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Nutrient availability in support of *Karenia brevis* blooms on the central West Florida Shelf: What keeps *Karenia* blooming?

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Abstract

Identifying nutrient sources, primarily nitrogen (N) and phosphorus (P), sufficient to support high biomass blooms of the red tide dinoflagellate, *Karenia brevis*, has remained problematic. The West Florida Shelf is oligotrophic, yet populations $>10^6$ cells L^{-1} frequently occur and blooms can persist for months. Here we examine the magnitude and variety of sources for N and P that are available to support blooms. Annual average *in situ* or background concentrations of inorganic N in the region where blooms occur range 0.02–0.2 μM while inorganic P ranges 0.025–0.24 μM. Such concentrations would be sufficient to support the growth of populations up to $\sim 3 \times 10^4$ cells L^{-1} with at least a 1 d turnover rate. Organic N concentrations average 1–2 orders of magnitude greater than inorganic N, 8–14 μM while organic P concentrations average 0.2–0.5 μM. Concentrations of organic N are sufficient to support blooms $>10^5$ cells L^{-1} but the extent to which this complex mixture of N species is utilizable is unknown. Other sources of nutrients included in our analysis are aerial deposition, estuarine flux, benthic flux, zooplankton excretion, N_2-fixation, and subsequent release of organic and inorganic N by *Trichodesmium* spp., and release of N and P from dead and decaying fish killed by the blooms. Inputs based on atmospheric deposition, benthic flux, and N_2-fixation, were minor contributors to the flux required to support growth of populations $>2.6 \times 10^4$ cells L^{-1}. N and P from decaying fish could theoretically maintain populations at moderate concentrations but insufficient data on the flux and subsequent mixing rates does not allow us to calculate average values. Zooplankton excretion rates, based on measured zooplankton population estimates and excretion rates could also supply all of the N and P required to support populations of 10^5 and 10^6 cells L^{-1}, respectively, but excretion is considered as "regenerated" nutrient input and can only maintain biomass rather than contribute to "new" biomass. The combined estuarine flux from Tampa Bay, Charlotte Harbor, and the Caloosahatchee River can supply a varying, but at times significant level of N and P to meet growth and photosynthesis requirements for populations of approximately 10^5 cells L^{-1} or below. Estimates of remineralization of dead fish could supply a significant proportion of bloom maintenance requirements but the rate of supply must still be determined. Overall, a combination of sources is required to maintain populations $>10^6$ cells L^{-1}.

Keywords: Red tides; Nutrients; Nutrient flux; Nitrogen; Phosphorus; Gulf of Mexico; West Florida Shelf; Tampa Bay; Charlotte Harbor

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1. Introduction

Blooms of harmful algal species are the result of complex interactions of environmental factors acting at both population and cellular levels and the ecophysiological responses of the individual species involved. While physical parameters (e.g. temperature, salinity) may determine the distribution or presence/absence of a Harmful Algal Bloom (HAB) species on a regional scale, nutrient availability regulates growth rate, biomass, and the duration of a bloom. Evaluating the significance of a single factor in regulating HABs is consequently a challenging task, and may require multi-bloom data sets.

Red tides caused by the toxic dinoflagellate, *Karenia brevis,* on the West Florida Shelf (WFS) originate in oligotrophic offshore waters, with subsequent transport inshore via winds and tidal currents (Steidinger and Haddad, 1981). Thermal and salinity fronts act as both barriers and transport mechanisms, concentrating normally disperse populations of *K. brevis* in shelf waters (Vargo et al., 2001). The combined result of these mechanisms is a *K. brevis* "bloom" which originated via concentration processes rather than from enhanced growth rates. Primary production rates within these blooms are 2–3 fold higher than background rates during non-bloom periods primarily because of elevated biomass in large patches (Vargo et al., 1987; Heil et al., 2004). However, inorganic nitrogen (DIN) and phosphate (DIP) concentrations within the bloom remain unchanged compared with non-bloom waters, reflecting the oligotrophic nature of the WFS (Heil et al., 2001).

The source of major nutrients, nitrogen (N) and phosphorus (P) required to maintain blooms (which can last up to18 months) has not been identified (Steidinger et al., 1998, Vargo et al., 2001). Nutrient input from N_2-fixation during *Trichodesmium* blooms (Walsh and Steidinger, 2001; Mulholland et al., 2004, 2006), remineralization of near bottom diatom blooms fueled by shelf-break upwelling (Walsh et al., in review), remineralization of fish and other vertebrates and invertebrates killed by brevetoxins during blooms (Walsh et al., in review) and estuarine flux of N and P when blooms reach coastal waters (Vargo et al., 2004) have all been proposed to support *K. brevis* blooms.

2. The historical *K. brevis* nutrient perspective

2.1. Phosphorus

Early research on the nutrient requirements of *K. brevis* blooms focused upon P. Based on 5 years of total phosphate (TP) data Dragovich et al. (1963, cited in Wilson, 1966) concluded that high P levels were not required to support *K. brevis* blooms. Few *K. brevis* cells were found in waters with TP $<0.2\,\mu M$ ($6.0\,\mu g\,L^{-1}$) or $>4\,\mu M$ ($\sim 120\,\mu g\,L^{-1}$). These values were confirmed in laboratory culture studies by Wilson (1966), and Wilson et al. (1975), and complemented earlier findings by Odum et al. (1955) and Bein (1957) that P is not a limiting nutrient for *K. brevis* blooms. Odum further stated that "in general P levels of coastal water seem similar in a red tide year to those of a non-red tide year" while Bien observed "waters of the west coast of Florida maintain a sufficient P concentration to support a red tide at all times of the year…".

Estimates of P requirements for growth, photosynthesis and cellular P-turnover rates calculated for a 1986 *K. brevis* bloom off the mouth of Tampa Bay (TB) confirm these earlier conclusions (Vargo, 1988; Steidinger et al., 1998). Population levels in this bloom ranged 6×10^4–6.5×10^5 cells L^{-1} and the measured standing stock of inorganic and hydrolyzable organic P ranged 28–120 mg m^{-2}. Based on growth rates of 0.2 doublings d^{-1} and primary production rates calculated from relationships developed by Shanley (1985), sufficient P was available in the water column to meet the daily requirements of this bloom provided that the turnover rate of the standing stock was once per day in order to maintain the measured concentration. The two stations with the highest population density (10^6 cells L^{-1}) would, however, deplete the water column supply within 1 d. Therefore, except at high population levels, sufficient P was available for bloom maintenance.

Based on the K_s value for growth over a range of phosphorus concentrations, Vargo and Howard-Shamblott (1990) concluded that *K. brevis* was adapted for growth in the low P oligotrophic waters of the WFS. They also calculated the cellular yield per unit of P and found that from 2 to 9×10^6 cells, depending on the size, can be produced per μmol of available P. This range is similar to that originally calculated by Wilson (1966). If we assume the lower cell concentration produced by 1 μM of P, then the ambient concentration of approximately 0.2 μM P will support about 20% of that value, or 4×10^5 cells. This population level corresponds to the observed cell concentrations supported by the standing stock of P during the 1986 bloom off TB (see Vargo, 1988; Steidinger et al., 1998). Therefore it is evident that additional unidentified sources of P

are required to support any increment in biomass during the near-shore blooms that may persist for months. Maintenance of these high biomass blooms would require rapid turnover of ambient P via remineralization processes or high fluxes from external sources.

2.2. Nitrogen

N, rather than P, is most often considered to be limiting in marine waters (Hecky and Kilham, 1988). It is curious, therefore, that there is far less information available about N metabolism for *K. brevis* than P metabolism. Dragovich et al. (1961) suggested there was never sufficient nitrate + nitrite ($NO_3 + NO_2$) in the water column to support *K. brevis* blooms. Less than 1% of their samples in neritic waters (>4000 samples) had N levels of 1 μM (14 μg N). Their ammonia (NH_4^+) levels were always higher than NO_3, especially after rains, which led to the suggestion that NH_4^+ would be the primary N source and that direct input from rain could be an important source.

If *K. brevis* requires approximately 0.48 μM P (15 μg L^{-1}) to support 10^6 cells L^{-1} (Wilson, 1966), which have a cellular molar ratio of N:P of 17.7:1 (Shanley and Vargo, 1993), a typical bloom concentration of 10^6 cells L^{-1} would require approximately 8.6 μM N (120 μg L^{-1}). Odum et al. (1955) did a similar calculation using a lower N:P ratio and estimated a value of 3 μM (45 μg L^{-1} N). Although a more realistic value, even Odum's estimated concentration is rarely found in west Florida coastal waters or bays.

Vargo et al. (2004) recently described the nutrient background and requirements for *K. brevis* blooms that occurred on the WFS between October, 1998 and January, 2002 and concluded that *in situ* levels of both N and P were insufficient to meet growth requirements. Calculated total nitrogen (TN) and TP fluxes from TB and Charlotte Harbor (CH) indicate that estuarine transport could supply from 5–20% of required N and from 4% to 90% of required P for the daily growth needs of a moderate population (3×10^5 cells L^{-1}) of *K. brevis* (Vargo et al., 2004). Therefore, fluxes of N and P from the two major West coast estuaries are barely sufficient to support moderate populations in near shore waters. Most blooms, however, have population levels greater than 3×10^5 cells L^{-1} and persist for months (Vargo et al., 2004). Supplying the nutrients required to maintain the biomass found during red-tide events has become a major dilemma. Inorganic and organic N released from *Trichodesmium* after N-fixation (Walsh and Steidinger, 2001; Lenes et al., 2001; Mulholland et al., 2004, 2006) may contribute sufficient quantities to maintain growth but processes such as N_2-fixation also require P and therefore exacerbate the potential for P-deficiency. Other than estuarine flux no other sources of P have been identified.

2.3. Possible sources of nutrients available to *K. brevis* blooms

Estuarine flux of N and P should be considered as a primary source for the growth and maintenance of blooms in nearshore coastal waters. This is particularly true for N sources. While DIN concentrations in both TB and CH are extremely low, TN concentrations are not (Fraser and Wilcox, 1981; McPherson and Miller, 1990; Vargo et al., 1991; KEA, 1992). The average concentration of $NO_3 + NO_2$ and PO_4 at the mouth of TB in 2001 was 0.6 and 0.9 μM, respectively, with a molar ratio of 0.7. However, the TN:TP ratio was 23.7, indicating that most of the TN is in the form of dissolved and particulate N. Dissolved organic nitrogen (DON) values in 2001 were typical and ranged 44–56 μM. These values represent approximately 93% of the TN at stations near the mouth of TB (source: HCEPC 2001). Similar concentrations and ratios of inorganic and total N and P are found in CH (Fraser and Wilcox, 1981). Thus, estuarine flux of inorganic N from both estuaries may not contribute significantly to the growth requirements of *K. brevis* blooms in coastal waters, but DON levels in both estuaries are quite high (Vargo et al., 2004) and must be considered as a source of N for coastal blooms.

Several potential offshore sources of DON exist. Centric diatom populations with high biomass (>5 μg chl *a* L^{-1}) develop in near bottom water during summer stratification seaward of the 20 m isobath (Heil et al., 2001), potentially fueled by NO_3 originating from offshore intrusions of shelf-break water associated with movement of the Loop Current (Walsh et al., 2006). This upwelled water does not reach the surface and has not been associated with elevated *K. brevis* populations. Decay and remineralization of the near bottom diatom populations combined with break down of the summer thermal stratification by vertical mixing during fall may be one possible source of required

nitrogen (N) for bloom development and growth. Elevated concentrations of DON in offshore waters coincide with the timing of increased vertical mixing and resuspension (Vargo et al., 2001; Lester et al., 2001).

Mid to late summer blooms of the diazotroph *Trichodesmium* spp., which often precede *K. brevis* blooms, appear to be stimulated by iron input from the Saharan dust events, and may also act as a source of NH_4^+ and organic N via excretion of amino acids (Walsh and Steidinger, 2001; Lenes et al., 2001). Elevated populations of *Trichodesmium* sp. coincide with elevated DON concentrations and non-detectable DIP and dissolved organic phosphorus (DOP) concentrations on the WFS and P limitation of *Trichodesmium* N_2-fixation has been reported (Sanudo-Wilhelmy et al., 2001). Therefore, as noted above, N-fixation as a potential N source for *K. brevis* bloom maintenance will only exacerbate the possibility of phosphorus limitation.

Atmospheric deposition is another possible source of nutrients to the coastal zone in this region. Studies of deposition rates in TB and the nearby coastal areas suggested that almost 30% of the annual N-loadings to TB were from wet and dry deposition. Values were on the order of $10^5\,kg\,yr^{-1}$ for N and $10^3\,kg\,yr^{-1}$ for P (TBNEP pers. comm.), but more recent (Pribble and Janicki, 1999) N and P deposition rates to the surface of TB are $760\,mg\,m^{-2}\,yr^{-1}$ for total wet and dry N ($148\,\mu M\ N\,m^{-2}\,d^{-1}$) and $5.87\,mg\,m^{-2}\,yr^{-1}$ for P ($0.00052\,\mu M\,m^{-2}\,d^{-1}$). When mixed into a 10 m water column, these rates yield fluxes of $0.0148\,\mu M\,d^{-1}$ N and $0.0000519\,\mu M\,d^{-1}$ P. The N input is comparable to estuarine flux of N (see below) while the P flux is negligible.

Paerl et al. (2002) calculated an N deposition rate of $6.5\,kg\,ha^{-1}\,yr^{-1}$ as an average rate out to the 200 m isobath for the Eastern Gulf of Mexico. This rate translates to an N flux of $127\,\mu mol\,m^{-2}\,d^{-1}$ which, if totally mixed in a 10 m water column would yield a daily input of ~0.013 μM. Such values are of the same order as measured *in situ* inorganic N concentrations.

Measurements of the δ^{15}PON from the WFS, including *K. brevis* populations, strongly suggest that several sources of N are used by *K. brevis* (Havens et al., 2004). The δ^{15}N range of particulates in *K. brevis* blooms (3–5) indicates that N derived from *Trichodesmium* remineralization, estuarine inputs, and seagrass-derived material may all be available to bloom populations (Heil et al., 2001, Vargo et al., 2001; Havens et al., 2004, Havens, 2004). No estimates are available for other potential sources of remineralized N and P such as vertebrates that have died from exposure to toxins, zooplankton excretion, benthic flux, and cell lysis. Additionally, the prior estimates of N- and P-flux from the estuaries, N_2-fixation, and atmospheric deposition must be re-examined in light of new measurements.

While remineralization processes via bacterial and higher trophic pathways are important sources of N and P for the maintenance of primary production in estuaries (Nixon and Pilson, 1983; Alongi and McKinnon, 2005) they are considered to be "regenerated" nutrients, *sensu* Dugdale and Goering (1967), and do not lead to increased biomass. Since growth (i.e. increased biomass) of *K. brevis* populations is the primary concern of this study and since there is little information available for the WFS for remineralization processes other than zooplankton excretion, we cannot address remineralization processes as a source of nutrients for bloom dynamics at this time.

In this paper we re-examine the seasonal and spatial distribution of inorganic and organic N and P within the ECOHAB:Florida study area, update our earlier estimates of N and P fluxes from west central Florida estuaries, examine N-fixation and atmospheric deposition, and add additional estimates of N and P from zooplankton excretion, benthic flux, and decay of fish killed by *K. brevis* blooms. The objective of this exercise is to constrain to the extent possible, the source or sources of nutrients that contribute to the maintenance of *K. brevis* blooms in nearshore waters of the WFS.

3. Methods

3.1. Hydrographic and chemical methods

Hydrographic data and water column samples were collected from 1998 to 2002 at 63 locations along three cross-shelf and two along shore transects (Fig. 1) during monthly quasi-synoptic cruises within an area on the WFS that extends from TB to Ft. Myers. This region historically experiences the highest incidence of *K. brevis* red tides. Continuous underway measurements of surface temperature, salinity, and chlorophyll *a* fluorescence were made on all cruises. Water column samples were taken with Niskin bottles mounted on a rosette sampler with a Seabird CTD. Inorganic nutrients (NO_2, NO_3, PO_4, SiO_4) were determined

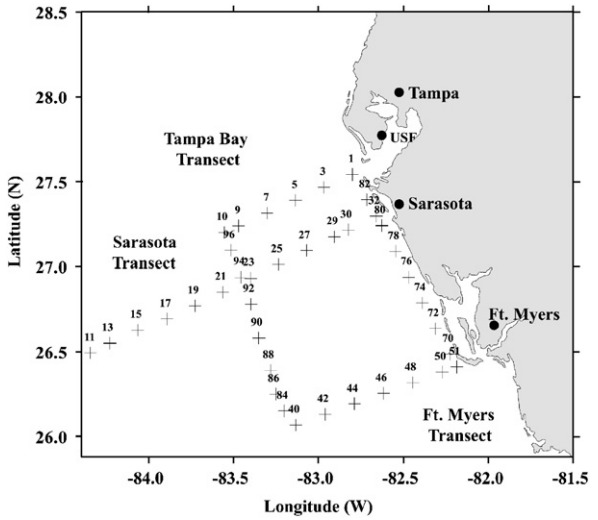

Fig. 1. Location of stations along the three cross-shelf transects and the 10 and 50 m isobaths that defined the ECOHAB:Florida control volume.

on frozen, unfiltered water samples taken at discrete depths and analyzed on an Alpkem RFA II segmented-flow nutrient analyzer. Particulate and total dissolved phosphorus (TDP) were determined following the methods described by Solorzano and Sharp (1980a, b). Total dissolved nitrogen (TDN) was determined with a modified procedure based on a combination of the oxidation step as described by Bronk et al. (2000) with the final NO_3 concentrations measured as described above for inorganic nutrients.

3.2. Nutrient input calculations

Calculation of estuarine flux of N and P from TB, CH, and the Caloosahatchee River is based on measured river flow data supplied by the USGS. Flow data from all of the major tributaries entering TB and CH was summed for each day to yield a daily total flow into each estuary, capturing respectively 45% and 63% of the two watershed drainage areas. Water flows for the Caloosahatchee River are based on the USGS gauge station at S-79, a water control structure at the freshwater end member of the river. The flow into each estuary was assumed to equal flow out and represents a lower boundary of estuarine flow since the effect of tidal mixing was ignored. Nutrient data were obtained from the monthly surveys conducted by the Hillsborough County Department of Environmental Protection and the Southwest Florida Water Management District for TB and CH with values for the Caloosahatchee based on measurements obtained only from lower river locations available in the STORET data base. Data on DON and DIN was available from the TB and CH databases but similar data in the STORET database for the Caloosahatchee River was incomplete, particularly during the bloom periods covered in this study. Therefore we were not able to calculate loadings and fluxes for DON and DIN for the Caloosahatchee River. For all three estuaries, the monthly measured samples were linearly interpolated to obtain daily nutrient concentrations. Nutrient loadings were then calculated by multiplying the daily flow rates by the concentration of N and P at the mouth of each estuary. Total loadings for TB and CH are based on the sum of the loadings for both estuaries, then averaging this sum for each month of bloom duration.

The average daily flux into the coastal zone was obtained by diluting the loadings into a volume described by the distance between TB and CH out to the 10 m isobath with a depth that varied at 2 km intervals from 0 to 12 km offshore. The final volume was estimated to be 7.642×10^{12} L. Our rationale for diluting all the estuarine flux into this volume is based on the location of salinity fronts and elevated *K. brevis* cell concentrations that occur between the shore and the 10–12 m isobath (Vargo et al., 2001) and the fact that the water column is isothermal and isohaline out to this depth when blooms are in their early stages (Vargo et al., 2001). Daily flux of N and P was then used for comparison with the N and P requirements of *K. brevis* populations for each month of each bloom.

Atmospheric deposition rates were calculated from the wet deposition data collected at the Gandy Bridge AIRMoN monitoring station (FL 18; 27.83N, 82.5W) available on the National Atmospheric Deposition Program web site. The rainfall concentrations of DIP and DIN ($NH_4^+ + NO_3$) were multiplied by the rainfall volume, summed for each month with an average calculated based on the number of days of actual rainfall. These values were then converted to a deposition rate per square meter of sea surface between TB and CH and diluted into the volume calculated above for estuarine flux. Daily fluxes were used for comparison with the N and P requirements of *K. brevis* for each month of each bloom.

Samples for zooplankton abundance and species composition were collected in October 1999 and September, October, and December 2001 at three

locations along the 10, 25 and 50 m isobaths. In 2004 excretion rates were measured in laboratory experiments at USF/CMS using animals collected in nearby coastal waters off TB. Animals were sorted on an Olympus dissecting scope. Animals were rinsed with filtered seawater and counted into 200 ml sealed chambers that contained either filtered seawater, natural seawater, or natural seawater with 10^4 cells L^{-1} of *K. brevis* added. Zooplankton were incubated in the sealed chambers for 2 h, then transferred onto 60 μm mesh net, rinsed with filtered seawater, and placed in filtered seawater in 60 ml BOD bottles. The BOD bottles were wrapped in aluminum foil, placed in the incubator, and allowed to incubate for 8 h. Controls consisted of BOD bottles filled with filtered seawater and incubated for 8 h. After the 8 h incubation period, the contents of the BOD bottles was filtered through a 60 μm mesh net into 60 ml acid cleaned bottles and frozen for nutrient analysis. Samples were analyzed for total ammonium and TP on a Technicon Auto-analyzer II continuous flow analyzer (Grasshoff, 1976; Gordon et al., 1993). Excretion rates per animal or per mg dry weight were multiplied by the measured abundance or dry weight of each dominant species as noted in the earlier collections (Lester, 2005).

Excretion by microzooplankton, which can be an important source of regenerated nutrients, particularly in coastal and estuarine waters (Lehrter et al., 1999) was not included in our calculations because there is no data available on the abundance, biomass or the excretion rates of microzooplankton in WFS waters.

N release rates from N_2-fixation by the cyanobacterium *Trichodesmium* spp. were taken from Mulholland et al. (2004, 2006). Their ranges of N release rates colony^{-1} were multiplied by the average number of colonies L^{-1} (data not shown), and converted to a daily flux rate. No distinction was made between N released as ammonia or DON.

No benthic flux measurements are known for the WFS. Marinelli et al. (1998) reported NH_4^+ flux rates measured on the shelf of the South Atlantic Bight and the continental shelf off Jacksonville, FL and Darrow et al., 2003) has estimated benthic N flux via a mathematical model of benthic microalgal growth and production. Marinelli et al.'s overall daily average NH_4^+ flux and Darrow et al.'s calculated flux rate were used to yield a range of values which were then diluted into a 10 m water column to estimate a final water column concentration.

Estimates of N and P re-mineralized from dead and decaying fish killed by elevated cell concentrations of *K. brevis* were taken from Walsh et al. (2006).

3.3. Bloom nutrient requirements

The N and P required to support observed *K. brevis* blooms was calculated based on the average cell concentration measured for each month of each bloom and a growth rate of 0.2 divisions d^{-1} and N and P cell content of 1.08×10^{-5} μg and 4.88×10^{-7} μg cell^{-1}, respectively (Heil, 1986; Shanley, 1985; Van Dolah and Leighfield, 1999). The average primary production rate for blooms in 2001 and 2002 (Bendis et al., 2004) was used to estimate N and P demands based on the commonly used Redfield molar ratio of 106:16:1. Additionally, N demand was also calculated from published uptake rates for NH_4^+, NO_3, glutamate, urea, and *Trichodesmium* excreted DON (Bronk et al., 2004). We used Bronk et al.'s bloom cell concentration of 9.5×10^6 cells L^{-1} and a 12 h photoperiod for uptake to calculate daily N demand.

4. Results and discussion

4.1. Bloom nutrient requirements

Surface concentrations of *K. brevis* were spatially and temporally dynamic during the 4 years in which blooms occurred during the ECOHAB:Florida program (Fig. 2). As noted by Vargo et al. (2004), blooms were typically first evident in the southern part of our study area off Ft. Myers in late summer/fall after breakdown of vertical stratification. Blooms then moved northward (1998–1999; 2000; 2001 blooms), often moving south again before dissipating (1999–2000; 2001–2002 blooms).

Calculated N and P requirements for these blooms (Table 1) range 0.056–20.5 and 0.003–0.927 μmol L^{-1} d^{-1}, respectively. For populations with $< 1 \times 10^6$ cells L^{-1}, N and P requirements are within the range of measured *in situ* NO_3 and PO_4 (Fig. 3). However, uptake to meet these requirements would then deplete *in situ* levels so a daily turnover or replacement rate is necessary to meet photosynthetic and growth requirements of the population.

We will examine standing stocks and fluxes of organic and inorganic N and P and all other potential sources to assess their potential to supply

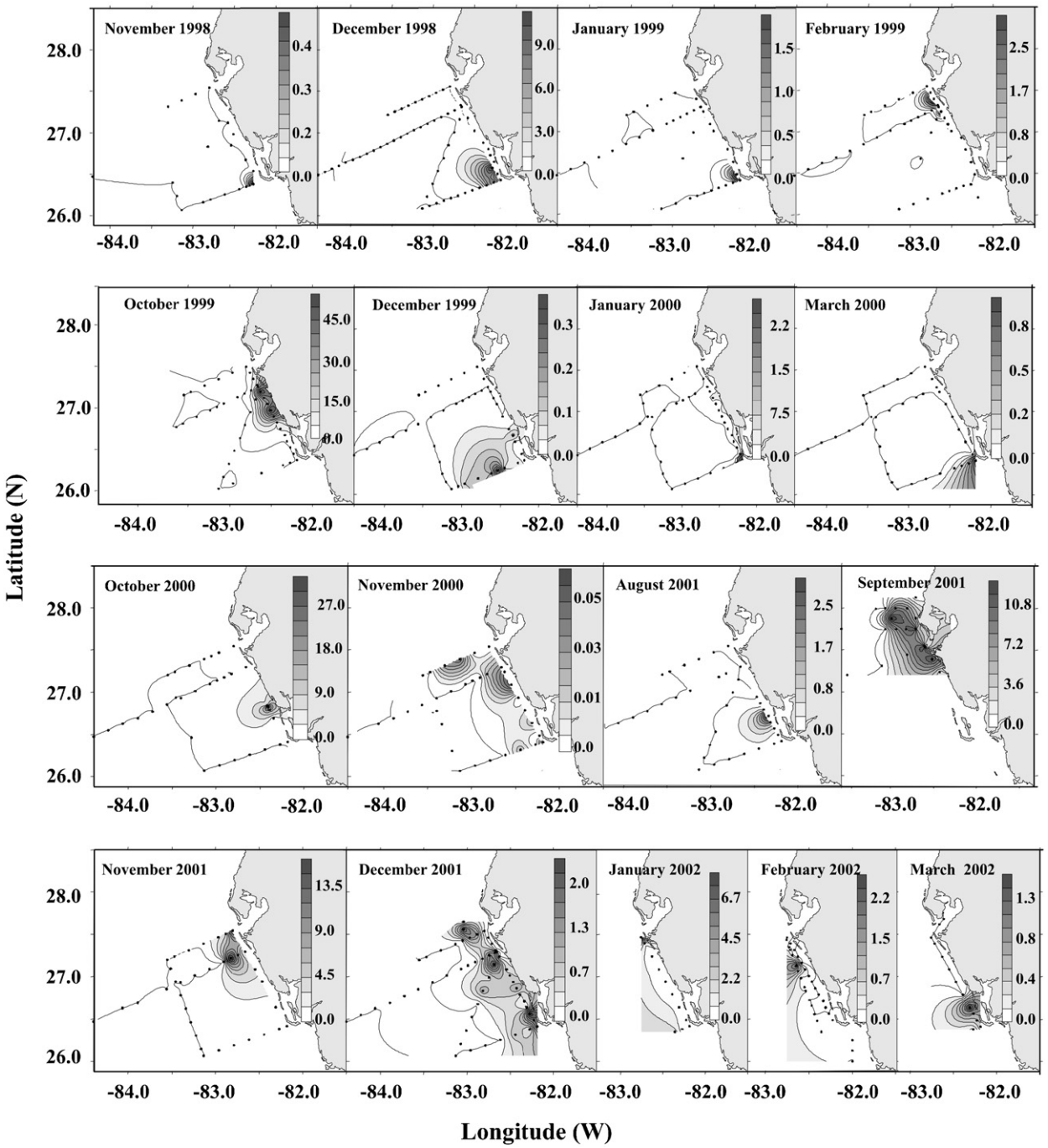

Fig. 2. The surface distribution of *Karenia brevis* (cells $L^{-1} \times 10^5$) in surface samples collected during each month of the four blooms encountered during the ECOHAB:Florida program.

the nutrients necessary to maintain *K. brevis* blooms on the WFS. However, it is understood that the standing stock of a nutrient is what remains after utilization and that maintenance of measurable concentrations is the result of the difference between uptake and the supply.

4.2. Inorganic nutrient supply

Typical concentrations of $NO_3 + NO_2$ in WFS coastal waters is $<0.5\,\mu M$. Seasonal and nearshore–offshore variability is typically $<0.3\,\mu M$ (Figs. 3(A), (B); Fig. 4) and average concentrations

Table 1
Average cell concentrations for *K. brevis* blooms within the ECOHAB:Florida control volume and the calculated N and P flux required to support growth of the observed cell abundance at an assumed growth rate of 0.2 divisions d^{-1}

Bloom	Abundance (cells $L^{-1} \times 10^4$)	N requirement ($\mu M\ d^{-1}$)	P requirement ($\mu M\ d^{-1}$)
Nov. 1998–Feb 1999	2.6	0.056	0.003
Oct. 1999–March 2000	12.4	0.267	0.012
Oct.–Nov. 2000	7.0	0.151	0.007
Assumed	100.0	2.160	0.098
Assumed	30.0	0.648	0.029
Oct. 2001[a]	950.0	20.520	0.927

Note that two assumed concentrations were added to extend the range of nutrient requirements within the limits of the observed populations.
[a]Data from Bronk et al. (2004).

at the 10, 25, and 50 m isobaths are essentially the same. Surface distributions of $NO_3 + NO_2$ at the start of each bloom were highly variable and were not related to estuarine outwelling (Fig. 4 and Vargo et al., 2001). The lack of estuarine outwelling of $NO_3 + NO_2$ is due to the elevated levels of PO_4 in both TB and CH. Both are highly P enriched due to tributaries which drain the Hawthorn phosphatic deposits located inland and between the two estuaries (see Vernon and Puri, 1965). Therefore, both estuaries are typically N-limited and display very low DIN:DIP ratios (Vargo et al., 2001; Heil et al., 2001). With the exception of June 2000, all ratios were well below the accepted Redfield molar value of 16:1. Thus neither TB nor CH can be considered a major source of DIN for coastal waters.

Estuarine outwelling of PO_4 and SiO_4 into coastal waters has a distinct seasonal pattern (Figs. 3(C), (D), and 4). Silicate (Si) concentrations in TB and CH are elevated since their tributaries often have fresh water values of $>800\,\mu M$ (Froelich et al., 1985, Vargo et al., 1991). Therefore, Si concentrations can be used as a non-conservative indicator for estuarine discharge into the coastal zone. Values for both P and Si are elevated at the 10 m isobath (Table 2) and display a distinct seasonal pattern with peaks occurring in late summer and fall during the normal rainy season (Figs. 3(C) and (D)). Concentrations of Si at the 25 m isobath are occasionally higher than the 50 m isobath, indicating estuarine influence and nutrient flux may extend to the 25 m isobath (Fig. 3(D), Table 2). These outwelling events may lead to the formation of salinity fronts that have been associated with elevated *K. brevis* populations (Vargo et al., 2001) and suggest that estuarine nutrient flux should play a key role in the turnover rates of dissolved nutrients in support of nearshore blooms. The areal extent of elevated Si concentrations (Fig. 4) was also used as an indication of the volume of coastal water into which estuarine nutrients were diluted for calculations of their availability for *K. brevis* growth (see below).

Infrequent upwelling events at the shelf break, which is approximately 180 nm offshore, may also serve as a source of nutrients. Slope water enriched with N, P, and Si can be seen in across-shelf transects seaward of the 30 m isobath (Heil et al., 2001; Vargo et al., 2001; Walsh et al., 2003) and leads to growth of diatom populations below the strong thermocline that develops during summer months. No surface manifestation of the elevated biomass is evident but chlorophyll concentrations in near-bottom waters may exceed $5\,\mu g\,L^{-1}$. As noted by Vargo et al. (2001), remineralization of this biomass may represent an N input of 5 μM DON upon resuspension.

Vargo et al. (2004) report that the average dissolved molar N:P ratios ($NO_3 + NO_2$, PO_4) at the start of each bloom were <1.0 indicating N depletion and P enrichment. However, P enrichment is a highly relative term since concentrations at the 10 m isobath rarely exceed 0.4 μM with annual averages of 0.2 μM (Table 2, Fig. 3). It is therefore enigmatic how such low absolute concentrations support *K. brevis* blooms of $>10^6$ cells L^{-1}. Indeed, cellular N:P and P:Chl ratios within large blooms are indicative of P-limited *K. brevis* populations (Vargo et al., 2004).

Seasonally, DIP and DOP at the 10 m isobath have similar concentration ranges with peaks in late

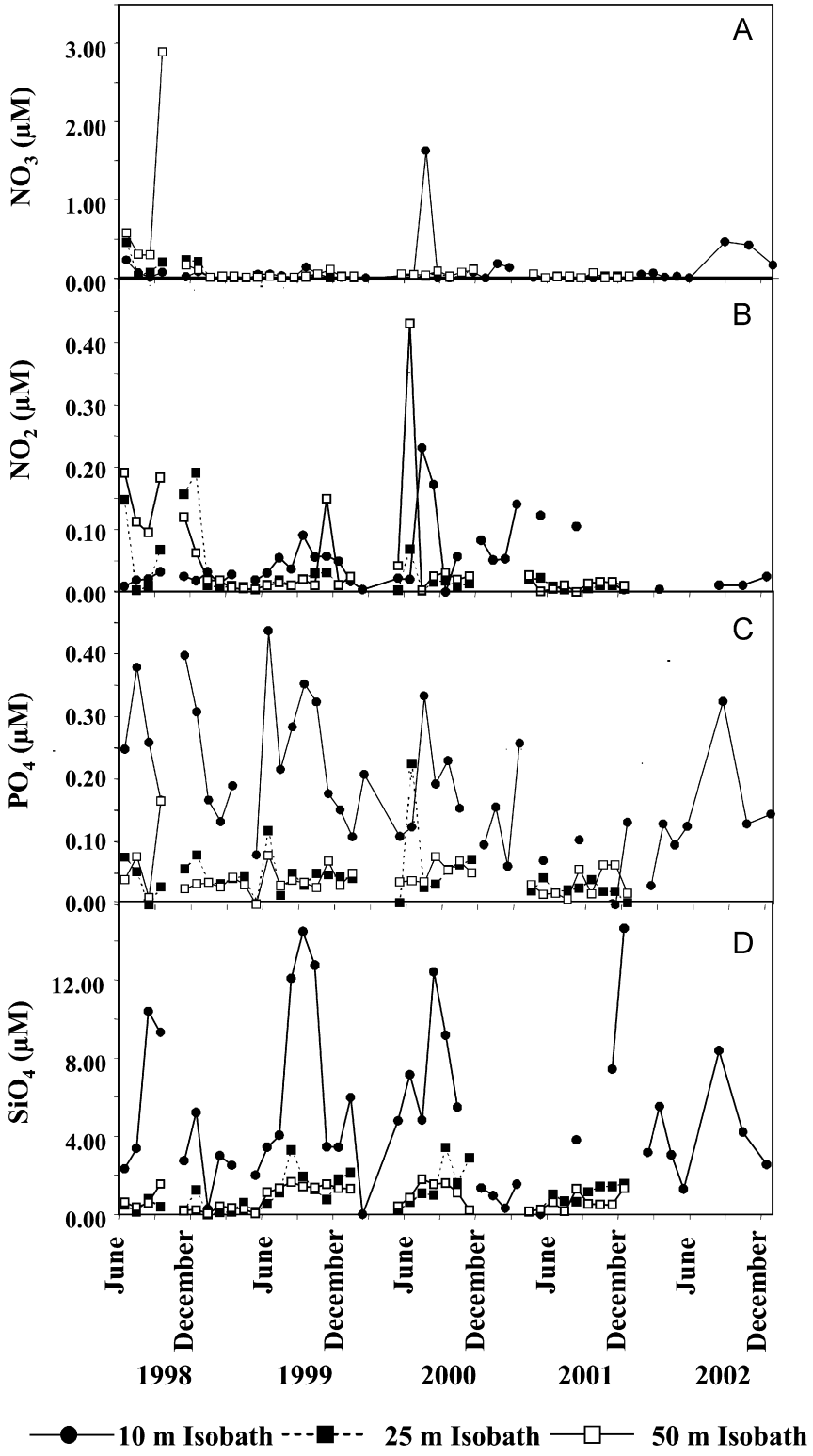

Fig. 3. The seasonal distribution of isobath averages of inorganic nutrients (μM) at the 10, 25, and 50 m isobaths. Nitrate (A), Nitrite (B), Phosphate (C), and Silicate (D).

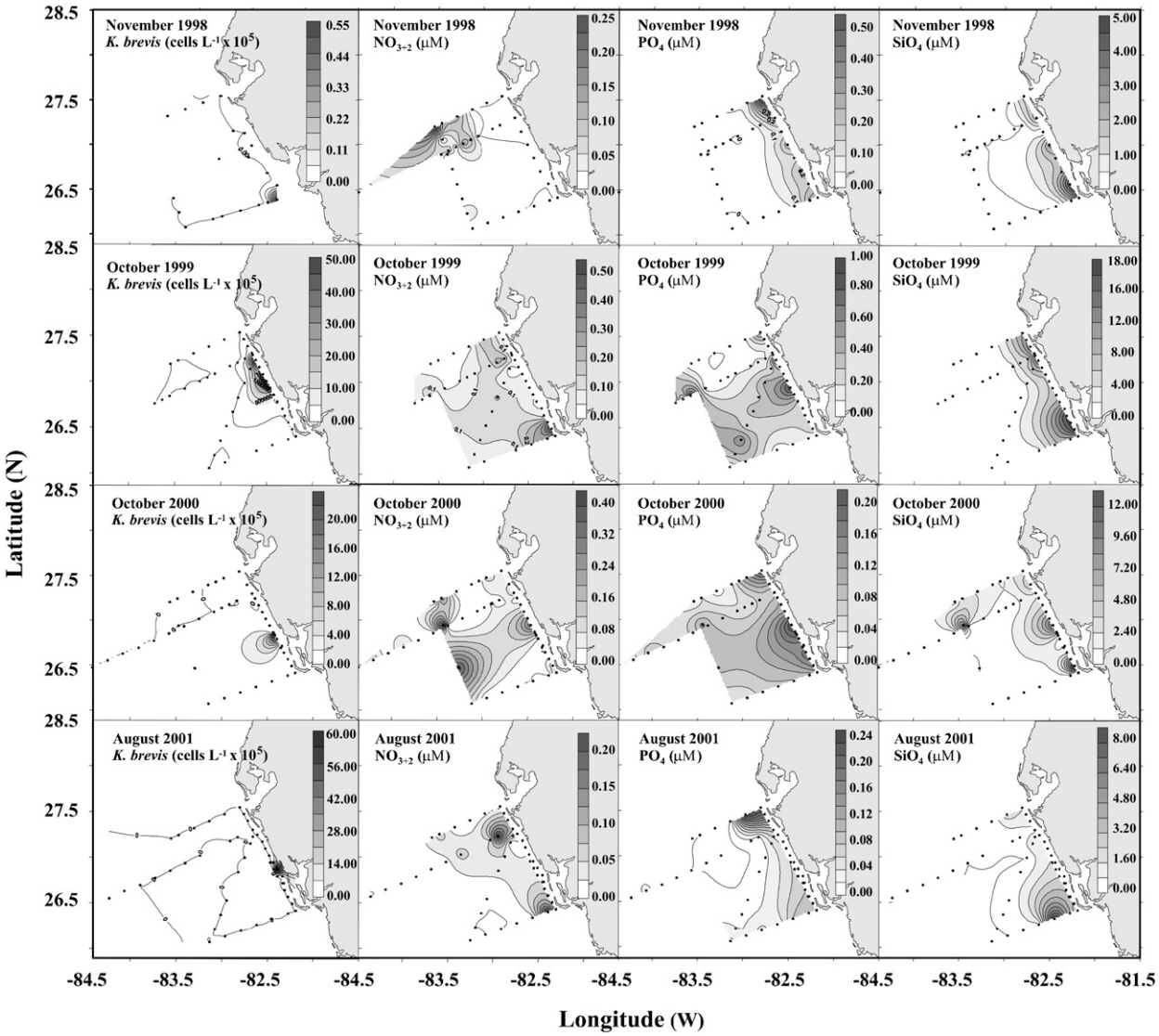

Fig. 4. Surface distributions of *Karenia brevis* abundance, nitrate, phosphate, and silicate concentrations during the first month of each bloom.

summer and fall (Figs. 5 and 6). Average values for DIP are approximately 50% of TDP (Table 2). Seaward of the 10 m isobath, DIP concentrations are extremely low with little seasonal change and average $<$ 20% of TDP. Therefore, as noted above, if 0.2 µM P would support approximately 4×10^5 cells L^{-1}, then the standing stock seaward of the 10 m isobath could only support 1/4–1/5 of that cell concentration or approximately 10^5 cells L^{-1}. Clearly the standing stock of P could not support more than one doubling of a moderate *K. brevis* population. However, since the standing stock of both N and P is relatively constant then either the turnover rate is equal to the uptake rate or uptake rates are minimal due to low concentrations. Indeed, Vargo and Howard-Shamblott (1990) found the K_s for growth on P was 0.18 µM—a value that is higher than many of the average water column concentrations found during the 1998–2001 blooms. Thus while *K. brevis* may be adapted for growth in oligotrophic waters, *in situ* P concentrations may be too low for efficient uptake.

4.3. Organic nutrient supply

DOP displays similar seasonal concentration patterns as DIP at the 10 m isobath (Fig. 5 and Table 2), although annual average concentrations

Table 2
Annual averages (μM) of dissolved inorganic and organic nutrients analyzed during the ECOHAB: Florida program at the 10, 25, and 50 m isobaths

	1998			1999			2000			2001		
Nutrient	10 m	25 m	50 m	10 m	25 m	50 m	10 m	25 m	50 m	10 m	25 m	50 m
TDN	11.183	10.877	11.993	13.718	11.399	9.338	14.553	8.632	9.369	14.083	9.215	7.872
DON	11.154	10.676	11.704	13.690	11.392	9.315	14.517	8.574	9.295	14.069	9.187	7.841
NO_3	0.020	0.0201	0.072	0.036	0.012	0.025	0.0199	0.050	0.055	0.050	0.021	0.021
NO_2	0.096	0.096	0.013	0.046	0.014	0.024	0.067	0.023	0.066	0.062	0.010	0.011
NO_{3+2}	0.092	0.255	0.073	0.069	0.027	0.048	0.247	0.061	0.101	0.079	0.023	0.024
TDP	0.518	0.275	0.270	0.522	0.347	0.322	0.630	0.196	0.179	0.404	0.241	0.237
DOP	0.255	0.226	0.211	0.286	0.303	0.284	0.457	0.137	0.131	0.288	0.216	0.203
PO_4	0.263	0.149	0.059	0.236	0.043	0.038	0.173	0.059	0.048	0.116	0.025	0.033
SiO_4	6.354	0.542	0.593	5.686	0.971	0.912	5.688	1.689	1.006	3.899	0.925	0.603

Values for each isobath are the averages of all depths and stations at that isobath.

are a factor of 4–5 times greater than DIP at the 25 and 50 m isobaths. Interannual variations were within two standard deviations (data not shown) and are not statistically different.

Peaks in DOP seen in November, 1998 and 1999, October, 2000, and November, 2001 (Fig. 6) all coincide with blooms (see Fig. 2). Since the proximate composition of *K. brevis* populations during these blooms suggested they were P-deficient, it is unlikely that they would excrete DOP. However, cell lysis or decay of fish killed during the bloom may result in increased P concentrations in the area (Walsh et al., in review). Interestingly, peaks in DOP concentration in November, 1998 and 1999 were found across the shelf (Fig. 6), even though blooms were generally confined to the region shoreward of the 20 m isobath (Figs. 2 and 3). The November, 1999 peak is of even greater interest since it is highest at the 25 m isobath, followed by the 50 and 10 m isobaths. While the peak in DOP does follow a peak period of river flow in CH (Fig. 7) that would not account for the higher concentrations at the 25 and 50 m isobaths. Similarly, the dense *K. brevis* bloom was found only in nearshore waters during the October–March time frame which might account for elevated DOP at the 10 m isobath but not further offshore. Hydrographic data from the 1999–2000 bloom showed a vertically homogeneous water column (data not shown) which could indicate intense vertical mixing or upwelling. Surface underway temperature values indicate that there was a strong thermal front with colder water nearshore out to about the 20 m isobath and was parallel to the shoreline from November, 1999 through January, 2000. Either upwelling or estuarine runoff of cooler water could set up and maintain this type of front. High surface SiO_4 concentrations in October, 1999 (Fig. 4) suggest estuarine flux occurred which could also contribute to elevated DOP. However, an offshore source of DOP must also have been present since, again, estuarine flux cannot explain the higher offshore DOP concentrations. Lenes et al. (2001) described elevated DOP levels and *Trichodesmium* spp. populations seaward of the 10 m isobath in September 1999. Carry over from this bloom may have contributed to the peak noted in October and November.

A second peak of DOP $>$ 1 μM occurred in March, 2000 but only at the 10 m isobath (Figs. 5 and 6) and results from only one value taken from St. 1 off TB. While there are no obvious errors that would indicate contaminated samples, TDP values for other stations at the 10 m isobath were in the range of 0.2–0.4 μM.

Since *K. brevis* has the ability to utilize monoesters of DOP via the cell surface enzyme alkaline phosphatase (Vargo and Shanley, 1985) the finding of DOP concentrations in the water column during blooms is enigmatic and suggests that despite particulate N to P ratios that suggest P limitation, *K. brevis* populations were either P sufficient or cellular P levels which regulate the production of alkaline phosphatase (Vargo and Shanley, 1985) were high enough to suppress the activity of this enzyme.

Inorganic N makes up less than 1% of the TDN in estuarine, coastal, and offshore waters (see Table 1). Therefore the bulk of the N standing stock is in the form of DON (Table 2, Fig. 8(A)). Although average concentrations tended to decrease

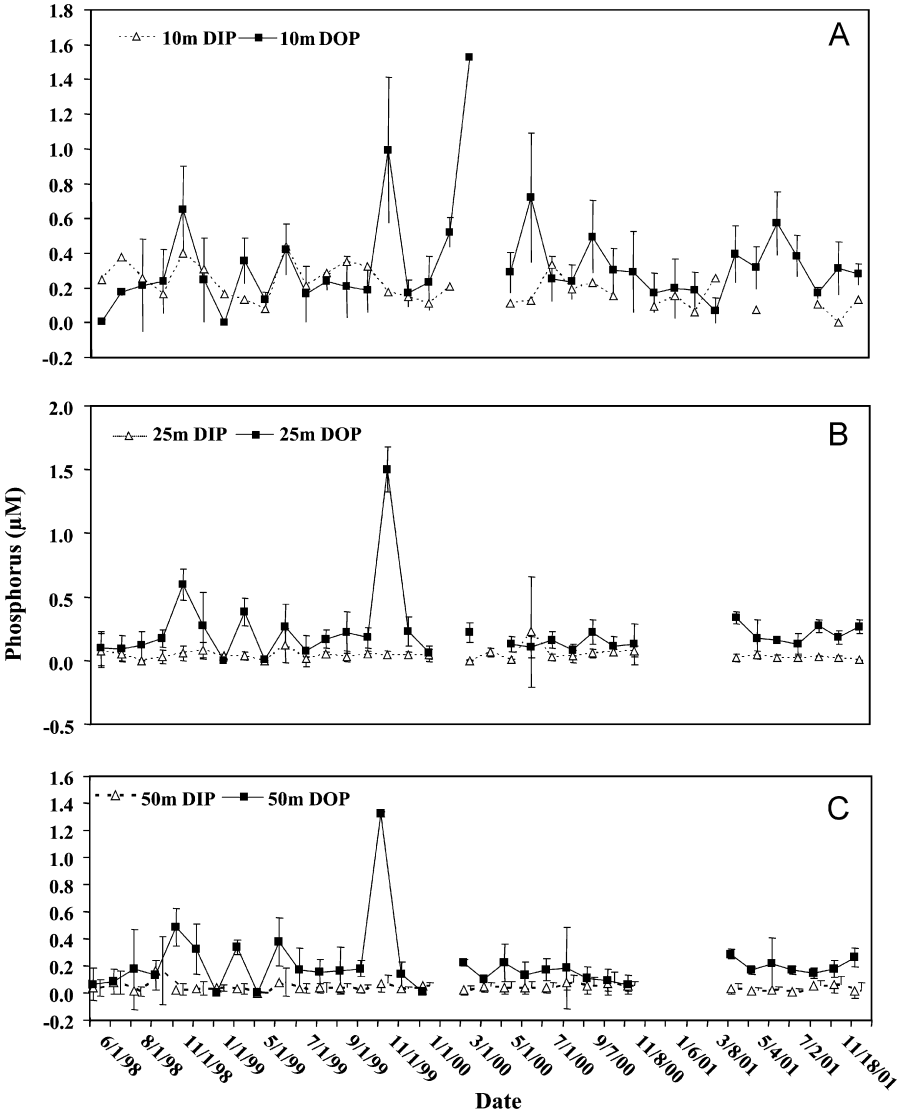

Fig. 5. The seasonal distribution of dissolved inorganic phosphate (DIP) and dissolved organic phosphate (DOP) at the 10 m (A), 25 m (B), and 50 m (C) isobath. Values are the average ± standard deviation.

from nearshore to offshore (Figs. 8(A), (B), Table 2), there were no statistically significant differences inter-annually or with depth (Fig. 8(B)). High variability during 1998 and 1999 masked seasonal changes but concentrations tended to be elevated during summer/fall as compared to winter/spring, particularly at the 10 m isobath.

4.4. Extent of estuarine impact

With the exception of DIN, the 3-y average of N and P species at the mouth of each estuary was higher in TB than in CH (Table 3). Greater urbanization in the Tampa–St. Petersburg area may account for these differences. TN, DON, and DIN concentrations at the 10 m isobath were, however, much lower than found at the mouth of TB and CH (Table 3). A combination of dilution and biological uptake reduced estuarine concentrations by almost 70% at the 10 m isobath. If we assume TN and TP are more conservative than the organic or inorganic fractions, then their concentrations were reduced by a factor of 3.0 by dilution into the coastal zone (Table 3). This difference in nutrient concentrations, assuming conservative behavior, should allow us to estimate an "impact

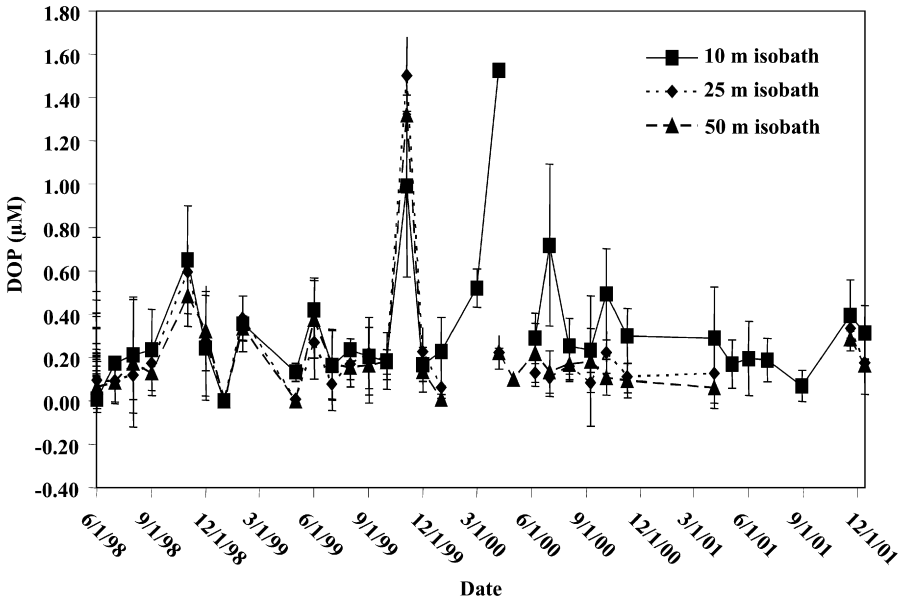

Fig. 6. The seasonal distribution of dissolved organic phosphate at the 10, 25, and 50 m isobaths. Values are the average ± standard deviation.

volume'' of estuarine nutrient flux. To estimate an impact volume we calculated the average daily volume of water flow from all tributaries in CH and TB (2.85×10^{10} and 1.32×10^{10} L, respectively; average $= 2.09 \times 10^{10}$ L) and further assumed that this volume of water would be displaced out of the mouth of each estuary carrying with it, the average value for N and P seen in Table 3. Using a simple dilution calculation, we estimate that the estuarine nutrient concentration found at the mouth requires a dilution into a final volume of approximately 6.5×10^{10} L to achieve the measured concentrations at the 10 m isobath (Table 2). With a total volume of 7.64×10^{12} L for the area from the shoreline to the 10 m isobath between TB and CH, diluting estuarine waters to achieve the measured nutrient concentrations accounts for $<1\%$ of the total volume. Therefore the impact of estuarine flows as a direct nutrient source for blooms should be confined to a relatively small area immediately offshore of the estuary. Surface distributions (e.g. Fig. 4) confirm the region of estuarine impact since it is apparent that elevated nutrient concentrations are confined to the region immediately seaward of the estuary except during periods of high river flow such as in October, 1999 and 2000 (Figs. 4 and 7A). However, since *K. brevis* populations are often found along the entire shoreline between CH and TB or beyond, we will base on calculations of nutrient flux on the premise that nutrients from the estuaries are instantaneously mixed into the entire volume between TB and CH (7.64×10^{12} L).

4.5. Estuarine nutrient flux

Flow patterns for the tributaries of TB and CH and for the Caloosahatchee River show the typical seasonal picture with elevated flow during the summer/fall rainy season (Fig. 7(A), (B)). There is some departure from the ''natural'' cycle in the Caloosahatchee River since regulatory water releases are made to maintain water levels in Lake Okeechobee and to flush out algal blooms and salt water that may contaminate potable water supplies (see Doering and Chamberlain, 1999).

Since nutrient flux rates are based on stream flow, peaks in N and P flux (Figs. 9 and 10) mirror river flow patterns and occur during the summer/fall rainy season. TN and TP flux for TB and CH for the three blooms described herein (Fig. 9) are high at the start of the bloom but decline rapidly thereafter. A similar pattern is seen for the Caloosahatchee River TKN and TP flux (Fig. 10) although the duration of flux during the 1999–2000 bloom was much greater than the preceding or following year. This pattern was not seen for flux from TB and CH.

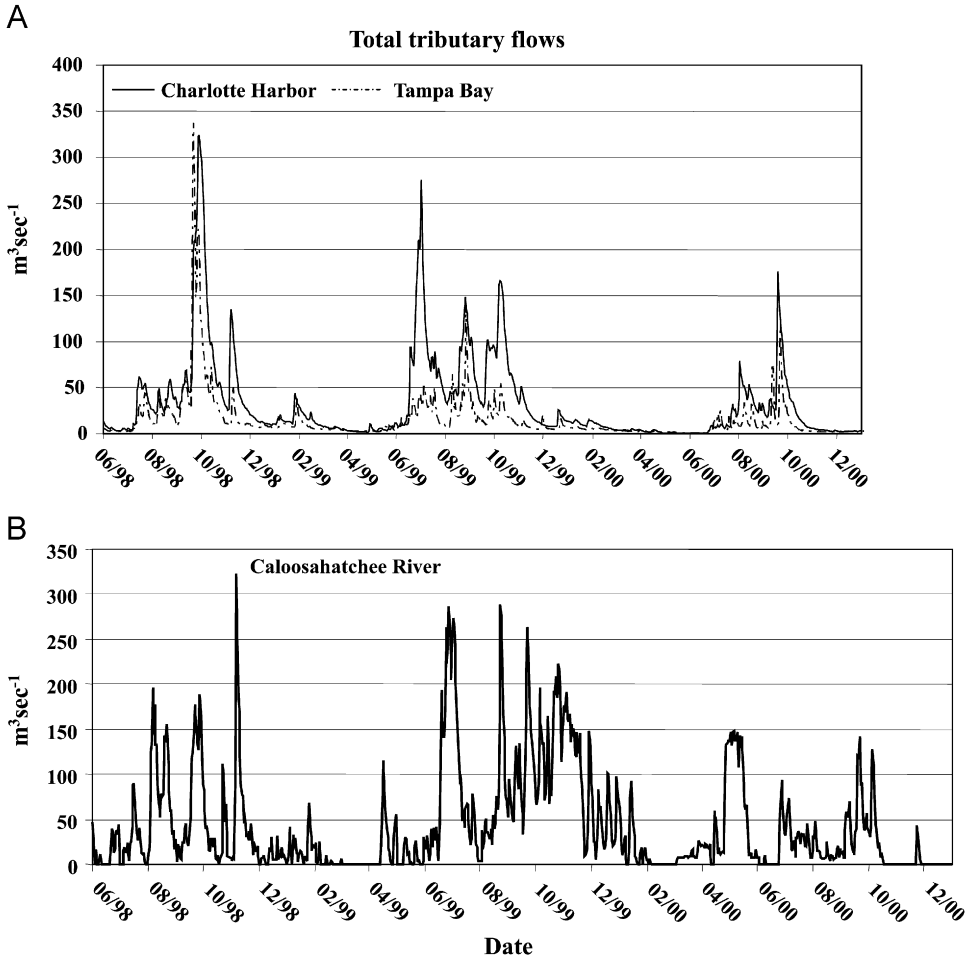

Fig. 7. Gauged river flow ($m^3 s^{-1}$) into (A) Tampa Bay, Charlotte Harbor and (B) the Caloosahatchee River.

Daily fluxes for N and P, summed for the three estuaries (Table 4) are quite low relative to the standing stocks of N and P in coastal water. Based on estuarine flux alone, the turnover time of the standing stop would be unrealistically high. As noted above, fluxes are calculated by diluting the loadings into the volume of well-mixed coastal water between TB and CH out to the 10 m isobath (7.64×10^{12} L). This volume was chosen because blooms typically occurred after breakdown of vertical stratification with an isothermal and isohaline water column present to approximately the 10 m isobath (Vargo et al., 2001). In addition, we used the concentration of N and P found at the mouth of the estuary to calculate loadings and flux rather than nutrient concentrations measured at the fresh water end member as is typically done (see Doering and Chamberlain, 1999; Doering, 2006). Therefore our flux rates are somewhat lower than those calculated by Doering (2006). His median loading values are 2618 kg d^{-1} for TN and 253 kg d^{-1} for TP based on discontinuous measurements made at the Franklin Lock and Dam from 1985 to 2003. Our loading values, based on the temporal duration for each of the three blooms and N and P concentrations measured at the mouth of the Caloosahatchee River ranged 1000–5000 kg d^{-1} for TKN and 155–400 kg d^{-1} for TP. While our average may be somewhat lower than Doering's median values, our results overlap and suggest that the overall flux from the river is similar over longer time frames.

The combined flux of N and P from TB, CH, and the Caloosahatchee River could theoretically supply 11–50% of the N and 11–57% of the P required to support growth of the measured population

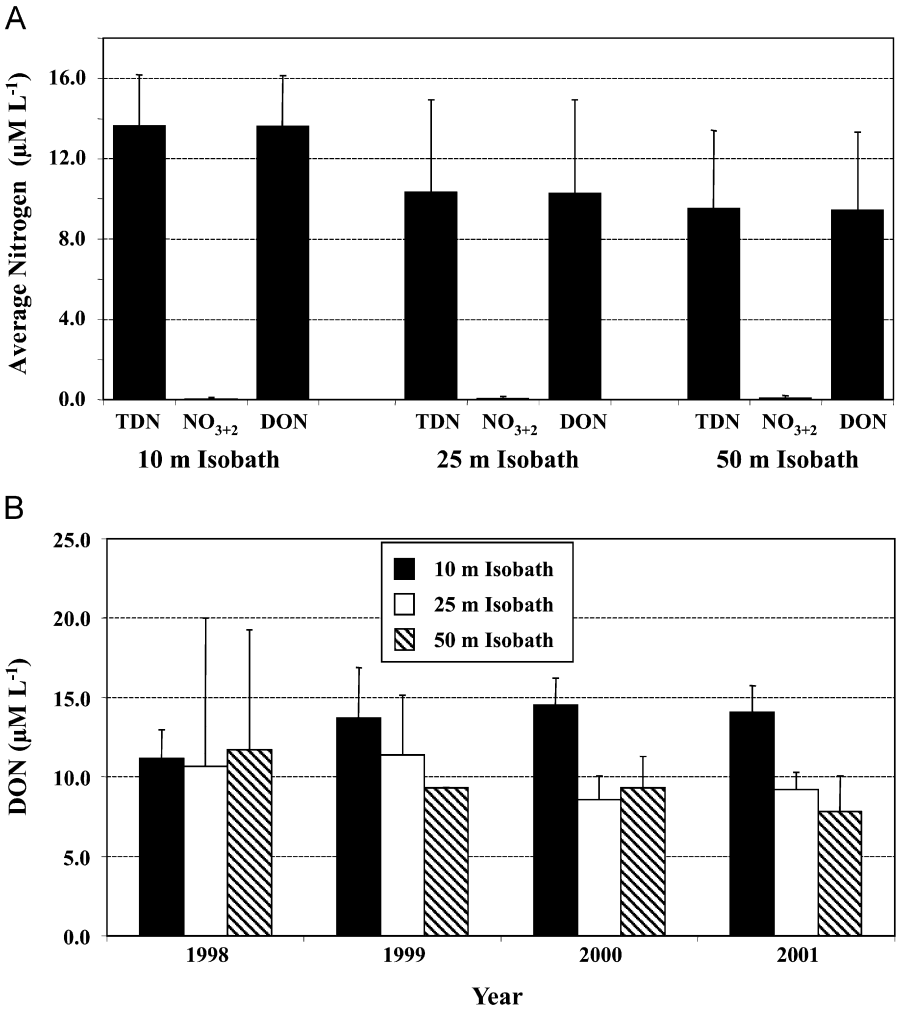

Fig. 8. (A) Average concentrations of total dissolved nitrogen (TDN), nitrate + nitrite ($NO_3 + NO_2$), and dissolved organic nitrogen (DON) at the 10, 25, and 50 m isobaths. Values are based on all samples collected during the ECOHAB:Florida cruises (1998–2001) and are the average ± standard deviation. (B) Interannual variation in the average DON concentration at the 10, 25, and 50 m isobaths. Values are the average ± standard deviation.

Table 3
Average (μM, ±SD) of N and P species for 1998 through 2000 measured at the mouth of Tampa Bay (TB) and Charlotte Harbor (CH) and at the 10 m isobath between both estuaries over the same time frame

	Estuary mouth		10 m isobath		
Nutrient type	TB	CH	1998–2000	% Estuary concentration	Reduction factor
TN	47.84 (18.87)	33.49 (28.62)	13.15	32.3	3.0
DIN[a]	1.69 (0.83)	2.17 (1.81)	0.21	10.1	9.2
DON[b]	46.15 (19.05)	31.42 (28.67)	13.12	33.8	2.9
TP	2.02 (0.62)	1.44 (0.97)	0.56	32.3	3.0
DIP	0.92 (0.49)	0.85 (0.93)	0.22	25.2	4.0
DOP[c]	1.10 (0.52)	0.59 (0.48)	0.34	40.0	2.5

Values at the 10 m isobath for the percent (%) of mean estuary concentration and the reduction factor are based on the grand average at the TB and CH concentrations (not shown).

[a]$DIN = \sum NO_3 + NO_2 + NH_3$.

[b]DON = TN–DIN.

[c]DOP = TP–DIP.

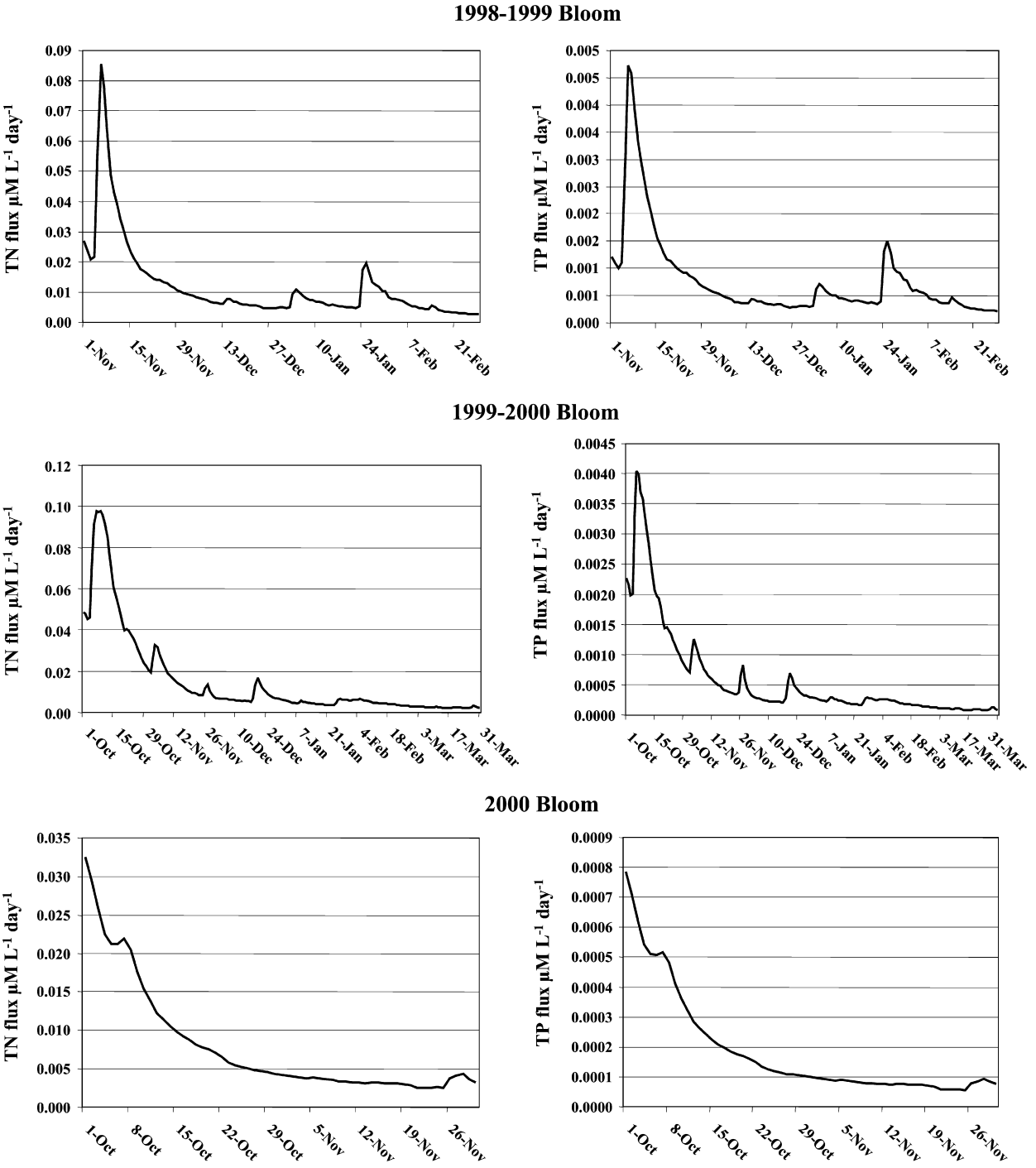

Fig. 9. The combined flux of TN and TP for Tampa Bay and Charlotte Harbor during three *Karenia brevis* blooms.

abundance for each of the three blooms (Table 5). Average N and P flux from the Caloosahatchee River is greater than that calculated for TB and CH (Table 5) as a result of higher average TN and TP concentrations and higher river flow. From June 1, 1998 through December 31, 2000, the period covered by all three blooms, average TKN and TP values were 62 and 3.7 µM, respectively, with an average river flow of 43.5 $m^3 s^{-1}$. TN and TP values for the same time period for TB and CH were 40.1,

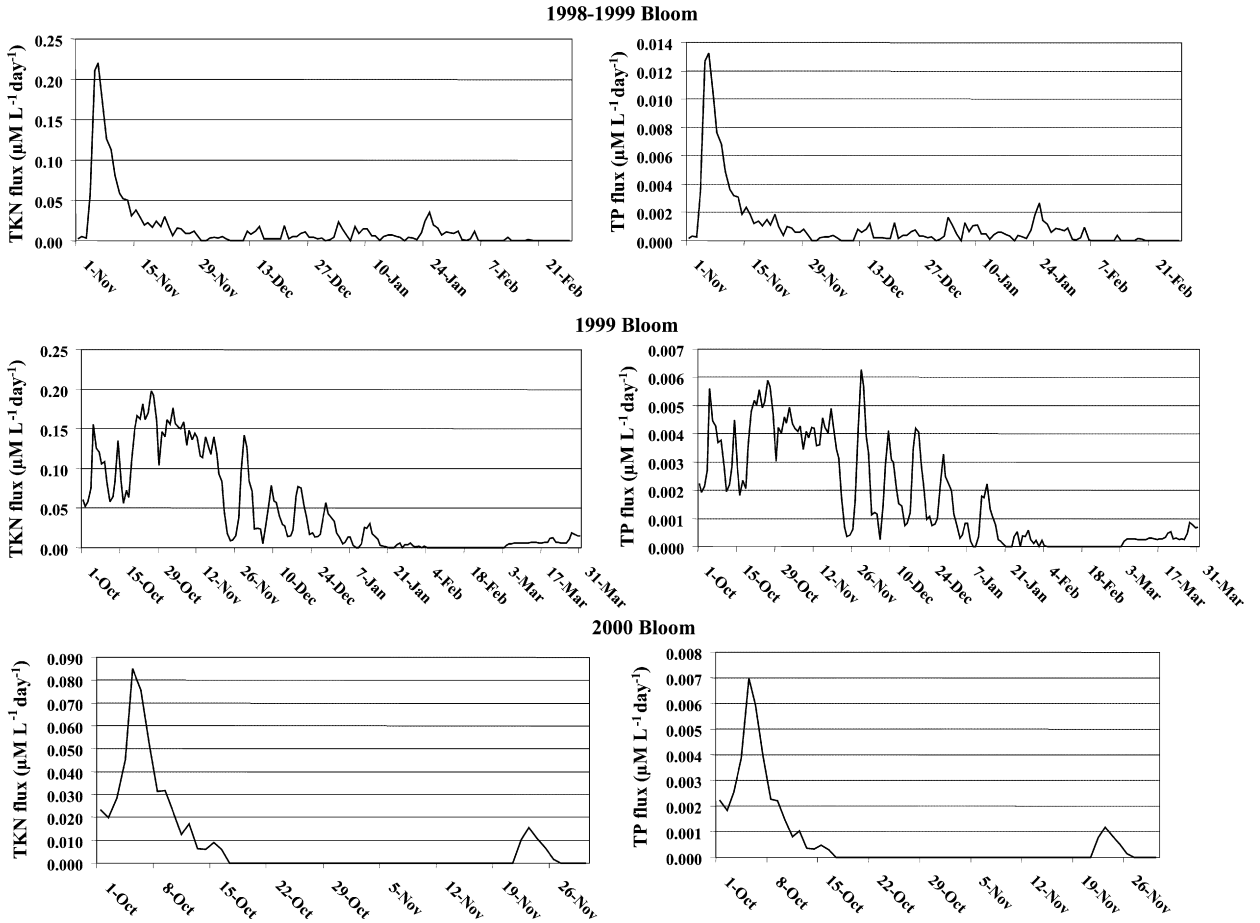

Fig. 10. The flux of TKN and TP for the Caloosahatchee River during three *Karenia brevis* blooms.

Table 4
Flux[a] as the sum of calculated values for Tampa Bay (TB), Charlotte Harbor (CH), and the Caloosahatchee River (CR) and the turnover time for the measured standing stock of nitrogen and phosphorus along the 10 m isobath

Bloom	N flux ($\mu M\,d^{-1}$)	P flux ($\mu M\,d^{-1}$)	Standing stock[b]		Turnover time (days)	
			TDN (μM)	TDP (μM)	TDN	TDP
1998–1999	0.028	0.0017	11.1	0.52	396	306
1999–2000	0.062	0.0026	13.7	0.52	221	200
2000	0.016	0.00075	14.6	0.63	913	840

[a]Flux calculated from loadings ($\mu M\,d^{-1}$) diluted into a volume between TB and CH out to the 10 m isobath (7.64×10^{12} L).
[b]Standing stock is based on the annual average of total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) at the 10 m isobath. The average at the isobath is based on all measured values at all depths for each station along that isobath.

1.9 and 31.7, 1.4 μM, respectively, with flow rates of 15.2 and 33 $m^3\,s^{-1}$, respectively. Thus higher concentrations and higher average river flow account for the elevated fluxes. However, the Caloosahatchee River does not discharge directly into the coastal zone. Flow is split between Pine Island Sound to the north and San Carlos Bay to the south with a majority of the flow discharging south through San Carlos Bay. Therefore, our adding all of the Caloosahatchee River flow to the coastal area from the mouth of CH to TB gives an overestimate of the true flux which would be less due to the additional

Table 5
Flux[a] of nitrogen and phosphorus from Tampa Bay (TB), Charlotte Harbor (CH), and the Caloosahatchee River (CR) and its relation to the N and P required to support growth of the *K. brevis* population measured in each of the blooms at a rate of 0.2 divisions d^{-1}

	TN flux ($\mu M\,d^{-1}$)				TP flux ($\mu M\,d^{-1}$)				Required ($\mu M\,d^{-1}$)		% Required met by total flux	
Bloom	TB	CH	CR	Total	TB	CH	CR	Total	N	P	N	P
1998–1999	0.004	0.008	0.016	0.028	0.0002	0.0005	0.001	0.0017	0.056	0.003	50	57
1999–2000	0.003	0.012	0.047	0.062	0.0002	0.0004	0.002	0.0026	0.267	0.012	23	22
2000	0.003	0.005	0.008	0.016	0.00005	0.0001	0.0006	0.00075	0.151	0.007	11	11

[a]Flux is calculated from loadings ($\mu mol\,d^{-1}$) diluted into a volume between TB and CH out to the 10 m isobath (7.64×10^{12} L).

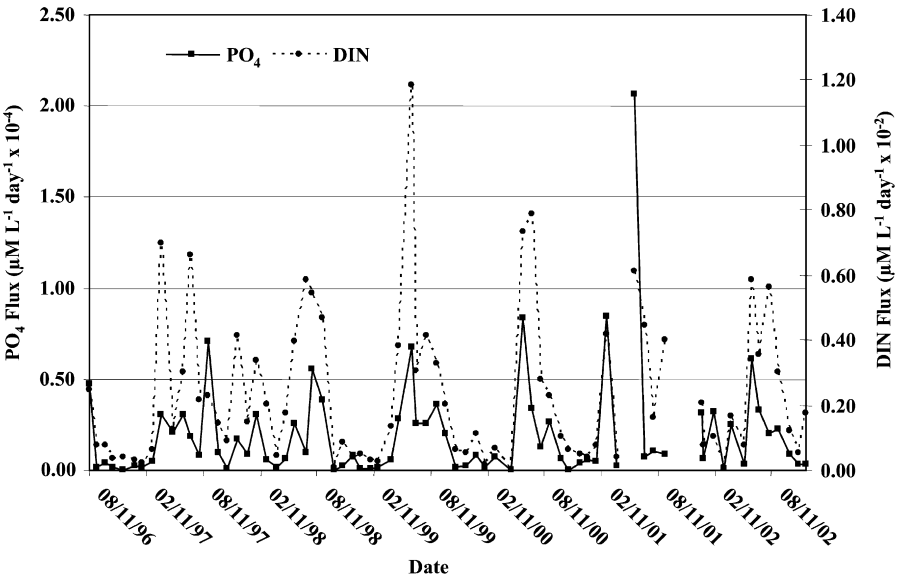

Fig. 11. Seasonal atmospheric deposition rates for inorganic phosphorus and inorganic nitrogen ($\mu mol\,L^{-1}\,d^{-1}$.

volume between the mouth of San Carlos Bay and CH out to the 10 m isobath. Overall, estuarine flux can be a significant source of N and P in support of the nearshore *K. brevis* blooms.

4.6. Atmospheric deposition of N, P, and Fe

Calculated wet deposition flux rates did not display an obvious seasonal pattern although winter–spring rates are typically lower than summer–fall (Fig. 11). Fluxes estimated over the time frames of the four blooms that occurred are typically low (Table 6) with DIN flux 1–2 orders of magnitude lower than estuarine flux and DIP flux 2 orders of magnitude lower (see Table 4). Unlike estuarine fluxes we cannot reduce the size of the box in which deposition occurs since no data is available for spatial patchiness. Dixon (pers. comm.) reports that bulk deposition at the Mote Marine Lab site for August, 2000 to October, 2002 was 3.69 kg $ha^{-1}\,yr^{-1}$ for DIN, 4.5 kg $ha^{-1}\,yr^{-1}$ for TN, and 0.43 kg $ha^{-1}\,yr^{-1}$ for TP. Wet deposition values at the Gandy site for the same time frame were

Table 6
Average atmospheric flux of N and P calculated for each *K. brevis* bloom examined

Bloom	DIN flux	PO_4 flux
1998–1999	4.64×10^{-4}	2.71×10^{-6}
1999–2000	6.27×10^{-4}	4.12×10^{-6}
2000	7.25×10^{-4}	3.69×10^{-6}
2001–2002	16.0×10^{-4}	15.0×10^{-6}

Flux measurements are based on inorganic N and P wet deposition rates measured at the Gandy Bridge AIRMoN monitoring station in upper Tampa Bay (TB). All flux rates are in $\mu M\,d^{-1}$ and are based on dilution of loadings into the volume of water between TB and Charlotte Harbor out to the 10 m isobath (7.64×10^{12} L).

4.18 kg ha^{-1} yr^{-1} for DIN and 0.11 kg ha^{-1} yr^{-1} for DIP. DIN wet deposition is approximately equal to bulk TN but DIP wet deposition is typically less than bulk TP. Therefore the DIN values are essentially comparable between the two sites, whereas our use of the Gandy site DIP rates may be an underestimate of P flux. Overall, atmospheric deposition of N and P rates are low relative to estuarine and other sources and would only supply a small fraction of the macronutrients required for *K. brevis* bloom maintenance.

4.7. Nitrogen fixation

N-fixation may be indirectly related to atmospheric deposition rates. Iron deposition on the WFS during Saharan dust events has been linked to the growth and N-fixation by *Trichodesmium* spp. (Walsh and Steidinger, 2001; Lenes et al., 2001) with subsequent release of ammonium, amino acids, and other DON compounds that may be utilized by *K. brevis* (Mulholland et al., 2004; Bronk et al., 2004). *Trichodesmium* N_2-fixation rates for the WFS ranged 0.62–1.02 nmol N colony^{-1} h^{-1} of which 20–80% of the gross N-fixation may be released as a variety of inorganic and organic N compounds (Mulholland et al., 2004). Using an average of 20 colonies L^{-1} measured during their study we estimate a daily flux of N via N_2-fixation of 0.032–0.164 μmol L^{-1} d^{-1}. While *Trichodesmium* spp. may contribute "new" N to the WFS, they are a competitor for available P. Recent studies at the BATS station off Bermuda indicate that *Trichodesmium* spp are typically P stressed and because they exhibit high alkaline phosphatase activity, they can be successful competitors for both inorganic and organic P (Ammerman et al., 2003). Therefore the presence of this N-fixing cyanophyte is a Janus figure: it supplies N but competes for P.

4.8. Zooplankton excretion

Lester (2005) measured zooplankton species abundance at three locations along three isobaths from 1999 through 2001. Using her abundance data for the dominant species at the collection locations combined with N and P excretion rates measured in laboratory experiments using the same species collected in coastal waters off TB, community excretion rates could supply all of the N and P required for blooms of approximately 10^4 cells L^{-1} (Table 7). The contribution of N excretion for growth requirements of populations $>10^5$ cells L^{-1} ranged 1–64% while, with two exceptions, most of the P demands could be met for blooms up to 10^6 cells L^{-1}.

Excretion rates were 1–2 orders of magnitude greater than estuarine flux (Table 4) and atmospheric deposition rates (Table 6). Based on the standing stocks of inorganic N and P (Table 2) zooplankton excretion rates would yield turnover rates for the water column standing stock of less than 1 d. A parallel study of zooplankton grazing also conducted by Lester (this volume) suggests that grazing losses are not sufficient to terminate the *K. brevis* blooms. Therefore zooplankton excretion appears to be a significant source of N and P for bloom maintenance and to the turnover rate of inorganic N and P on the WFS but as noted earlier it is "regenerated" N and P and will not result in additional biomass.

4.9. Other potential nutrient sources

We might expect that benthic fluxes of remineralized N and P must also be important for an area such as the broad, shallow WFS that supports a diverse autotrophic and heterotrophic benthic community (Lyons and Collard, 1974; Phillips et al., 1990). Unfortunately there are no measurements available for benthic inorganic flux for this region of the shelf. Literature data for shelf sediment fluxes in general are also sparse. A recent paper by Marinelli et al. (1998) contains the only available flux data from a nearshore region of the continental shelf off St. Augustine, FL and the South Atlantic Bight. They found no net flux of P and ammonia flux ranged from uptake (−13.9 μmol m^{-2} h^{-1}) to an efflux of 59.5 μmol m^{-2} h^{-1}. We chose to use their overall average for light and dark chambers without their two high values. This value was 16 μmol m^{-2} d^{-1} and was then diluted into the 10 m overlying water column to yield a daily rate of 0.0016 μmol L^{-1} d^{-1}.

Darrow (pers. comm. and Darrow et al., 2003) also provides a second value based on a model being developed for his Ph.D. dissertation on sediment–water column interactions on the WFS. His results also indicate there is no net P flux with ammonia efflux rates up to 1 mmol m^{-2} d^{-1}. When diluted into a 10 m water column this rate yields a flux of 0.10 μmol d^{-1} or two orders of magnitude higher than that measured by Marinelli et al. (1998).

Based on modeled values, however, benthic flux of inorganic P cannot be considered as a source for bloom maintenance, whereas N flux meets the

Table 7
Measured zooplankton community ammonia and phosphorus excretion rates and the fraction of *K. brevis* nitrogen and phosphorus requirements met by N and P excretion

Date and station	*K. brevis* (cells $L^{-1} \times 10^3$)	Ammonium excretion rate	Phosphorus excretion rate	Nitrogen bloom requirements	Phosphorus bloom requirements	% Bloom	
						N requirements provided by excretion	P requirements provided by excretion
Oct. 1999							
51	7.5	0.364	0.498	0.016	0.001	>100	>100
1	16.0	0.383	0.636	0.035	0.002	>100	>100
80	5270.0	0.445	0.676	11.383	0.514	3.9	>100
Sept. 2001							
70	8.0	0.362	0.016	0.017	0.001	>100	>100
72	200.0	0.129	0.027	0.432	0.020	29.9	>100
73	500.0	0.014	0.030	1.080	0.049	1.3	61.0
74	75.0	0.040	0.007	0.162	0.007	24.7	100
75	15.0	0.258	0.356	0.032	0.002	>100	>100
Oct. 2001							
6	1268.0	0.008	0.006	2.739	0.124	0.3	4.7
10	742.0	0.208	0.175	1.603	0.072	12.9	>100
16	1320.0	0.056	0.016	2.851	0.129	1.9	12.2
21	1078.0	0.880	0.879	2.328	0.105	38.0	>100
5	744.0	1.024	1.153	1.607	0.001	64.0	>100
Dec. 2001							
1	16.0	0.035	0.024	0.035	0.002	100.0	>100
32	68.0	0.260	0.218	0.147	0.007	>100	>100
70	176.0	6.819	3.088	0.380	0.017	>100	>100

Excretion rates and bloom requirements are in units of μmol N or P $L^{-1} d^{-1}$.

growth requirements of populations up to approximately 2.6×10^4 cells L^{-1} (see Table 1). Since this is the population level at which the standing stock of inorganic N and P will meet growth requirements, then benthic flux of N may be sufficient to maintain the inorganic N standing stock.

Access to benthic nutrient sources requires proximity. It has long been known that this species does not display the migration pattern typical of most dinoflagellates in which cells actively migrate to depth during darkness (Heil, 1986). Cells are positively phototactic and negatively geotactic during daylight hours and collect in surface waters, but vertical position during the night is related to dispersal (Heil, 1986) but with some depth regulation related to cellular physiological status (Heil, 1986; Kamykowski et al., 1998). In addition to the lack of oriented downward migration, it is apparent from annual averages of surface and bottom PO_4^{3-} and NO_3^- at the 10, 25, and 50 m isobaths (Table 8) that near bottom PO_4^{3-} or NO_3^- concentrations on the WFS are extremely low. Only during upwelling years such as 1998 were PO_4^{3-} or NO_3^- bottom concentrations elevated at the 25 m isobath, and even then only to 0.10 ($\pm$0.12) and 0.55 ($\pm$0.73) μmol L^{-1}, respectively. Cells would be expected to derive minimal nutritional benefit from active downward migration under these conditions and in fact, of the 60 vertical profiles of *K. brevis* concentrations collected synoptically over the course of 4 blooms in a variety of locations and bloom stages, 48 profiles exhibited surface maxima, one profile exhibited a mid-depth maxima, and 10 profiles exhibited bottom maxima. All but one of the profiles with bottom maxima were documented in well mixed water columns of less than 10 m depth, with half of these occurring during the light period and half during the dark period. Vargo et al. (2001) documented that *K. brevis* blooms occur after the water column becomes well mixed during the late summer–early fall period. This observation, combined with the shallow depth and lack of relationship with photoperiod, suggests that these bottom maxima were the result of physical mixing rather than active downward migration.

Table 8
Average annual surface and bottom concentrations of PO_4^{3-} and NO_3^- (as μmol L^{-1}) at the 10, 25, and 50 m isobaths

		PO_4^{3-}			NO_3^-		
		10 m	25 m	50 m	10 m	25 m	50 m
1998[a]	Surface	0.29 (0.17)	0.03 (0.04)	0.03 (0.05)	0.06 (0.09)	0.10 (0.12)	0.13 (0.21)
	Bottom	0.32 (0.15)	0.10 (0.12)	0.22 (0.19)	0.09 (0.10)	0.55 (0.73)	2.75 (3.54)
1999	Surface	0.24 (0.13)	0.06 (.009)	0.03 (0.04)	0.04 (0.06)	0.01 (0.02)	0.01 (0.02)
	Bottom	0.21 (0.13)	0.05 (0.02)	0.11 (0.08)	0.03 (0.04)	0.02 (0.03)	0.10 (0.19)
2000	Surface	0.16 (0.18)	0.08 (0.13)	0.04 (0.03)	0.16 (0.51)	0.07 (0.16)	0.05 (0.09)
	Bottom	0.14 (0.11)	0.05 (0.04)	0.08 (0.05)	0.11 (0.34)	0.06 (0.12)	0.19 (0.22)
2001[a]	Surface	0.11 (009)	0.02 (0.02)	0.03 (0.04)	0.13 (0.30)	0.01 (0.02)	0.01 (0.03)
	Bottom	0.12 (0.10)	0.04 (0.04)	0.16 (0.30)	0.10 (0.25)	0.03 (0.05)	0.08 (0.25)

Each value is the average of monthly measurements (±SD).
[a]Values for these years consist of measurements made from June through December.

4.9.1. Fish kills

More recently Walsh et al. (2006) suggest that decay and remineralization of dead fish killed by *K. brevis* blooms may also be a source of N and P to enhance and maintain existing blooms. Based on a spacing of ~1 individual m^{-3}, they estimate decaying fish may supply 160 mmol N m^{-2} and 5 mmol P m^{-2} and with vertical mixing in the first meter these values may be diluted to 160 μM N and 5 μM L^{-1}. With horizontal mixing they estimate a 30-fold dilution to 5.3 μM N and 0.17 μM P. Unfortunately rates of decay cannot be estimated, so their values must serve as standing stocks available within close proximity to fronts. Indeed, Walsh et al. report that measured N and P stocks on the order of 6 μM NH_4 and 0.35 μM TDP were measured close to dead fish in October, 2001. It is rare, however, to find NH_4 concentrations >1 μM within blooms, so if dead fish are a source the N is taken up rapidly. The estimated concentrations of P are, however, of the same order as the measured standing stocks (see Table 2) and could theoretically supply the P necessary to maintain growth. Certainly P from decaying fish must be considered given the lack of sources for this required nutrient. It should further be noted that floating, decaying fish are typically seen at fronts and windrows which are also accumulation mechanisms for *K. brevis* populations (Steidinger and Haddad, 1981; Vargo et al., 2004) which would keep the nutrient source and its sink in close proximity.

5. Summary/conclusions

All potential inputs of N and P in support of *Karenia brevis* blooms are summarized in Table 9. Based on these estimates, both estuarine flux and zooplankton excretion estimates could meet the N and P requirements for growth and production of blooms on the order of 10^5 cells L^{-1} at 0.2 divisions d^{-1} or production rates of 3 gC m^{-2} d^{-1}. For blooms at higher population levels, up to 10^6 cells L^{-1}, zooplankton excretion of phosphorus may be a significant source for bloom maintenance. Otherwise there is no single source with sufficient flux rates to support maintenance of high biomass blooms. We use the term maintenance since, based on the concept of regenerated nutrients *sensu* (Dugdale and Goering, 1967) zooplankton excretion produces regenerated nutrients that can maintain biomass rather than fuel an increase in biomass.

Rates of N-uptake (Steidinger et al., 1998; Bronk et al., 2004) yield high and unrealistic daily requirements which cannot be met by any of the potential N sources. Bronk et al. (2004) based their rates on a measured population of 9.5×10^6 cells L^{-1}, which was also used to estimate the rate calculated from the V_{max} for NO_3 reported in Steidinger et al. (1998). These high populations are typical of surface aggregations and, when combined with the high uptake rates, suggest that cells in these surface aggregations must use stored nutrients to meet their metabolic demands or rely on very rapid turnover rates of the standing stocks to meet nutrient demands. However, recent estimates of nitrogen demand based on actual uptake rates and not V_{max} values, range 0.15–1.15 μmol L^{-1} d^{-1} (Mulholland et al., 2004) and are more in line with our estimates based on growth.

Since the standing stock of inorganic N and P (Table 2) must, as noted above, turnover at least

Table 9
Summary of potential N and P sources for *K. brevis* blooms, their estimated flux rates ($\mu mol\,L^{-1}\,d^{-1}$) and the *K. brevis* uptake requirements from measured N kinetics in the October 2001 bloom and growth requirements for blooms reported in Table 1

	Flux rate			
	Nitrogen		Phosphorus	
Nutrient source/sink	Bloom	Study average	Bloom	Study average
Source				
Atmospheric deposition		8×10^{-4}		6.38×10^{-6}
1998–1999	4.64×10^{-4}		2.70×10^{-6}	
1999–2000	6.27×10^{-4}		4.12×10^{-6}	
2000	7.25×10^{-4}		3.64×10^{-6}	
2001–2002	1.60×10^{-3}		1.50×10^{-5}	
Trichodesmium N_2 fixation		0.032–0.164		NA
Estuarine flux		0.035		0.0016
1998–1999	0.028		0.002	
1999–2000	0.062		0.002	
2000	0.016		0.0008	
Zooplankton Excretion		0.71		0.49
Measured[a] range	0.01–6.8		0.006–3.1	
Benthic Flux				
	0.10[b]		0[b]	
	0.0016[c]		0[c]	
Dead fish				
10 m depth	5.33		0.17	
Required				
K. brevis uptake				
Bronk et al.[d]	14.3–83.6			
Steidinger et al.[e]	14.8			
Mulholland et al. 2004	0.15–1.15			
K. brevis growth[f]	0.056–20.5		0.003–0.927	
K. brevis production[g]	3.8		0.24	

[a]From Lester (2005).
[b]Estimated from Darrow model.
[c]Estimated from Marinelli et al. (1998).
[d]For 9.5×10^6 *K. brevis* cells L^{-1}, units are in $\mu mol\,L^{-1}\,h^{-1}$ (from Bronk et al., 2004).
[e]Based on V_{max} for NO_3; units are $\mu mol\,L^{-1}\,d^{-1}$.
[f]See Table 1 for the range of biomass and N and P requirements at a growth rate of $0.2\,d^{-1}$.
[g]Based on an average primary production rate of $3\,gC\,m^{-2}\,d^{-1}$ for 2001 and 2002 blooms (Bendis et al., 2004) and the Redfield ratio of C:N:P of 106:16:1.

once per day to meet the requirements of relatively low population density blooms, multiple or as yet unidentified sources must be available to meet such high uptake rates since there is little change in the standing stock of dissolved inorganic nutrients during blooms, throughout the year, or across the shelf (Fig. 3).

We cannot evaluate the importance of the high standing stock of organic N and P in coastal waters as yet. DON concentrations are several orders of magnitude greater than DIN and DOP concentration is of the same magnitude as DIP. Utilization of organic nutrients by *K. brevis* is well documented (Baden and Mende, 1979; Shimizu and Wrensford, 1993). Recent work by Mulholland et al. (2004) verifies that *K. brevis* can use DON produced by the cyanobacterium *Trichodesmium*. Based on the analysis by Lenes et al. (2001) and the low δN values of particulates collected in offshore patches of *K. brevis* blooms during the ECOHAB:Florida study (Havens, 2004) we can infer that some fraction of the DON we measured within coastal waters results from N-fixation and excretion by *Trichodesmium* and should contribute to the N-pool as a source in support of coastal red tide blooms. However, the average particulate δN value for samples collected during *K. brevis* blooms with populations $>50{,}000\,cell\,L^{-1}$ ranged 3.12–5.11 over 3 yr and

suggests that recycled N makes up the major source of N for bloom growth and maintenance.

Although there is a slight seasonal pattern to DON concentrations (Fig. 10(B)), the overall annual averages are remarkably consistent (Table 2) despite large differences in the magnitude of annual blooms. Thus it is unclear to what extent DON contributes in support of blooms. Either uptake is a small fraction of the available concentration or turnover rates are rapid enough that relatively constant levels are maintained.

The argument for DOP is equally unclear. Early work by Vargo and Shanley (1985) and Vargo and Howard-Shamblott (1990) demonstrated that natural populations and cultures of *K. brevis* use DOP for growth. Yet DOP concentrations are always measurable and of the same magnitude as DIP (Table 2, Fig. 5). The combined standing stock of inorganic and organic P is sufficient to meet growth requirements for $>5\times10^6$ cells L^{-1} (Table 1) but P is never depleted, so its turnover rate must be similar to the uptake rate. However, bloom populations have composition ratios that indicate P-limitation (Vargo et al., 2004). The elevated C:P and N:P ratios noted by Vargo et al. (range: 170–1149; 21–489, respectively) are also characteristic of populations growing at low specific and relative growth rates (Goldman et al., 1979). One would expect low growth rates under P-limitation but population growth rates for *K. brevis* are typically 0.2 divisions d^{-1} (Heil, 1986; Van Dolah and Leighfield, 1999). While this is a low growth rate it may be near maximal for *in situ* populations. If so, then based on Goldman et al. (1979) our proximate ratios should be close to Redfield. While this is true for populations early in the bloom, ratios increase toward the end of the bloom when biomass is high (Heil et al., 2001).

Our estimated flux rates for P do not contribute sufficient amounts to maintain standing stocks or growth although P excretion by zooplankton may be sufficient to maintain standing stocks if high zooplankton populations are present and actively feeding. The level of P limitation and the sources that contribute to the maintenance of growth and standing stocks is an enigma that remains to be solved.

Estuarine flux of both N and P, when confined to a relatively small area near the mouth of the estuary, could support high populations. Indeed, Martin and Kim (1977) as cited in Froelich et al. (1985) suggest that P-flux from the Peace River into Charlotte Harbor and efflux through Boca Grande into coastal waters correlate with outbreaks of red tides in the area. We would then expect to find high populations at the mouths of the estuaries with abundance patterns tapering off N and S of the mouth. Such population distributions were found during the1999, 2000, and 2001 blooms. Estuarine efflux, particularly of P may therefore provide the necessary levels for increased biomass and maintenance of blooms when trapped by nearshore fronts (Vargo et al., 2001, 2004).

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