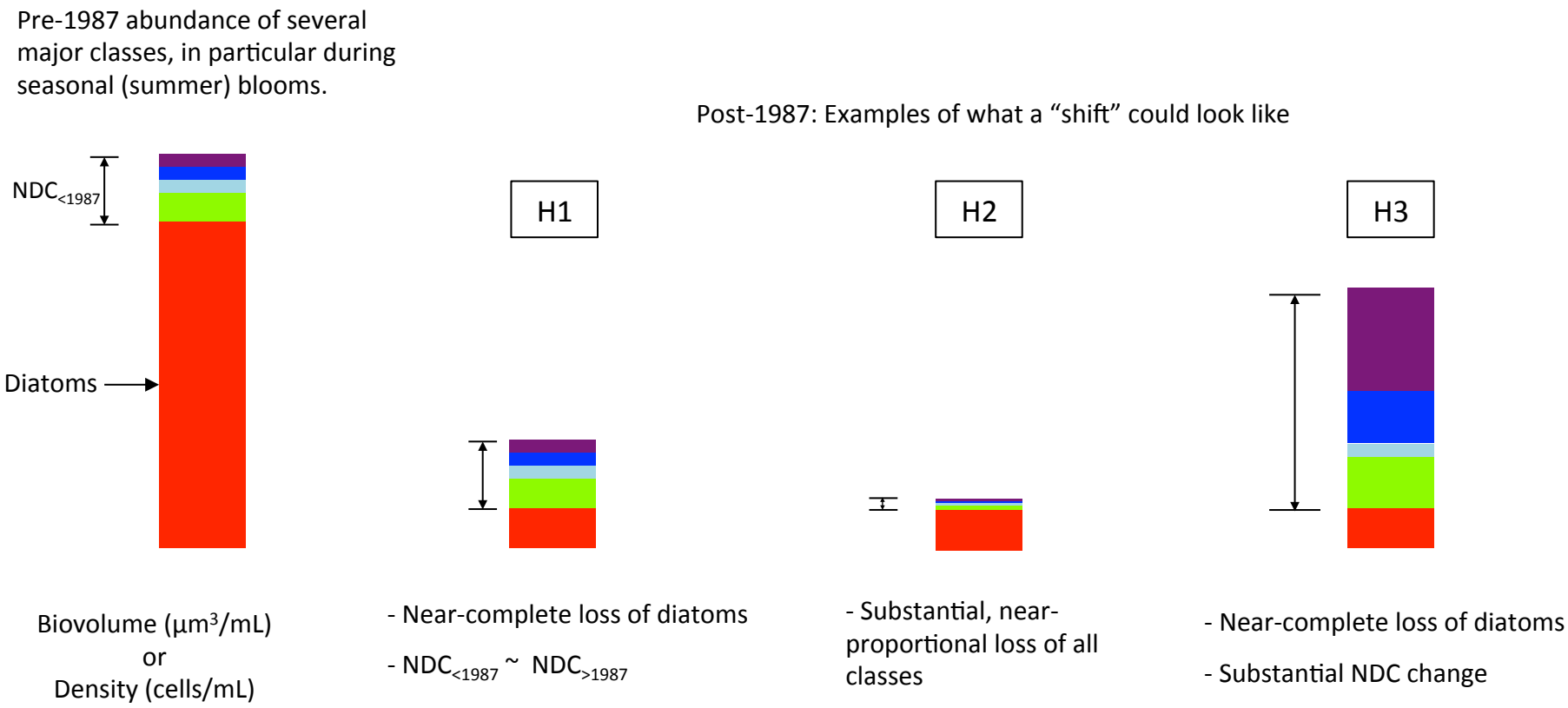
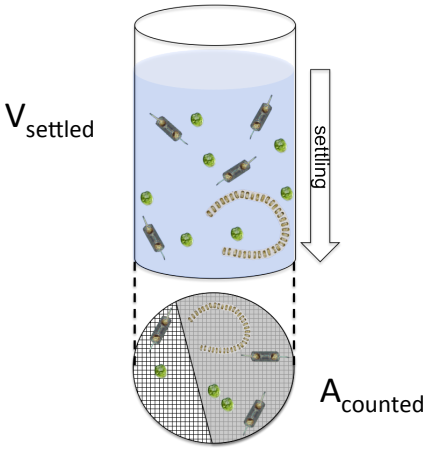
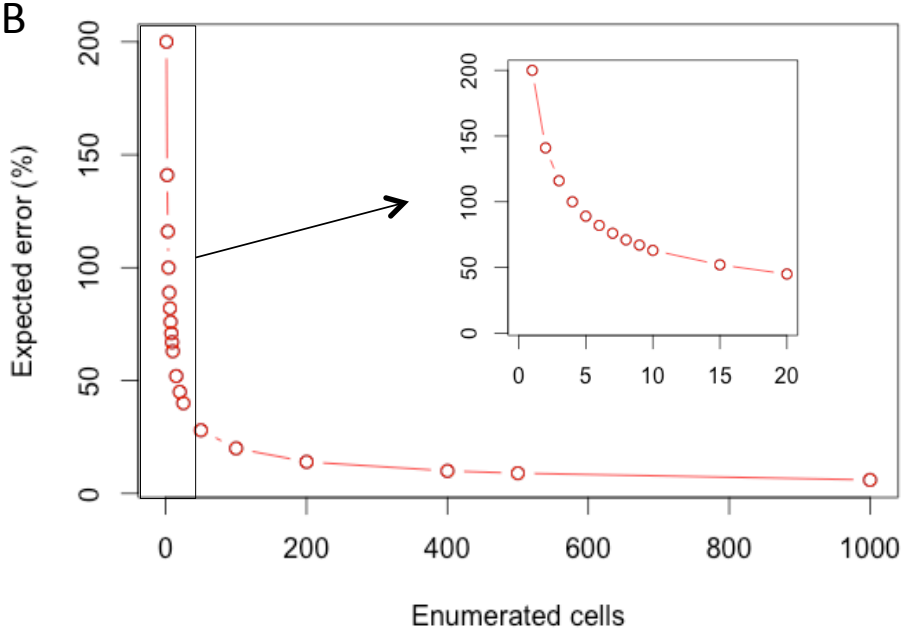


Figure 2 Schematic conceptualizing different representations of a phytoplankton community composition shift. The nature of a shift (to H1, H2, or H3) is important because it may point toward some mechanistic explanations over others. The stacked columns are intended to qualitatively depict densities (cells mL^{-1}).

A



B



$$D = \frac{C}{V_{settled} \cdot \frac{A_{field} \cdot G}{A_{total}}}$$

$$A_{counted} = \frac{A_{grid} \cdot G}{A_{total}}$$

$$V_{counted} = V_{settled} \cdot \frac{A_{grid} \cdot G}{A_{total}}$$

Figure 5.A. Schematic of counting procedure and conversion of cell counts to estimated density; **B.** Relationship between the number of enumerated cells and the expected error (in %) for the density estimation (95% confidence interval).

$V_{settled}$	water volume allowed to settle prior to counting (mL)
A_{total}	total area onto which sample was allowed to settle (mm^2)
A_{grid}	area of a single grid cell (mm^2)
G	# of grid cells counted
C	enumerated cells over for a given taxa, summed across G grids (cells)
$A_{counted}$	the fraction of A_{total} actually counted (unitless)
$V_{counted}$	the volume of sample actually counted (mL)
D	estimated density of cells of a given taxa (cells mL^{-1})

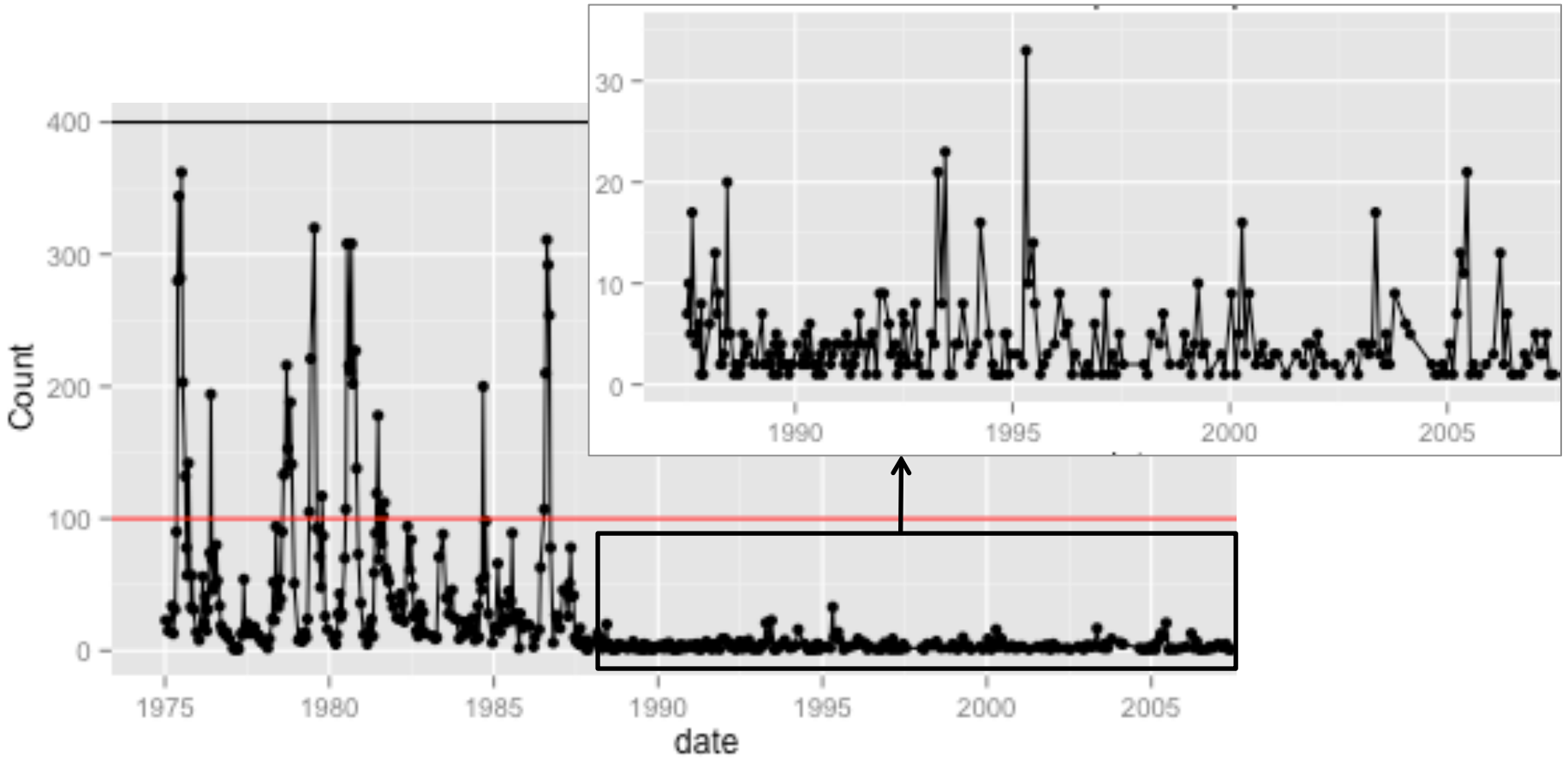


Figure 6 Total number of cell enumerated by microscopy at the Suisun Bay stations D7: 1975-2007. The black horizontal line at 400 counts denotes the recommended minimum number of counts for the most abundant taxa to yield ±10% uncertainty

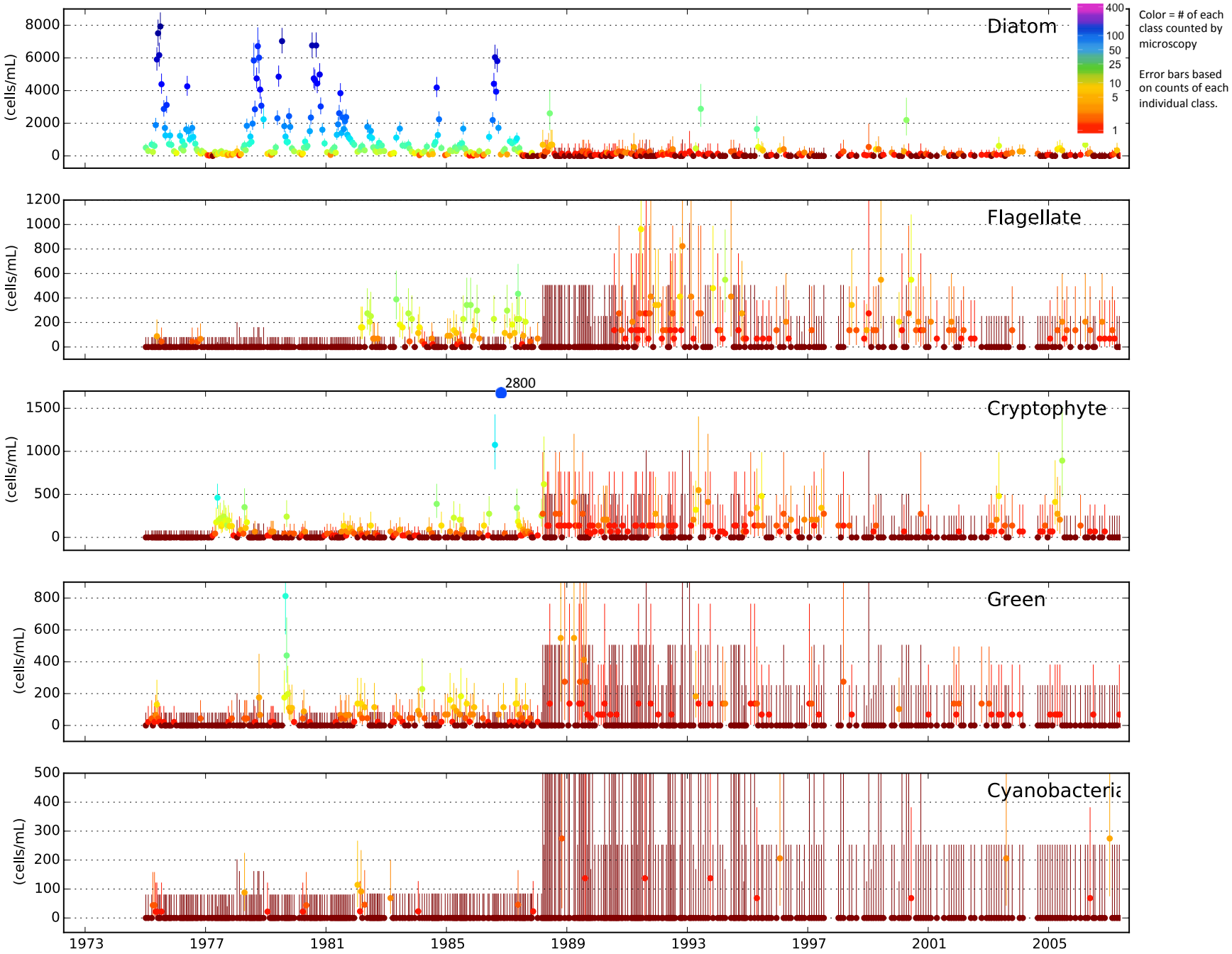
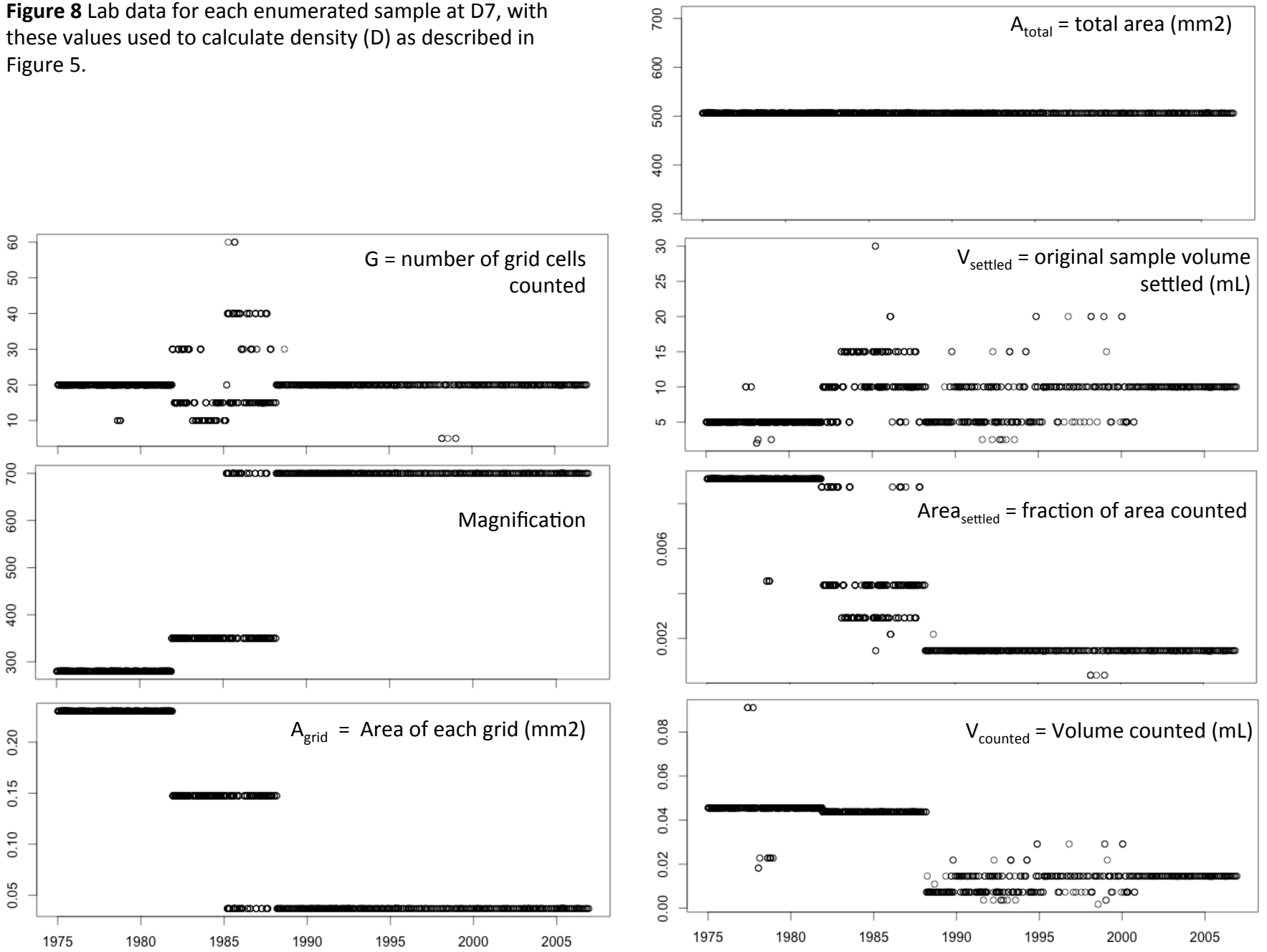


Figure 7 D7 densities data presented again (same as Figure 4) with confidence intervals computed based on maximum likelihood and a Poisson distribution. Color indicates the number of cells counted.

Figure 8 Lab data for each enumerated sample at D7, with these values used to calculate density (D) as described in Figure 5.



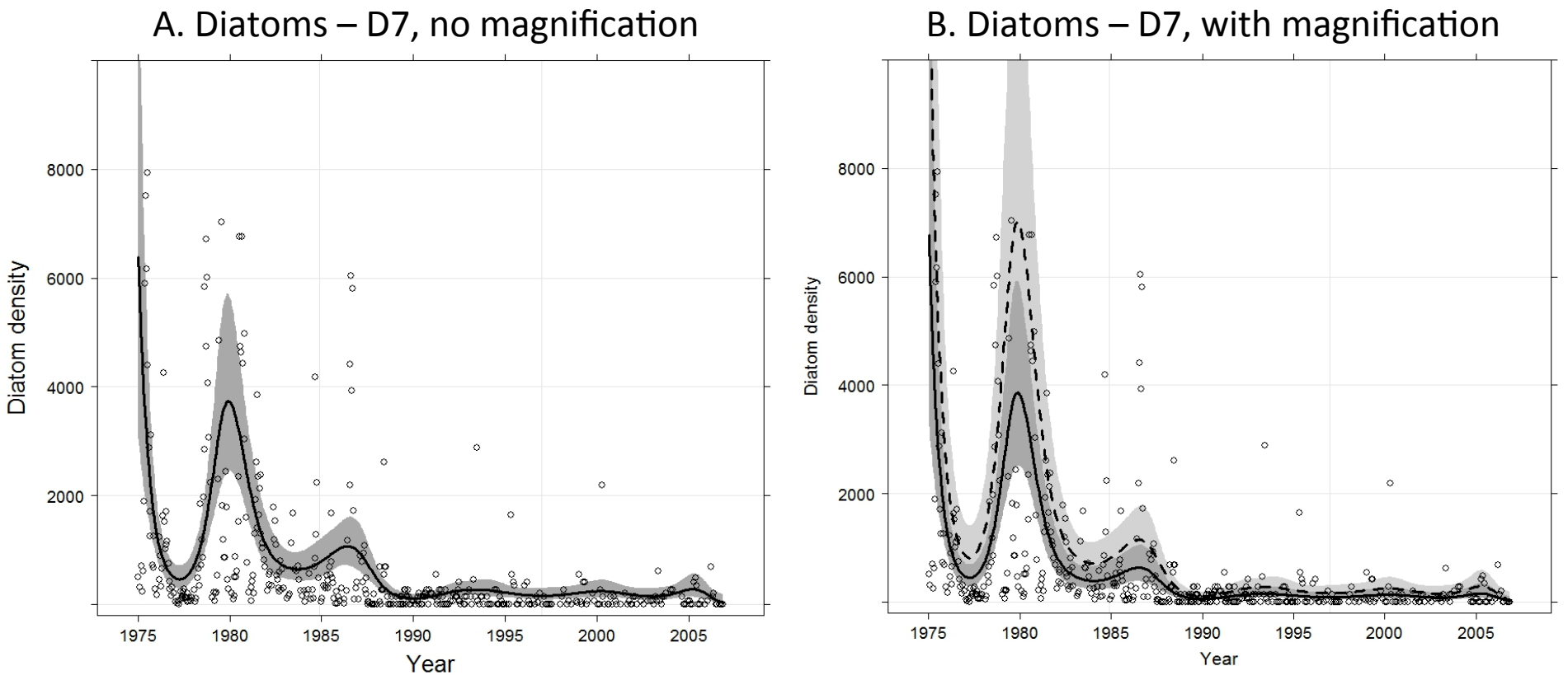


Figure 9. Trends in diatom density at D7 showing raw data (points), and model predictions from a model without magnification (A) and a model in which predictions are adjusted for the magnification effect (B). In B, the solid line and dark grey polygon represent predictions for magnification 280x and the dotted line and light grey polygon represent predictions for magnification 700x. Polygons represent 95% confidence intervals. Model predictions are taken from generalized additive models (GAMs) including spline functions for covariates year and day of the year for each station, with magnification either included or excluded.

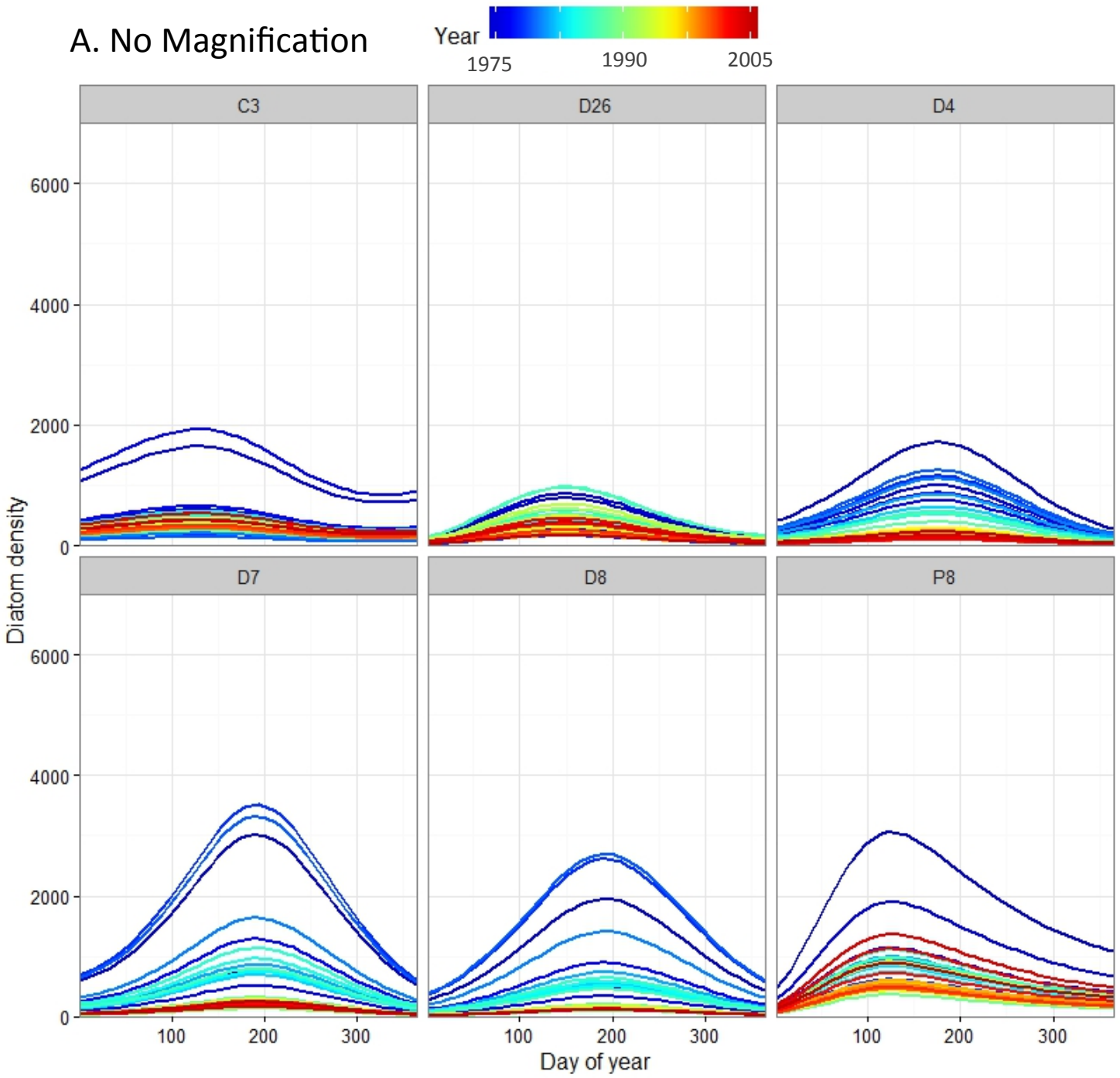


Figure 10.A Predicted diatom density (cells mL^{-1}) seasonal variability over the period 1975-2006 (colors), for the six stations with complete phytoplankton records. See Figure 1 for locations. Curves represent predictions for a model without magnification.

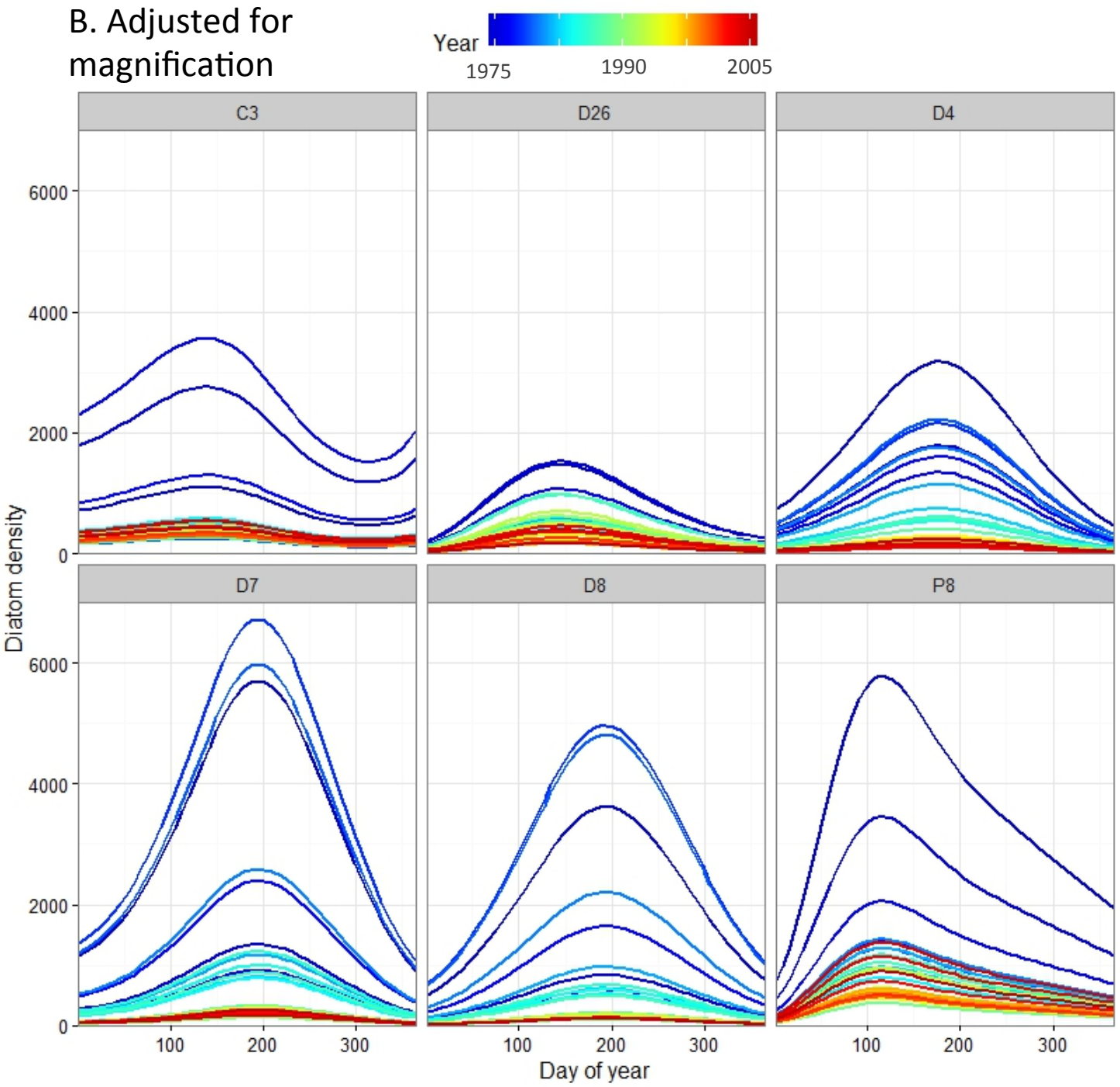


Figure 10.B Predicted diatom density (cells mL^{-1}) seasonal variabilityover the period 1975-2006 (colors), for the six stations with complete phytoplankton records. See Figure 1 for locations. Curves represent predictions for a model that included magnification, with densities adjusted to magnification = 700.

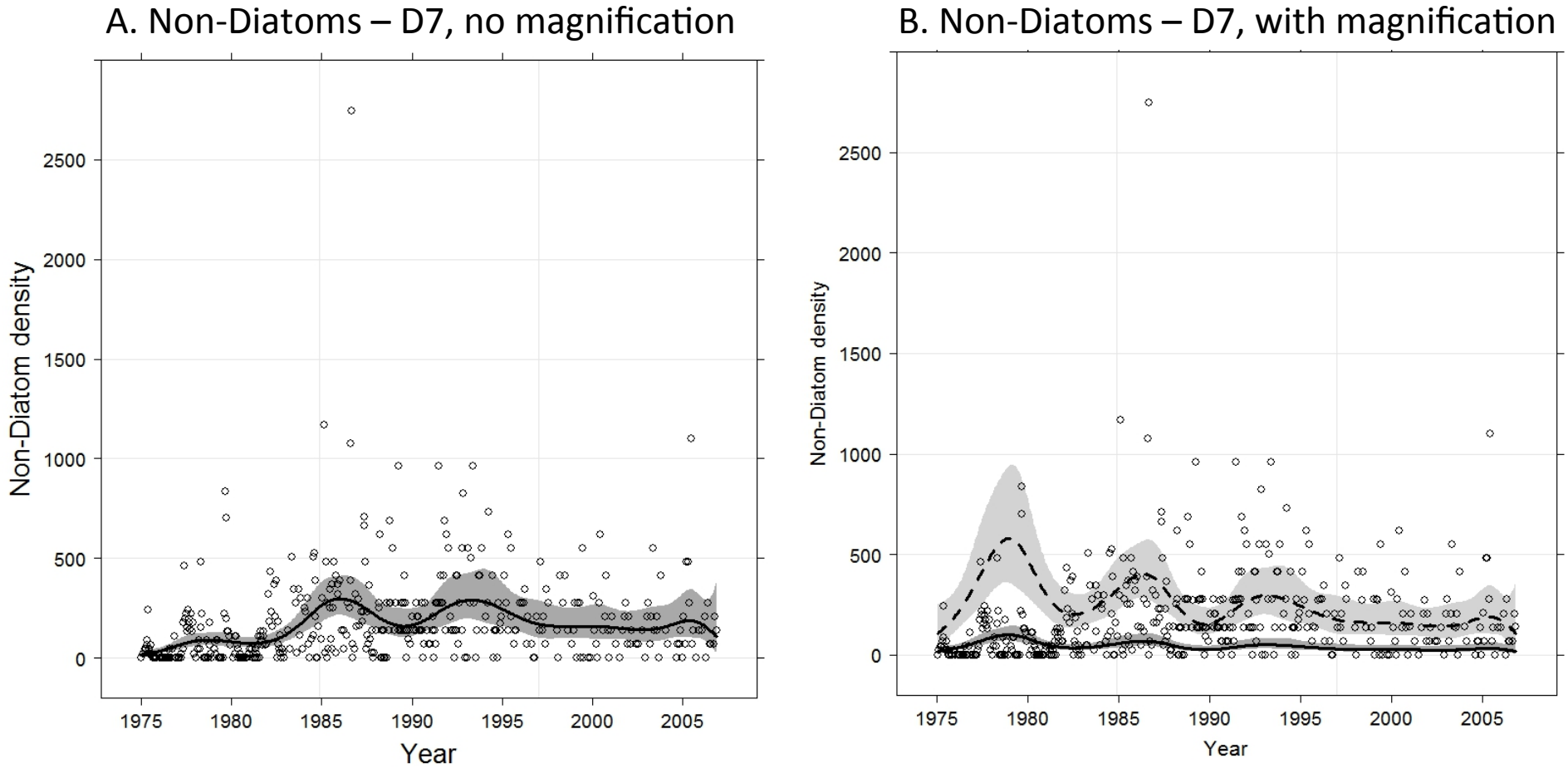


Figure 11. Trends in non-diatom density at D7 showing raw data (points), and model predictions from a model without magnification (A) and a model in which predictions are adjusted for the magnification effect (B). In B, the solid line and dark grey polygon represent predictions for magnification 280x and the dotted line and light grey polygon represent predictions for magnification 700x. Polygons represent 95% confidence intervals. Model predictions are taken from generalized additive models (GAMs) including spline functions for covariates year and day of the year for each station, with magnification either included or excluded. The non-diatom grouping includes the classes chlorohytes (greens), cryptophytes, and flagellates.

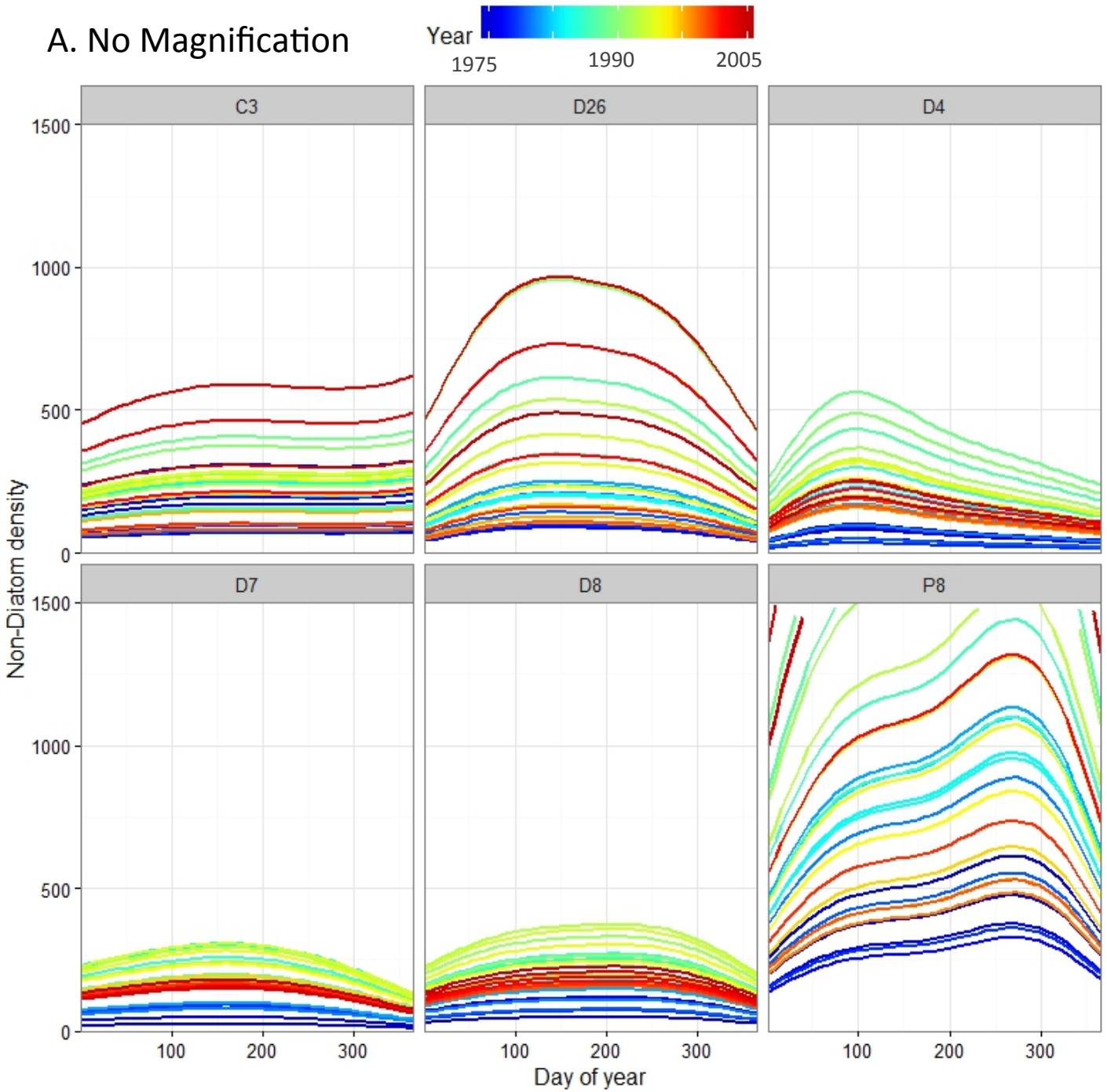


Figure 12.A Predicted non-diatom density (cells mL^{-1}) seasonal variability over the period 1975-2006 (colors), for the six stations with complete phytoplankton records (Figure 1). Curves represent predictions for a model without magnification. Note: Different y-axes between 13.A and 13.B

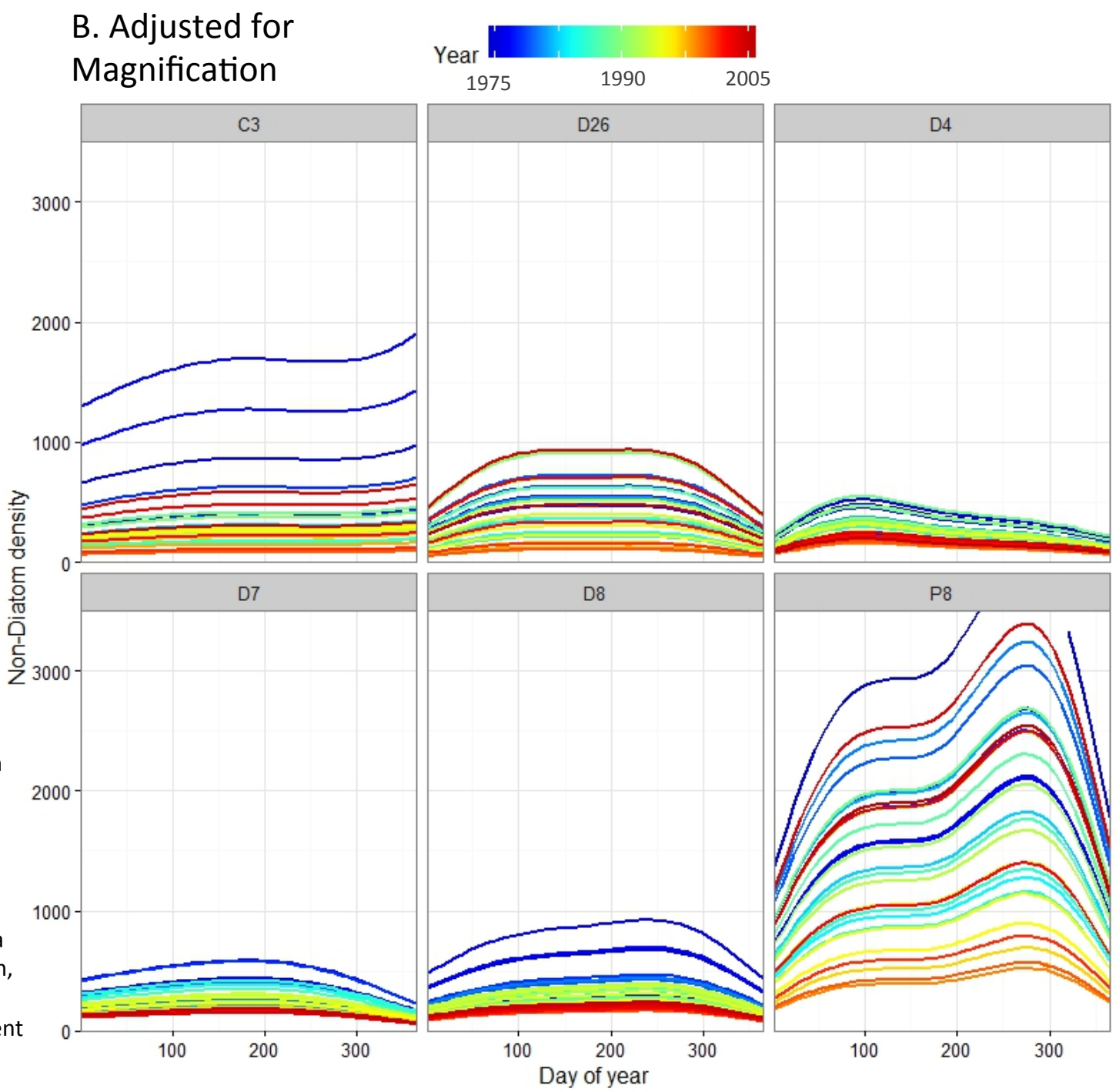


Figure 12.B Predicted non-diatom density (cells mL^{-1}) seasonal variability over the period 1975-2006 (colors), for the six stations with complete phytoplankton records (Figure 1). Curves represent predictions for a model that included magnification, with densities adjusted to magnification = 700. Note: Different y-axes in 13.A and 13.B

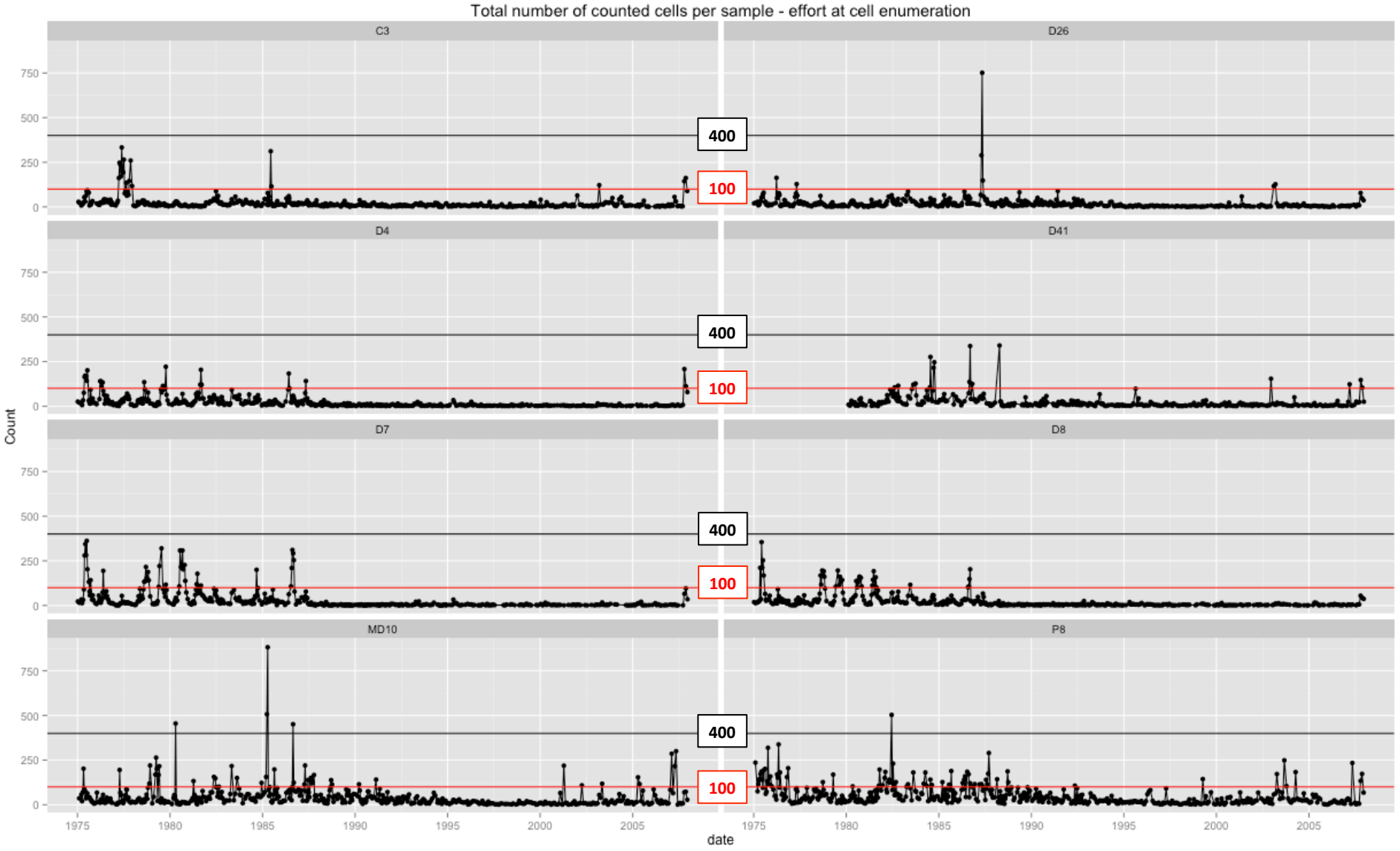


Figure A.1: Total number of cell enumerated at all DWR-IEP stations with complete records from 1975-2007

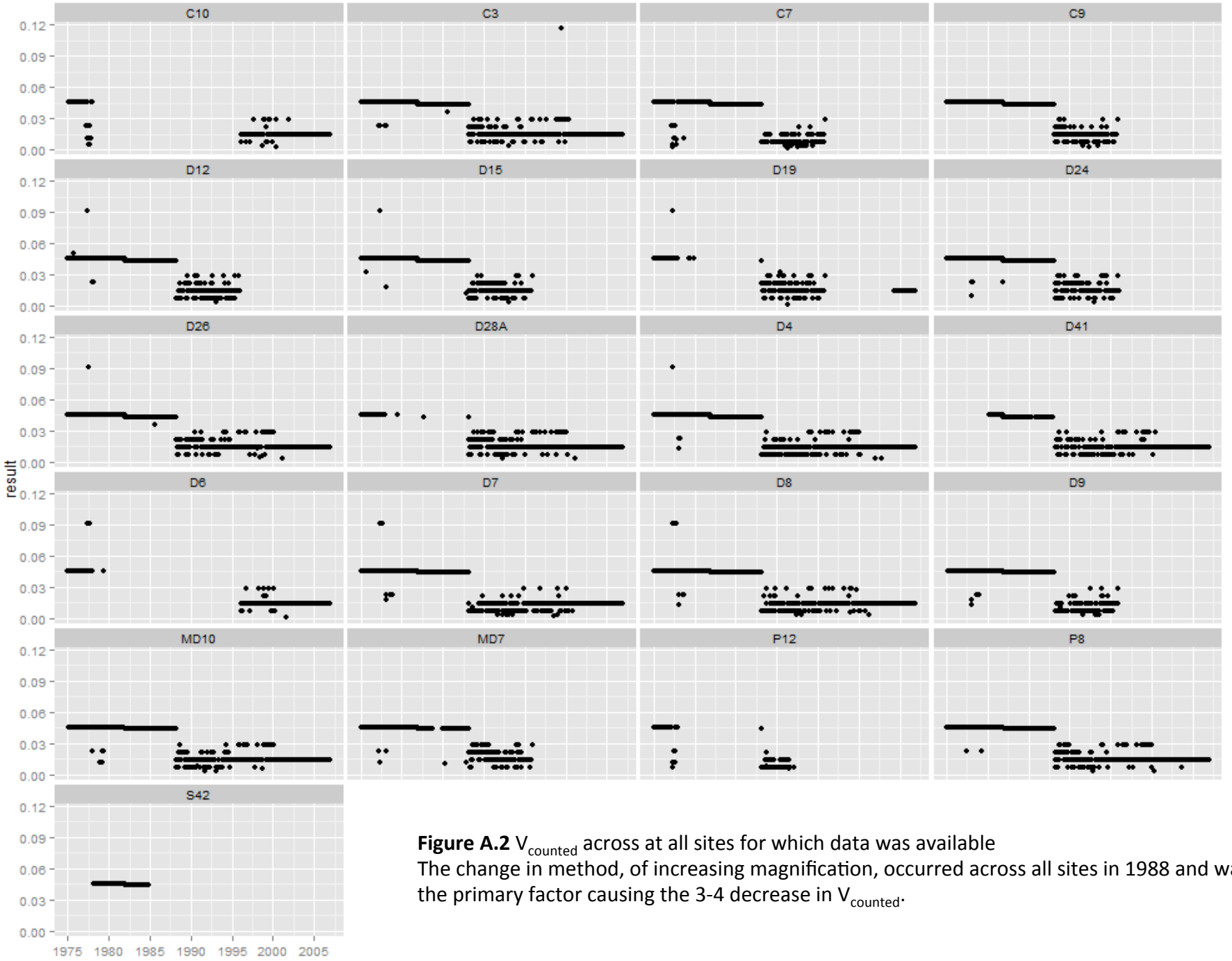


Figure A.2 $V_{counted}$ across at all sites for which data was available
The change in method, of increasing magnification, occurred across all sites in 1988 and was the primary factor causing the 3-4 decrease in $V_{counted}$.

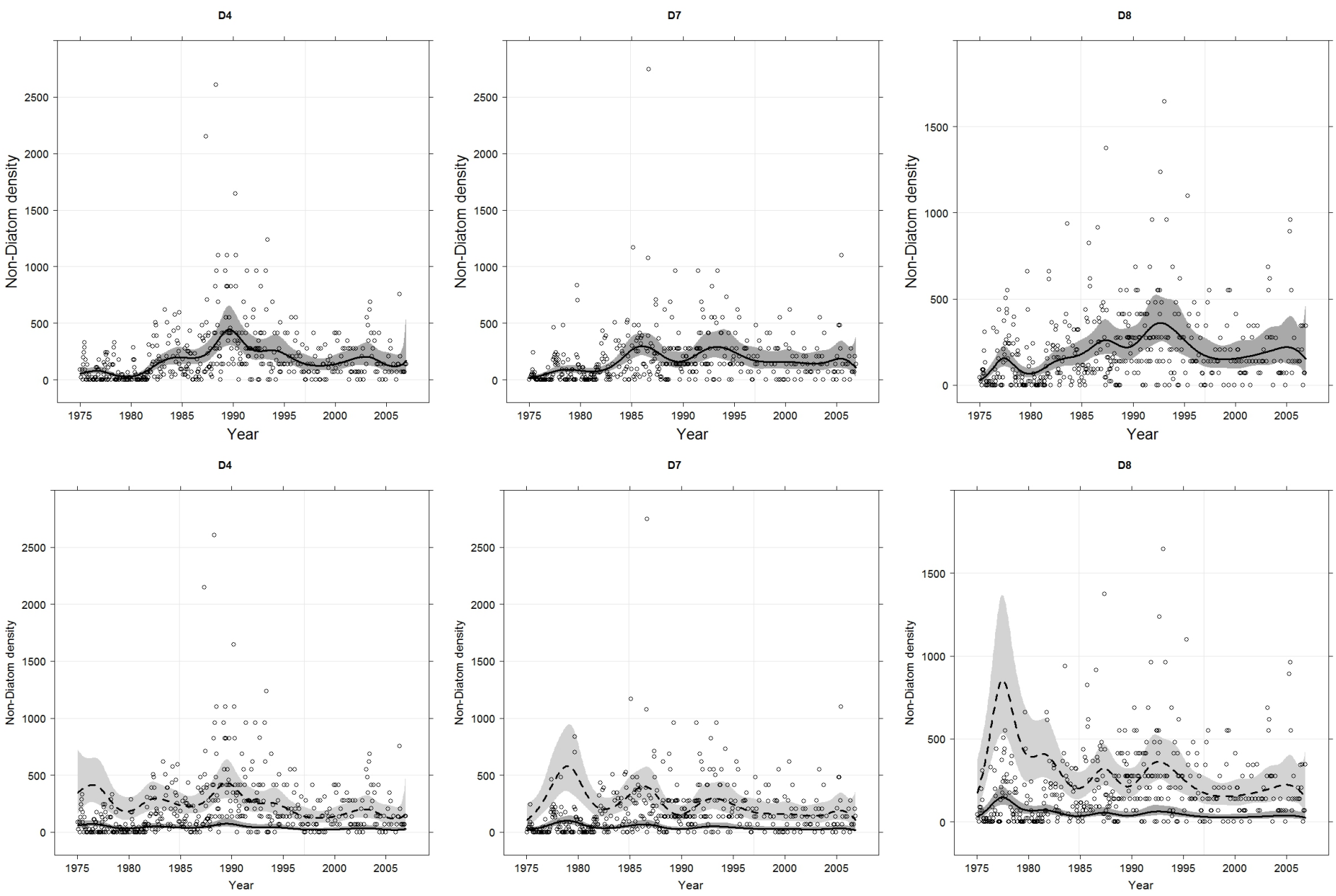


Figure A.3 Non-diatom densities, Delta+Suisun model with magnification. Top row is model with no magnification. Each curve represents magnification-adjusted model predictions: solid line = 280x, dashed line = 700x.

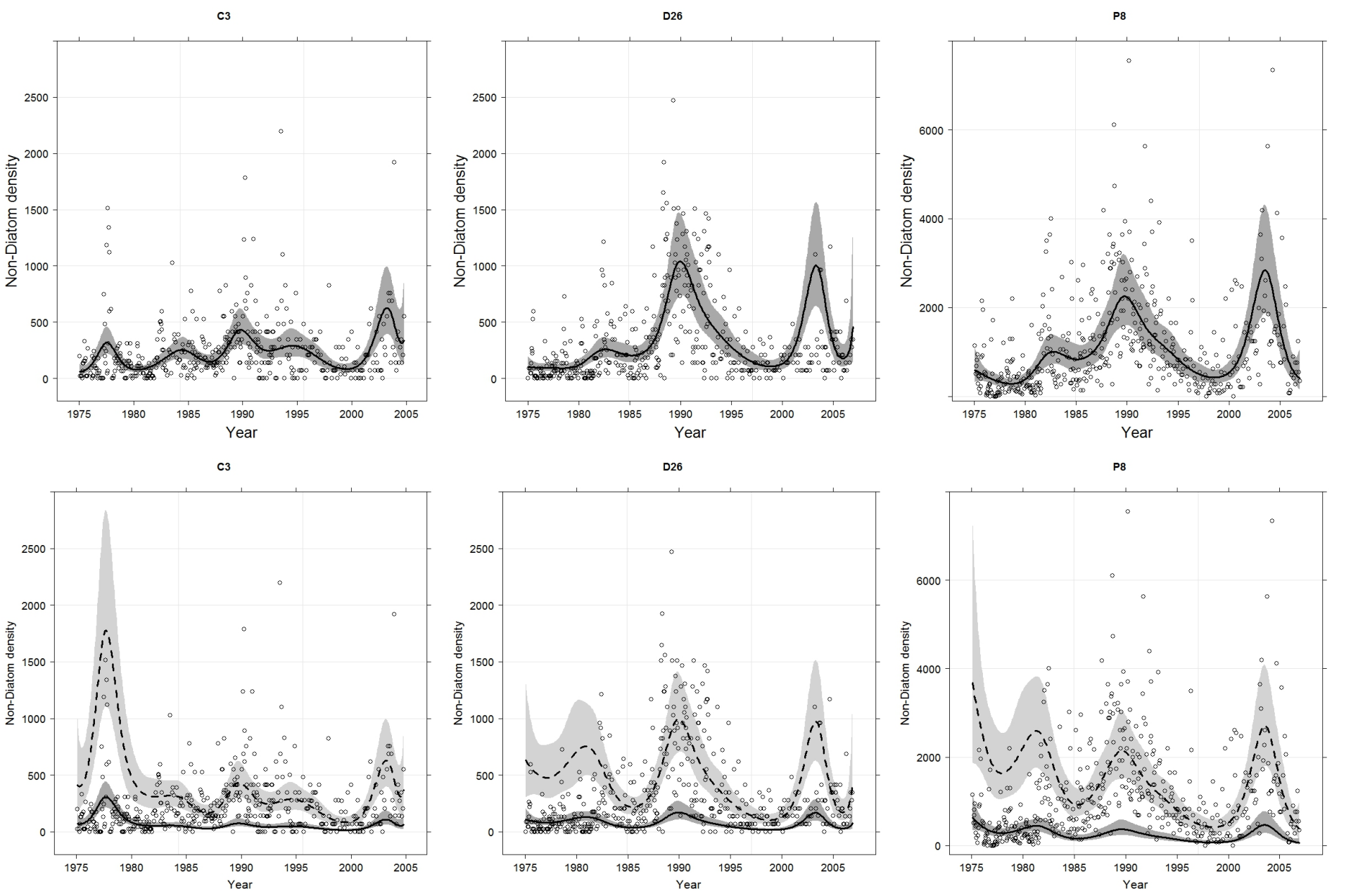


Figure A.3 cont'd Non-diatom densities, Delta+Suisun model with magnification. Top row is model with no magnification. Each curve represents magnification-adjusted model predictions: solid line = 280x, dashed line = 700x.

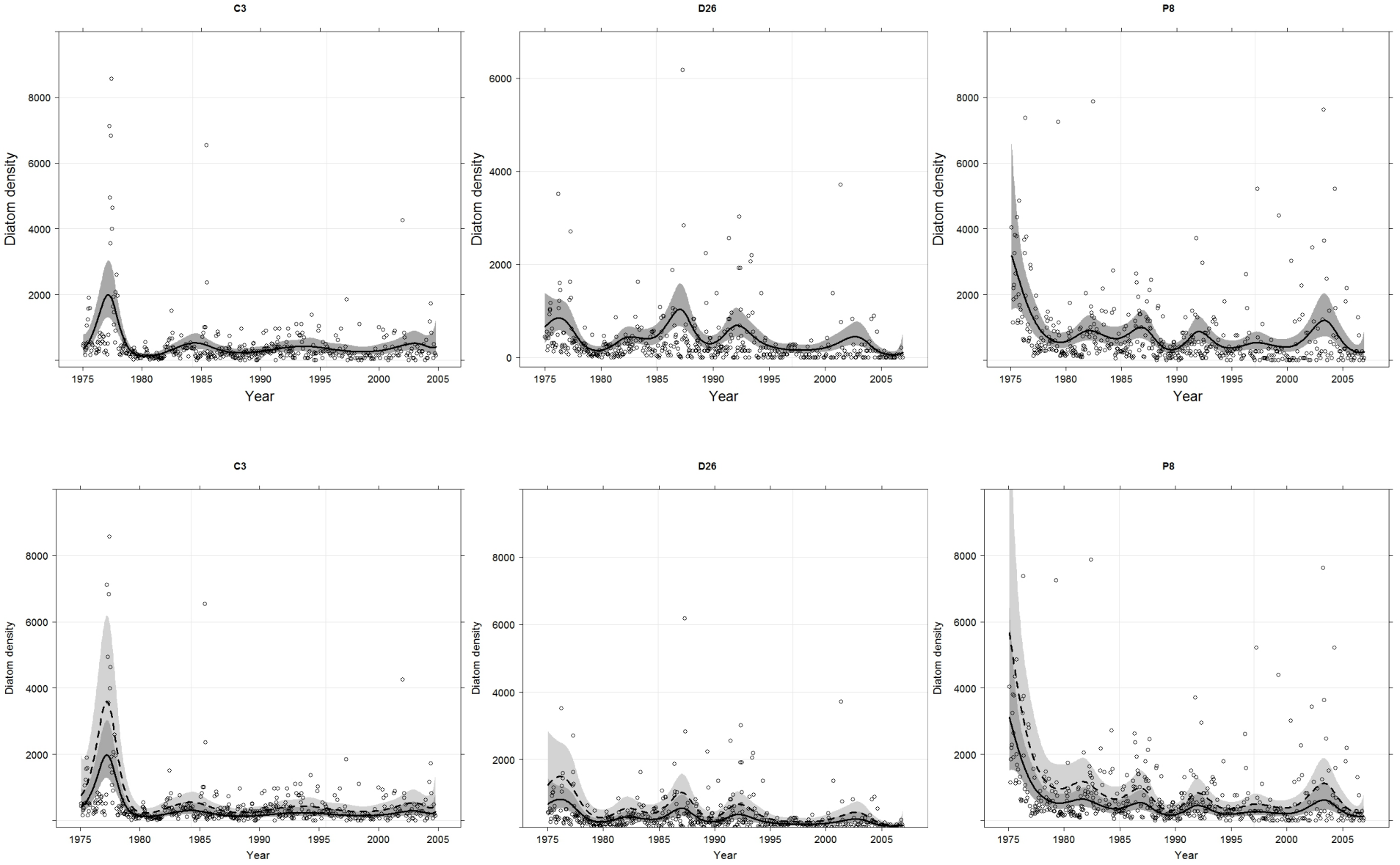


Figure A.4 Diatom densities, Suisun + Delta model. Top row is model with no magnification. Bottom row is magnification model; each curve represents magnification-adjusted model predictions: solid line = 280x, dashed line = 700x.

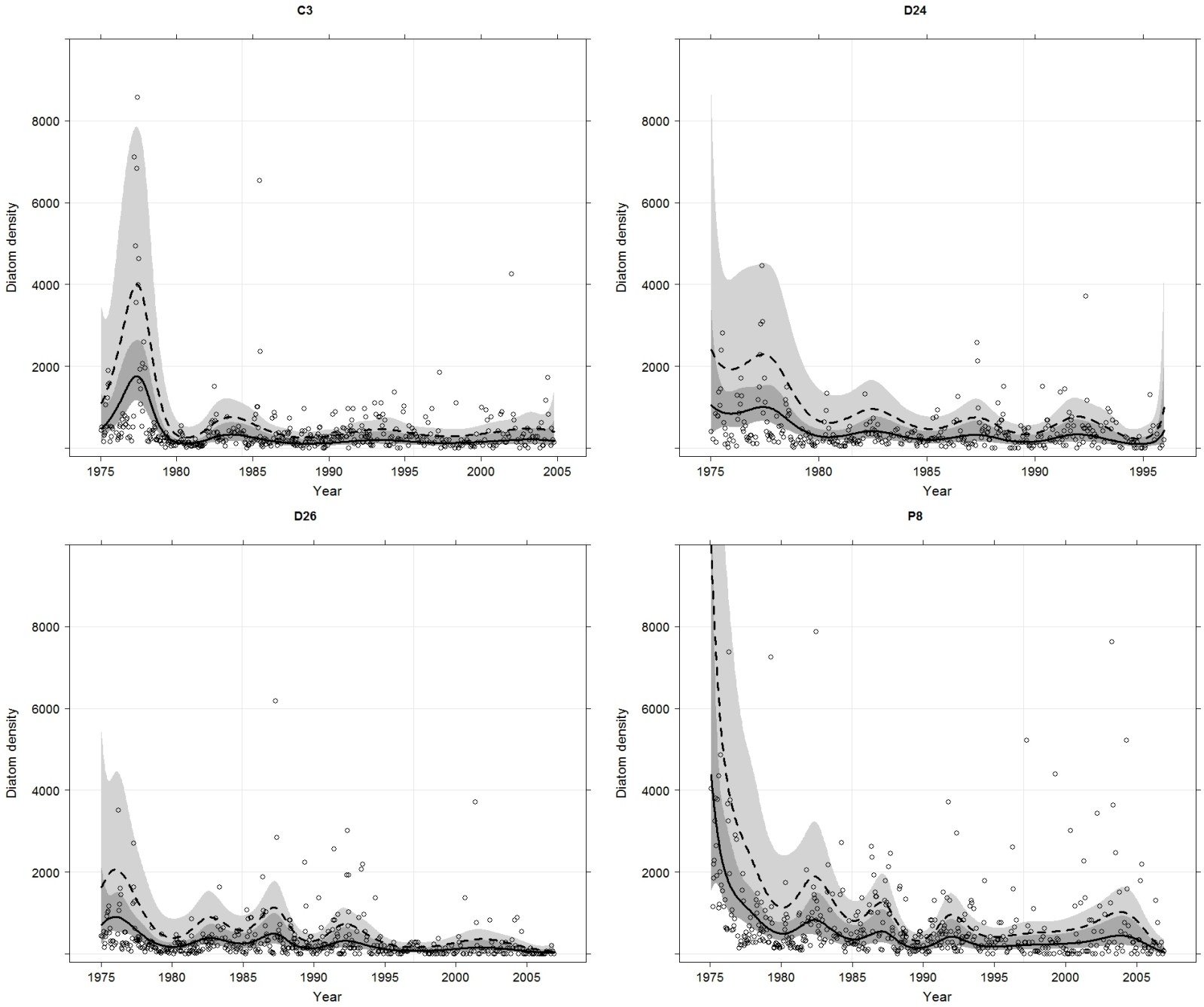


Figure A.5 Diatom Delta-only model with magnification. Each curve represents magnification-adjusted model predictions: solid line = 280x, dashed line = 700x.

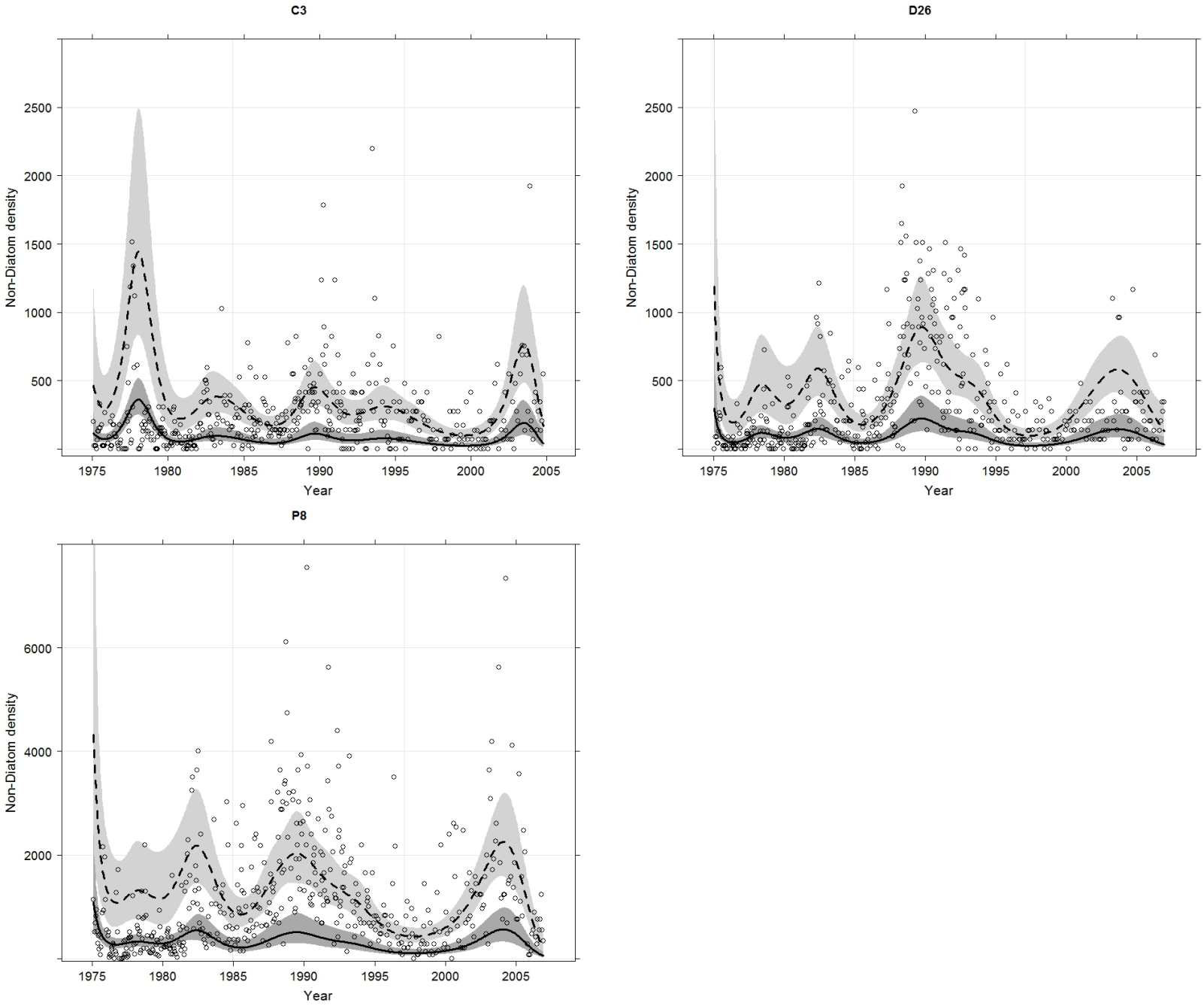


Figure A.6 Non-diatom Delta-only non-diatom model with magnification. Each curve represents magnification-adjusted model predictions: solid line = 280x, dashed line = 700x.

Section 6

The Suisun Bay Problem: Food Quality or Food Quantity?

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James E. Cloern, U.S. Geological Survey, 345 Middlefield Rd., Menlo Park, CA 94025
Email: jecloern@usgs.gov

Anthony Malkassian, San Francisco Estuary Institute and Ocean Sciences Department, University of California-Santa Cruz

Raphael Kudela, Ocean Sciences Department, University of California-Santa Cruz

Emily Novick, San Francisco Estuary Institute

Melissa Peacock, Ocean Sciences Department, University of California-Santa Cruz

Tara Schraga, U.S. Geological Survey

David Senn, San Francisco Estuary Institute

Introduction

Data collected by the Interagency Ecological Program's (IEP) Environmental Monitoring Program (EMP) have documented remarkable restructuring of biological communities in Suisun Bay over the past four decades. Manifestations of change include: establishment of the invasive clam *Potamocorbula amurensis* as a keystone species that is a potent consumer of phytoplankton (Kimmerer and Thompson 2014) and copepod nauplii (Kimmerer et al. 1994); significant reduction of phytoplankton biomass and primary production (Alpine and Cloern 1992); restructuring of the zooplankton community through replacement of rotifers, cladocerans and calanoid copepods by non-native cyclopoid copepods having lower nutritional value for fish (Winder and Jassby 2011); and population collapses of multiple species of fish including indigenous species at risk of extinction (Sommer et al. 2007). The scientific and policy communities have both contributed major efforts to understand and address the significant environmental declines seen in the Sacramento-San Joaquin Delta. Scientific clarification on the relative roles of the contributing factors could help focus ongoing large-scale planning efforts, and lead to more reasonable expectations of management outcomes.

The consensus of the broad scientific community, including local experts (Baxter et al. 2010, Hanak et al. 2013) and outside experts (Meyer et al. 2009, NRC 2012), is that population declines in the estuary across multiple trophic levels have been caused by multiple human disturbances. When queried about steps toward rehabilitation, strong majorities of local scientists recommended actions to restore more natural processes in the estuary, giving highest priority to restoring flows and habitat (Hanak et al. 2013). An alternative hypothesis has emerged recently that attributes many of these biological changes to another dominant causative factor -- changes in nutrients due to increased inputs from wastewater treatment plants, the largest of which is the Sacramento Regional Wastewater Treatment Plant (SRWTP). The proposed mechanisms are ammonium (NH_4) suppression of the fast growth potential of diatoms (Dugdale et al. 2007), and selection for different species at all trophic levels as NH_4 loads and the nitrogen (N) to phosphorus (P) ratio increase (Glibert 2010, Glibert et al. 2011). At the trophic level of the primary producers, increased NH_4 inputs have been suggested to contribute to a decrease in phytoplankton biomass due to lower production rates (Dugdale et al. 2007), and a shift in the phytoplankton community through a decrease in diatoms (Glibert 2010, Glibert et al. 2011) and increases in green algae and cyanobacteria (Glibert 2010), dinoflagellates and other flagellates (Glibert 2010, Glibert et al. 2011). Therefore the alternative hypothesis is that a root cause of restructured

biological communities and fish population collapses has been increased wastewater inputs, leading to increased NH_4 concentration and N:P ratio (Glibert et al. 2011).

This hypothesis has important management implications and is now being considered in policies to protect, restore and enhance the Delta ecosystem. California's Delta Plan (Delta Stewardship Council 2011) makes specific reference to it: "Dugdale et al. (2007) has determined that ammonium concentrations may be having a significant impact on phytoplankton composition and open-water food webs because of suppression of diatom blooms in the Bay-Delta. ", and "Ratios of nutrients in Delta waters are thought to be a primary driver in the composition of aquatic food webs in the Bay-Delta (Glibert et al. 2011)." Our goal here is to examine the ecosystem-scale evidence to determine whether or not it is consistent with the nutrient-focused hypothesis. To do this we asked if patterns of change detected in IEP-EMP monitoring data are consistent with four patterns of change that would be expected if the increase of NH_4 loading and corresponding increase in N:P over the past 3 decades are important drivers of ecological change in Suisun Bay:

(1) A pattern of decreasing phytoplankton biomass that tracked the pattern of NH_4 increase – either a steady trend of decline or a step decrease after NH_4 concentration exceeded the proposed threshold of 4-10 μM (Dugdale et al. 2007),

(2) A pattern of decreasing diatom abundance that tracked the patterns of increasing NH_4 concentration and N:P,

(3) Trends of increasing abundances of green algae, cyanobacteria, dinoflagellates, and other flagellates,

(4) A phytoplankton community having poor food quality as a proposed outcome of elevated N:P (Glibert et al. 2011).

We used IEP-EMP phytoplankton data collected from 1975-2009 to compare measured changes in Suisun Bay against these expected patterns. Similarities between observed and expected patterns of change would provide evidence supporting the proposition that nutrient forms and ratios are important regulators of biological communities; alternatively, differences between observed and expected patterns would provide evidence against this proposition, supporting the broad consensus that ecosystem damage has been caused by multiple human disturbances and cannot be attributed to a single factor.

Data and Analyses

Using IEP-EMP data (http://www.water.ca.gov/iep/products/data.cfm), we focused our analyses

on Suisun Bay where the transformation from a high-chlorophyll diatom-dominated state to one of low chlorophyll and dominance by small phytoplankton cells has been attributed to wastewater inputs of NH_4 (Glibert et al. 2011, Dugdale et al. 2013). From water-quality data (e.g., file 'WQ1975-2012/Lab Data 1975-1984x.csv') we computed mean annual chlorophyll *a* and NH_4 concentrations, and N:P as the ratio of dissolved inorganic nitrogen (DIN) to total phosphorus (TP), in samples collected at stations D7 and D8 (Figure 1) over the period 1975-2012. We computed SRWTP loadings of ammonium-N from measured NH_4 concentrations in plant effluent and effluent discharge for the period of record, 1985 through 2013 (Mussen, personal communication, see "Notes"). We used data from the IEP-EMP benthos program to calculate mean annual abundance of *Potamocorbula amurensis* at site D7-C, the only long-term benthos monitoring station in Suisun Bay (Peterson and Vayssieres 2010). We extracted phytoplankton abundance data from IEP-EMP files 'CommonNameData2007.xls' and '2008_2010_ Phyto.xlsx'. This record includes 1054 samples collected monthly at stations D7 and D8 between 8 January 1975 and 11 December 2009.

Before proceeding, we want to first communicate important information about the reliability of the IEP-EMP data for assessing changes in phytoplankton community composition. A standard practice of microscopic analysis is to count a minimum of 400 phytoplankton cells per sample, which yields estimates of total cell abundance having an accuracy (95% confidence limit) of ± 10% (Karlson et al. 2010). Abundances of individual species would have lower accuracy, depending on number of cells counted of each species. The number of cells (or colonies) counted in Suisun Bay samples collected from 1975-2007 never reached the standard of 400 (Figure 2). Cell counts were exceptionally low between 1988 and 2007 (Figure 2) when a mean of only 5 cells were counted per sample. These analyses yield estimates of cell abundance with extremely large uncertainty -- the span of the confidence interval (± 89%) is nearly double the value of reported cell abundances. Errors in estimated abundances of subsets of the community, such as diatoms or flagellates, are even larger. Therefore, cell abundances have not been measured with sufficient accuracy to provide reliable estimates of phytoplankton community change over time. However, important policy-shaping conclusions have been drawn from this data set (Glibert 2010, Glibert et al. 2011) so we proceed to use it as a test of the expected phytoplankton responses to changing nutrient inputs. Recognizing the data have errors too large to detect trends of change, we use them nonetheless for consistency with past studies and to determine if we reach similar, or different, conclusions. We note that IEP-EMP samples collected after 2007 were

analyzed with a different method, and accuracy of population abundances has improved (2007-2009 mean = 368 cells counted/sample; Figure 2).

In order to increase the power of statistical tests, we aggregated the phytoplankton data by averaging cell abundances from all samples collected each year at the two Suisun Bay stations, and then further aggregated the data by binning cell abundances into six phytoplankton groups: diatoms, dinoflagellates, green algae, cyanobacteria, cryptomonads, and other flagellates (e.g., Prasinophytes, Chrysophytes, Haptophytes, Euglenoids). Even this level of aggregation, however, yields population estimates with errors too large for detecting changes. For example, counts of green algae, which have been reported to increase, averaged fewer than 2 cells per sample from 1975-2007. We are preparing a manuscript to explain why trends derived from these kinds of data are highly suspect and do not provide a reliable basis for making policy decisions.

Observed vs. Expected Phytoplankton Patterns

Phytoplankton Decrease Tracked Increasing Ammonium-N Loading?

We used two simple approaches to identify patterns in the IEP-EMP data: the Mann Kendall (MK) test in **R** package *wq* (Jassby and Cloern 2012) to detect trends over time; and the CUSUM test in R package *changepoint* (Killick et al. 2014) to determine if trends were the result of abrupt step changes. The MK test is a nonparametric method for measuring trends and their significance in series of non-normal variables such as population sizes. The CUSUM test was designed to identify segments of a series that have significantly different means. The MK test confirmed significant increases in NH_4 loading from SRWTP ($p < 0.001$), and NH_4 concentration ($p = 0.001$) and N:P ($p < 0.001$) downstream in Suisun Bay (Figure 2). The smaller rate of NH_4 increase in Suisun Bay (1.5%/year) compared to NH_4 loading upstream (2.6%/year) reflects within-estuary processes of NH_4 consumption, such as nitrification, as wastewater NH_4 is transported downstream.

Next we measured patterns of change in phytoplankton biomass as chlorophyll *a* concentration (Figure 2). The MK test revealed a highly significant decline ($p = 0.001$) over the period 1975-2012, and the CUSUM test identified a change point in 1987 that divides the series into two eras: 1975-1986 (mean chlorophyll *a* concentration = 9.9 μg/Liter) and 1988-2012 (mean chlorophyll *a* concentration = 2.0 μg/Liter). The MK test detected no significant trends of chlorophyll *a* change in the eras before or after 1987, so the phytoplankton decline in Suisun Bay occurred as a step change rather than a trend over time. If phytoplankton biomass in Suisun Bay has been altered by changes in NH_4 or N:P then we

would expect (1) a steady decrease of phytoplankton biomass that mirrored the steady increase of NH_4 loading from SRWTP, and (2) a significant biomass decrease during the period 1988-2012 when NH_4 loading increased from 7.5 to 12.8 tons N/day. However, neither pattern was observed (Figure 2). Instead of a steady decline over time, the 1987 change point signaled an abrupt regime shift when phytoplankton biomass and primary production decreased five-fold (Alpine and Cloern 1992). This regime shift coincided with the population explosion of *Potamocorbula amurensis* within the first year of its appearance in Suisun Bay (Figure 2). However the abrupt decline of phytoplankton biomass was not associated with an equivalent step-change in NH_4 concentration (the CUSUM test revealed no significant change in NH_4 concentration around 1987). And the chlorophyll *a* decline occurred before the 1988-2012 period of largest NH_4 loading increase from SRWTP (Figure 2).

These observed patterns of change suggest that the phytoplankton decline in Suisun Bay was caused by an abrupt and permanent increase in grazing mortality rather than to the expected steady decrease in growth rate associated with increased NH_4 loading. This conclusion is supported by measurements demonstrating that *Potamocorbula* filtration is fast enough to control phytoplankton biomass growth (Cole et al. 1992), and disappearance of the large summer diatom bloom that was characteristic of Suisun Bay during the pre-*Potamocorbula* era (Figure 12, Cloern and Jassby 2012).

Diatom Decrease Tracked Increasing NH_4 Loading?

The MK test detected a large and highly significant ($p < 0.001$) diatom decrease in Suisun Bay over the period 1975-2009. However, the pattern of diatom decrease (Figure 3) did not track the increase of NH_4 loading (Figure 2). Instead of a steady loss, the diatom loss was abrupt and it occurred in synchrony with the chlorophyll *a* decline (Figure 2). The CUSUM test identified a 1987 change point separating a 1975-1986 era of high diatom abundance (mean 1101 cells/mL) and a 1988-2009 era of low diatom abundance (mean 107 cells/mL). One tenet of the alternative hypothesis is that the Suisun Bay diatom loss began after NH_4 inputs from SRWTP started to increase in the early 1980s (Dugdale et al. 2007, Glibert 2010). However, the MK test showed no significant trend of diatom decrease during the 1975-1986 era ($p = 0.11$). This result doesn't discount the possibility of a process that would drive a diatom decline, but any effect of that process was overwhelmed by hydrologic variability during 1975-1986, including the two wettest consecutive years (1982 and 1983) and two driest years (1976 and 1977) on record. The MK test also did not reveal a significant diatom decline during the 1988-2009 era ($p = 0.13$) and this is an important departure from expectations because NH_4 inputs nearly doubled during

that period (Figure 2). Therefore, patterns of change in the 35-year IEP-EMP record do not provide evidence that the loss of diatoms from Suisun Bay can be attributed to wastewater inputs of NH_4.

Patterns in the IEP-EMP data do provide strong evidence that the loss of diatoms was a manifestation of the regime shift toward chronic low phytoplankton biomass after *Potamocorbula* became established. The mechanism of this regime shift is confirmed by measurements showing that phytoplankton grazing losses exceed phytoplankton production in Suisun Bay (Kimmerer and Thompson 2014). Early evidence of the power of clam grazing was provided during the 1977 drought when diatom abundance was unusually low (Figure 3). This low-diatom anomaly coincided with salt intrusion that facilitated colonization of Suisun Bay by the marine clam *Mya arenaria* (Nichols 1985). This event previewed the state of low-diatom abundance that has persisted in Suisun Bay since the *Potamocorbula* invasion. Diatoms might be more susceptible to clam grazing than other algae because they sink (Cloern et al. 1983). Sinking transports diatoms to the sediment-water interface where clam filtration occurs, and it could explain why the diatom loss after 1987 (Figure 3) was larger than the chlorophyll *a* loss (Figure 2). Similar losses of diatoms have occurred in other ecosystems, such as Lake Michigan after invasion by the quagga mussel *Dreissena rostriformis* bugensis (Fahnenstiel et al. 2010).

The collective weight of evidence – synchrony of an abrupt diatom decline with the *Potamocorbula* arrival, absence of the expected trend of a diatom decrease mirroring the trend of increased NH_4 loading, measurements showing that clam grazing is faster than phytoplankton production, and precedents of bivalve invasions leading to diatom declines in other ecosystems – is inconsistent with the proposition that the diatom decline was caused by changing nutrient forms or ratios.

Increasing Abundances of Non-diatoms?

We used the MK test to look for the expected trends of increasing abundances of other algal groups (Figure 3), and found no significant trends of increasing or decreasing abundances of cryptomonads ($p = 0.98$), other flagellates ($p = 0.24$), green algae ($p = 0.25$), dinoflagellates ($p = 0.47$), or cyanobacteria ($p = 0.89$) over the period 1975-2009. Thus, the IEP-EMP data set does not support the proposition that other algae have outcompeted diatoms because their growth is favored by high NH_4 and/or high N:P.

Poor Food Quality?

We tested the expectation of low phytoplankton food quality by computing an index of food quality from measurements of phytoplankton biovolume in 152 samples collected as part of the USGS research program (Cloern and Dufford 2005, Sobczak et al. 2005). Surface samples were collected irregularly during 1992-2014 in Suisun Bay and in the lower Sacramento River below the SRWTP discharge (Figure 1). Phytoplankton biovolume is computed as measured cell volume (μm^3/cell) times abundance (cells/mL) of all phytoplankton species (Cloern and Dufford 2005). The food-quality index is based on laboratory experiments showing that growth efficiency of crustacean zooplankton is highest when they are fed algae enriched in highly unsaturated fatty acids (cryptomonads and diatoms), and lowest when fed algae poor in these essential fatty acids (cyanobacteria) (Brett and Müller-Navarra 1997). For each USGS sample we computed:

(1) $$\text{Food Quality Index} = 0.2*P_{cy} + 0.525*P_{gr} + 0.7*P_{di} + 0.95*P_{cr}$$

where P_{cy}, P_{gr}, P_{di}, and P_{cr}, are the proportions of phytoplankton biovolume in a sample contributed by cyanobacteria, green algae, diatoms, and cryptomonads. The food values of each algal group are from (Park et al. 2003). Similar analyses cannot be applied to the IEP-EMP dataset because phytoplankton biovolume was not consistently measured or reported.

The food quality index ranged from 0.28 to 0.95. It was low (< 0.5) during blooms of cyanobacteria (e.g. *Oscillatoria*, *Aphanizomenon*) or green algae (e.g. *Spirogyra*), but these were rare, occurring in only 5 of 152 samples (Figure 4). The food quality index was high (> 0.7) in 114 of 152 samples where cryptomonads contributed a substantial fraction of biovolume. Phytoplankton biovolume in Suisun Bay and the lower Sacramento River was composed mostly of diatoms (overall mean 62%) and cryptomonads (mean 24%). Cyanobacteria, dinoflagellates and green algae were minor (but episodically important) components. As a result of this community composition, the mean quality of the phytoplankton food resource downstream of SRWTP was high (0.73), and virtually identical to that of a pure-diatom community (0.7). Thus, the USGS data set does not support the proposition that quality of the phytoplankton food resource is impaired by high NH_4 and/or high N:P.

Conclusion and Management Implications

Independent data sets collected by IEP-EMP and USGS do not provide evidence to support the hypothesis that increased NH_4 or changes in N:P have altered phytoplankton community composition in

Suisun Bay or selected for algal species having poor food quality. This conclusion has important management implications. First, it reminds us that phytoplankton populations in Suisun Bay are regulated by many factors, including light limitation of growth by high turbidity (Alpine and Cloern 1988, Jassby 2008), grazing losses to clams and zooplankton (Kimmerer and Thompson 2014), and by variability of freshwater inflow (Cloern et al. 1983, Jassby 2008, Dugdale et al. 2013). Sewage inputs of nutrients may play a role, but the empirical record indicates that its role is overwhelmed by these other factors. Second, this result extends up the food chain because fish populations and their supporting ecosystem functions are also regulated by many factors. Food supply plays a role, but its role in population losses of native fishes is unclear given the effects of other factors such as habitat loss (Whipple et al. 2012) and fragmentation (Sommer et al. 2001), flow modifications (Meyer et al. 2009, Moyle et al. 2010), fish entrainment by water diversions (Rose et al. 2013), changes in salinity and turbidity (Mac Nally et al. 2010, Hasenbein et al. 2013), disruption of food webs by introduced species (Winder and Jassby 2011), and contaminant effects (Brooks et al. 2012).

As we work to unravel the enormous complexity of the San Francisco Bay-Delta ecosystem, it's essential for us to listen to the estuary. The estuary has been telling us for decades that, from an energetics perspective, the Suisun Bay problem (chronic food limitation of consumers) is one of low quantity, not poor quality of the phytoplankton food supply. But more importantly, from a more holistic ecosystem perspective, the estuary has been telling us that the Suisun Bay (and Delta) problem spreads far beyond the single issue of food supply (Baxter et al. 2010). The broad scientific community has reached a strong consensus that the estuary has been damaged over many decades by multiple disturbances, and they have advised that recovery will be difficult and require steps to mitigate each disturbance where mitigation actions are feasible.

The nutrient-focused hypothesis has led some to conclude that recovery of the estuary might be achieved or accelerated by a single action – implementation of advanced wastewater treatment. These conclusions emerge from propositions that: "...a clear management strategy is the regulation of effluent N discharge through nitrification and denitrification. Until such reductions occur, other measures, including regulation of water pumping or manipulations of salinity, as has been the current strategy, will likely show little beneficial effect. Without such action, the recovery of the endangered pelagic fish species is unlikely at best" (Glibert 2010); and "An understanding of the critical role of anthropogenic NH_4 input could provide a powerful tool for management of estuarine productivity, since typically the

proportion of the anthropogenic input/loading of NH_4 in these regions can be controlled by changes in water treatment practices and water allocation (dilution)" (Dugdale 2007).

Improvements in wastewater treatment have clear environmental benefits by reducing inputs of nutrients, toxic contaminants, and the oxygen demand of wastewater (e.g. Cloern and Jassby 2012). However, if we accept the proposition that nutrients (forms and ratios) function as a master regulator of the estuary then we face two risks. First, we risk disappointment if the projected outcomes of advanced wastewater treatment, including increased primary production (Dugdale et al. 2007) and return of biological communities to an earlier state (Glibert 2010, Glibert et al. 2011), are not realized. Second, we risk missed opportunities to address the root causes of ecosystem degradation and fish population declines. Our primary purpose here is to remind resource managers of the consistent guidance given by the broad scientific community: "Consideration of the large number of stressors and their effects and interactions leads to the conclusion that efforts to eliminate any one stressor are unlikely to reverse declines in the listed species." (NRC 2012).

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Notes

Dr. Timothy D. Mussen, Sacramento Regional County Sanitation District, provided NH_4 concentrations in plant effluent and effluent discharge from the Sacramento Regional Wastewater Treatment Plant (personal communication, 21 April 2014).

Figure 1. Map showing locations of IEP-EMP stations D7 and D8 and USGS stations in the lower Sacramento River (657, 649) and Suisun Bay (3, 4, 6, 8, 415, location of X2) where phytoplankton were sampled from 1975-2009 and 1992-2014, respectively.

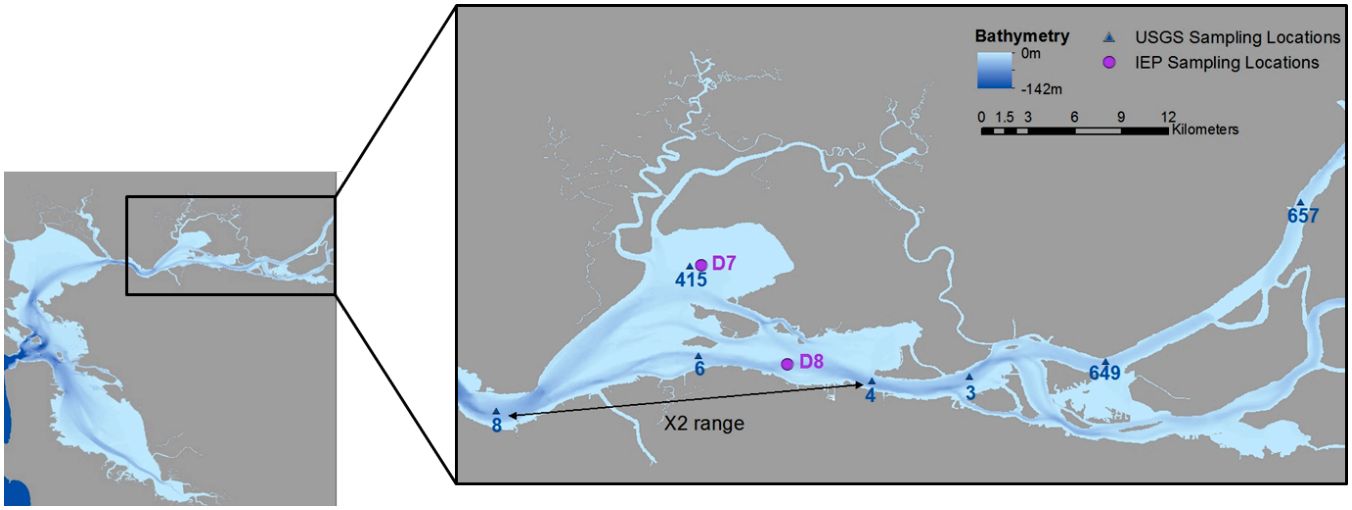

Figure 2. Top panel: number of cells or colonies counted in phytoplankton samples collected at IEP-EMP station D7. Bottom panels show mean annual: NH_4 loading from SRWTP; NH_4 concentration and N:P ratio in Suisun Bay (means of measurements at stations D7 and D8); chlorophyll *a* concentration in Suisun Bay (means of measurements at stations D7 and D8); and *Potamocorbula amurensis* abundance (station D7-C). The orange lines in the chlorophyll *a* panel demarcate a 1987 change point in mean chlorophyll *a* concentration, synchronous with the establishment of *Potamocorbula* in Suisun Bay. There was no significant trend of chlorophyll *a* concentration before or after 1987, nor was there a decadal trend in chlorophyll *a* mirroring the increases in NH_4 concentration and N:P ratio. ND means not determined.

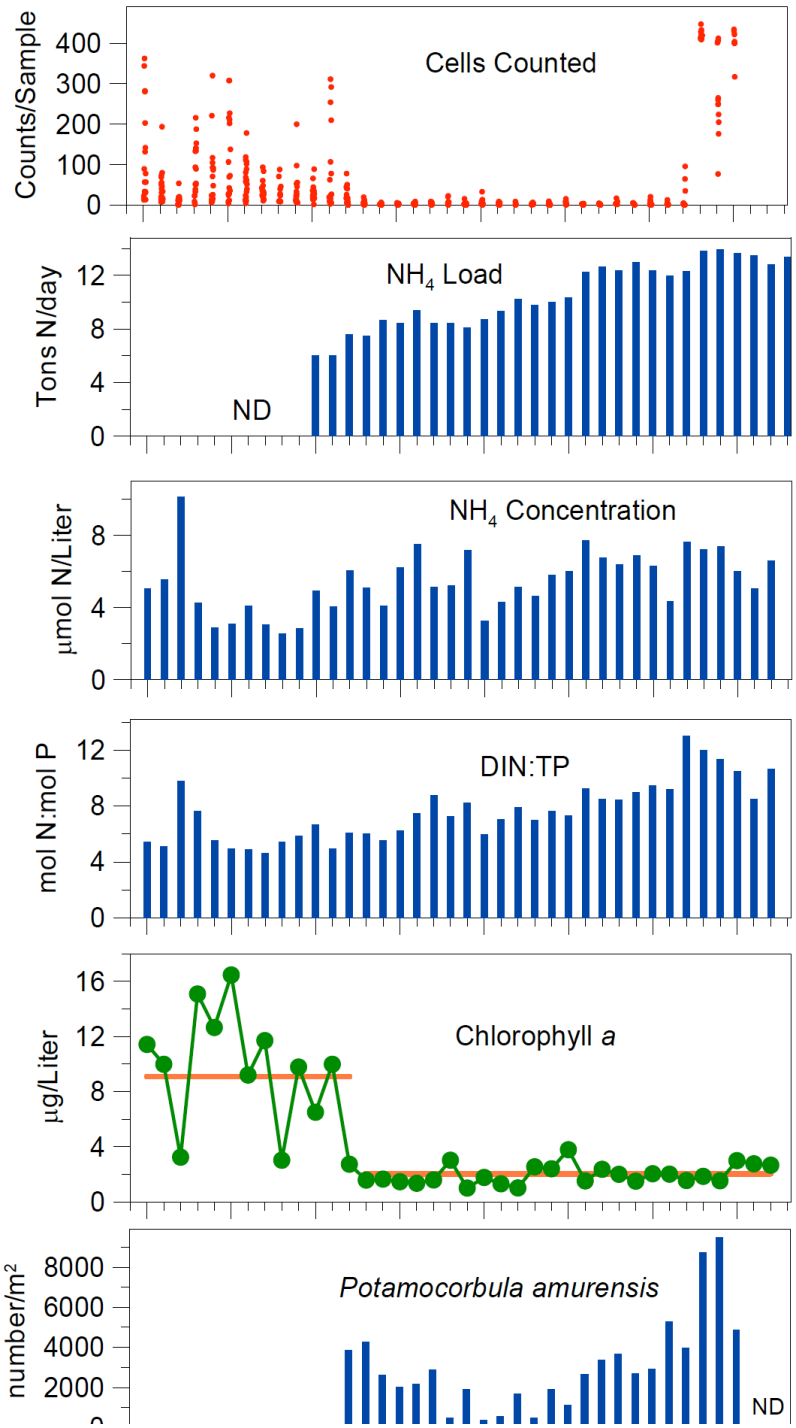

Figure 3. Mean annual abundances of six phytoplankton groups in Suisun Bay (means of measurements at IEP-EMP stations D7 and D8). The orange lines in the top panel demarcate a change point in mean diatom abundance in 1987. There was no trend of diatom abundance before or after 1987, and no trend, upward or downward, for the other phytoplankton groups.

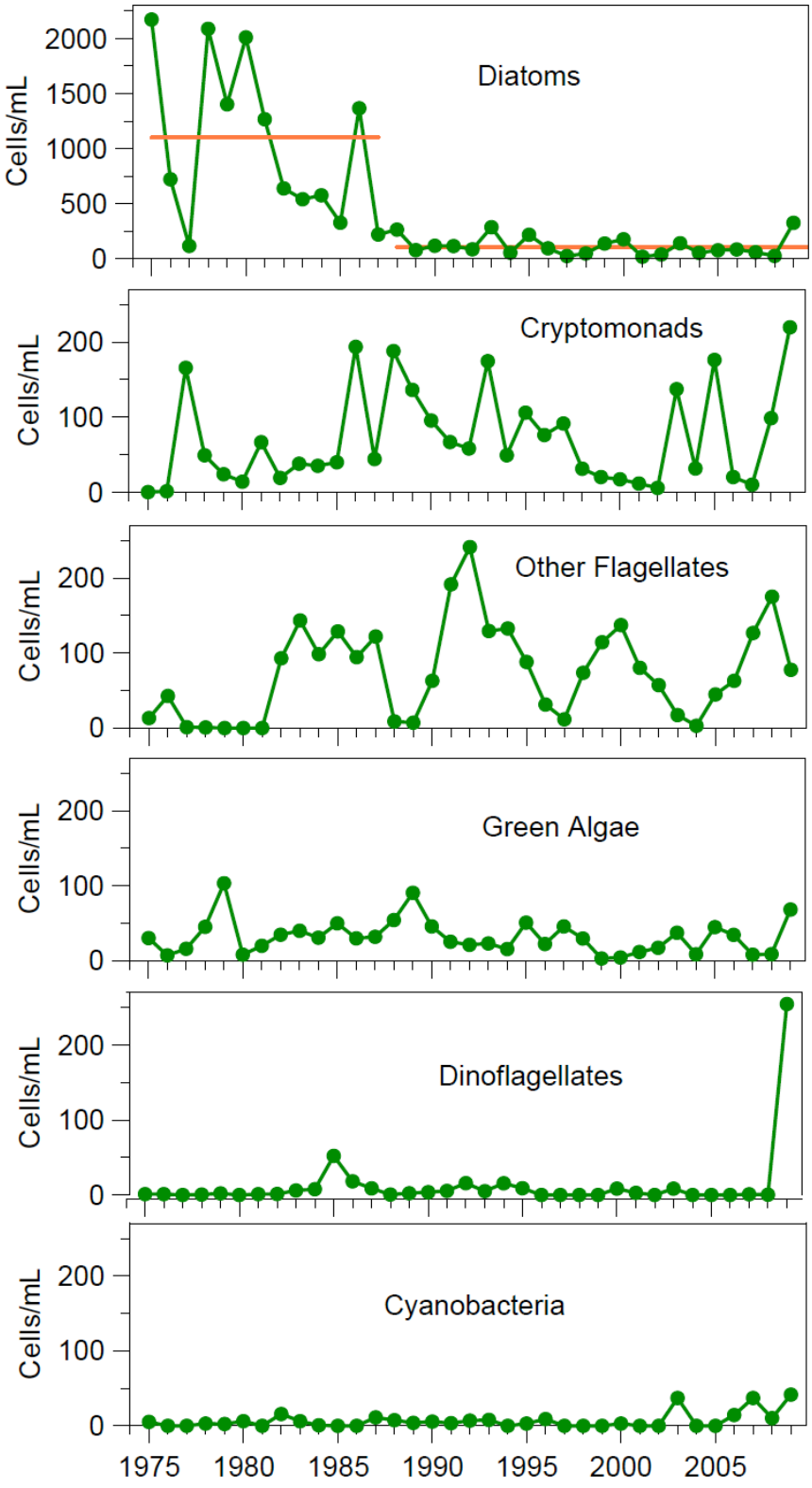

Figure 4. Pie chart shows the mean proportions of phytoplankton biovolume contributed by diatoms (62%), cryptomonads (24%), green algae (6%), cyanobacteria (4%), chrysophytes (2%) and dinoflagellates (2%) in 152 USGS samples collected in Suisun Bay and the lower Sacramento River from 1992-2014 (see map, Figure 1). Bottom graph shows the food quality index of each sample computed from the proportional contributions of diatoms, cryptomonads, green algae, and cyanobacteria to total biovolume. Green horizontal line is the food-quality value for diatoms (0.7).