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The growth dynamics of *Karenia brevis* within discrete blooms on the West Florida Shelf

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Abstract

As part of the ECOHAB: Florida Program, we studied three large blooms of the harmful bloom forming dinoflagellate *Karenia brevis*. These blooms formed on the West Florida Shelf during Fall of 2000 off Panama City, and during Fall 2001 and Fall 2002 off the coastline between Tampa Bay and Charlotte Harbor. We suggest that these blooms represent two different stages of development, with the 2000 and 2001 blooms in an active growth or maintenance phase and the 2002 bloom in the early bloom initiation phase. Each bloom was highly productive with vertically integrated primary production values of 0.47–0.61, 0.39–1.33 and 0.65 g C m^{-2} d^{-1} for the 2000, 2001 and 2002 *K. brevis* blooms, respectively. Carbon specific growth rates were low during each of these blooms with values remaining fairly uniform with depth corresponding to generation times of 3–5 days. Nitrogen assimilation by *K. brevis* was highest during 2001 with values ranging from 0.15 to 2.14 μmol N L^{-1} d^{-1} and lower generally for 2000 and 2002 (0.01–0.64 and 0.66–0.76 μmol N L^{-1} d^{-1} for 2000 and 2002, respectively). The highest *K. brevis* cell densities occurred during the 2001 bloom and ranged from 400 to 800 cells mL^{-1}. Cell densities were lower for each of the 2000 and 2002 blooms relative to those for 2001 with densities ranging from 100 to 500 cells mL^{-1}. The 2000 and 2001 blooms were dominated by *K. brevis* in terms of its contribution to the total chlorophyll *a* (chl *a*) pool with *K. brevis* accounting generally for >70% of the observed chl *a*. For those populations that were dominated by *K. brevis* (e.g. 2000 and 2001), phytoplankton C biomass ($C_{p,0}$) constituted <30% of the total particulate organic carbon (POC). However, in 2002 when diatoms and *K. brevis* each contributed about the same to the total chl *a*, $C_{p,0}$ was >72% of the POC. The fraction of the total chl *a* that could be attributed to *K. brevis* was most highly correlated with POC, chl *a* and salinity. Nitrogen assimilation rate and primary production were highly correlated with a greater correlation coefficient than all other comparisons.

Keywords: Phytoplankton; Algal blooms; Biomass; Pigments; Growth rate; Nitrogen assimilation

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1. Introduction

Over the past 20–30 years, coastal environments have experienced an increasing frequency of blooms of various harmful algae (HAB; Anderson et al., 2002). In these bloom events, phytoplankton increase in number to much higher cell densities than would normally be the case, often leading to discoloration of coastal waters, and thus providing the rationale for their common name of "red tides." These HAB events can lead to a wide range of environmental, health and societal or economic problems. Mostly, the harmful impacts are due to the common HAB characteristic of production of various toxins and the bioaccumulation of these toxins within the food web. Some of the more usual impacts of HAB events include fish and shellfish kills, human illnesses or potentially human deaths from consumption of contaminated fish or shellfish, or respiratory distress though inhalation of aerosols that contain either the toxins or the cells containing the toxins. In some cases, HABs have been associated with mortalities in aquaculture pens or ponds (Hoagland et al., 2002). Fish kills may also be the result of severe oxygen depletion that derives from the respiration that occurs within these dense blooms, often in combination with warm surface water temperatures and water column stratification. Often these dense blooms are associated with surface foams and slicks that have impacts on tourism and beach or boating-related recreational activities (Hoagland et al., 2002; Van Dolah et al., 2001). At the same time as we were observing the increase in frequency of HABs, the scientific community observed a trend toward increased coastal eutrophication (Howarth et al., 2002; Rabalais and Nixon 2002; Seitzinger et al., 2002). It has become clear that the two issues are not unrelated (Anderson et al., 2002). However, the relationships between coastal eutrophication and HAB formation, development and maintenance are not yet well understood (Anderson et al., 2002) and are currently the subject of a significant body of ongoing research. The National Science Foundation (NSF), the Office of Naval Research (ONR), the Environmental Protection Agency (EPA), and the National Oceanic and Atmospheric Administration (NOAA), in recognition of the critical nature of our lack of understanding of HABs and their relationships to coastal eutrophication, initiated a national research initiative entitled the Ecology and Oceanography of Harmful Algal Blooms (ECOHAB) in the mid-1990s. The overall goal of ECOHAB is "To develop an understanding of the population dynamics and trophic impacts of harmful algal species which can be used as a basis for minimizing their adverse effects on the economy, public health, and marine ecosystems" (ECOHAB: A National Research Agenda). This research initiative has supported a variety of research programs and provided for the development of regional ECOHAB programs that can address the specific HAB forming species and the associated suite of health, fisheries and social/economic issues appropriate for each region. In addition, the ECOHAB program has focused on three specific elements: the HAB organisms, environmental regulations of blooms, and food web and community interactions.

The Gulf of Mexico is one such region that has experienced substantial blooms of the toxic dinoflagellate *Karenia brevis* (C.C. Davis) G. Hansen & Ø. Moestrup (*K. brevis*; formerly *Gymnodinium breve* Davis) throughout recorded history of the Gulf region (Steidinger et al., 1998). Blooms of this dinoflagellate along the West Florida Shelf have been associated with massive fish kills through the activity of brevetoxins that are produced by the organism. However, there remain significant questions in regard to bloom initiation, maintenance and export of *K. brevis* on the West Florida Shelf. In addition, there is uncertainty as to the sources of inorganic and organic nutrients that support the growth and maintenance of such large blooms in the shelf waters. The ECOHAB: Florida program was supported by the ECOHAB Program to address these questions and to assess how environmental forcing factors impact cellular, behavioral and community processes during different stages of bloom development.

Typically, blooms of *K. brevis* begin to form offshore in oligotrophic waters of the mid-shelf during Fall. As cells grow and divide, the bloom intensifies and can be concentrated or transported by physical features such as density fronts (Walsh et al., 2003). Often these physical mechanisms not only serve to concentrate the bloom organisms but also transport the bloom towards shore, particularly when conditions support downwelling, where they may persist for periods from a few months to as much as 18 months (Walsh et al., 2003). Physical mechanisms, such as entrainment and advection offshore, and prevailing winds that support upwelling conditions may also serve to dissipate the bloom during Winter. This sequence of bloom

development is consistent year to year in West Florida Shelf waters (Steidinger et al., 1998) but may not be characteristic of *K. brevis* blooms that occur elsewhere in the Gulf of Mexico.

As part of the ECOHAB: Florida program, we examined the growth dynamics of blooms of *K. brevis* that occurred along the West Florida Shelf over three consecutive years: 2000, 2001 and 2002. The overall ECOHAB: Florida strategy involved combining survey cruise legs with process cruise legs to address several of the program questions. As a whole, the ECOHAB: Florida program has focused on addressing the following objectives: (1) model all phases of the blooms with the goal of predicting landfall; (2) describe the physical environment of the shelf including those mechanisms that transport or concentrate blooms; (3) determine how blooms respond to physical and chemical forcing on the cellular, behavioral and community levels over the various stages of bloom development; (4) determine the source and types of nutrients that support growth and development of blooms, both inshore and offshore, and how these blooms can persist over time; and (5) explore the fate and effects of toxins derived from *K. brevis* blooms, including bioaccumulation within the food web. The research presented here addresses the growth dynamics of *K. brevis* in the blooms studied during process cruises in West Florida Shelf waters during 2000, 2001 and 2002. Our study focused on elements of program objectives 3 and 4 and is not intended to be a synthesis of bloom dynamics for the ECOHAB: Florida program as a whole. Specifically, we addressed the following questions as they pertain to these overall program objectives: (1) what are the levels of primary production within blooms in various stages of development; (2) what are the carbon specific growth rates and the rates of total nitrogen assimilation of *K. brevis* in these blooms; and (3) what are the abundances of *K. brevis* in the blooms in terms of cell densities and carbon biomass and as a fraction of the observed chlorophyll *a* (chl *a*) biomass. The results of this study will contribute to the efforts of the ECOHAB: Florida Program scientific team to achieve overall program goals.

2. Materials and methods

Process cruises were conducted during each of the three field years (2000, 2001 and 2002) that are addressed in this study during the Fall bloom periods for *K. brevis* (generally September through November or December). These process cruises were segmented into 2–4 legs with different specific process studies conducted during each leg. However, each leg followed a similar strategy—conduct a rapid synoptic survey of an area that had been identified as having a bloom currently in progress followed by determining the location for the initiation of the set of process experiments that were to be conducted during that leg. The primary area of interest was the shelf region between Tampa Bay to the north and Charlotte Harbor to the south. Although in 2000, due to an approaching tropical storm, the cruise was conducted on the shelf to the south of Panama City along the Florida panhandle. In 2000, Process Cruise Leg D was conducted on the R/V Pelican over October 1–6, with productivity incubations conducted on three consecutive days (24 h in situ incubations initiated on October 2, 3 and 4). The 2001 Process Cruise Leg B was conducted on the R/V Suncoaster over October 20–26 in our primary sampling region off Tampa Bay. Twenty-four hour in situ incubations were conducted on three consecutive days with experiments initiated on October 22, 23 and 24. During the 2002, only a single Process Cruise leg was conducted on board the R/V Suncoaster over September 1–7. Only one experiment was conducted on this leg, with the 24-h in situ incubation initiated on September 4, 2002.

During each Process Cruise, standard grid surveys included stations at set intervals where CTD casts were conducted and samples were collected for determination of the abundance of *K. brevis* using both a Coulter electronic particle counter equipped to provide size-fractionation of the sample and by microscopic examination and enumeration. Once a suitable bloom patch was identified, a surface tracking Lagrangian drifter was deployed so that process studies could be conducted within a discrete bloom patch over the course of the cruise leg. A CTD rosette cast was conducted prior to sunrise for several consecutive days. Each morning samples were collected from three depths from the surface to just above the bottom (shelf depths in the study regions were generally less than 15 m) for determination of ^{14}C primary production, carbon specific growth rates and phytoplankton carbon biomass, total nitrogen assimilation, HPLC pigments, and for particulate organic carbon and nitrogen. Samples were attached to a vertical array and resuspended to their sampling depth with incubations conducted in situ from sunrise to the

following sunrise. The array was connected using a 20 m line to a spar buoy incubation system that was equipped with a VHF radio transmitter and a strobe light so that it could be tracked throughout the day and following night for ease in location and recovery at the end of the 24-h incubation. The in situ productivity array generally tracked the Lagrangian drifter that was used to track the bloom patch under study.

During the 2001 Process Cruise, we conducted profiles with a Biospherical PNF 300 profiling natural fluorometer to obtain vertical profiles of photosynthetically available radiation (PAR) at local noon on each day that a productivity experiment was conducted. Total daily incident irradiance was estimated for these experiments from surface PAR using the following equation:

$$\text{Maximum daily irradiance} = Q_s = 2NE_m/\pi, \quad (1)$$

where Q_s is in units of mol quanta $m^{-2} d^{-1}$, N is in $s\,d^{-1}$ and E_m is the surface irradiance at local noon in units of mol quanta $m^{-2} s^{-1}$. This equation yields an estimate of daily irradiance and is based on a cloud-free sky (Kirk, 1983), and thus may be an overestimate of actual daily irradiance. During the 2002 Process Cruise, daily integrated irradiance was measured with a Li-Cor LI-1000 integrator. It should be noted that the values determined using the Biospherical PNF 300 are scalar irradiance while those determined with the Li-Cor LI-1000 are downward irradiance (e.g. a cosine collector is used in this instrument), and thus the values are not directly comparable. The values provide information for daily comparisons within each Process Cruise. No irradiance data were obtained during the 2000 Process Cruise.

Primary production, carbon specific growth rates, phytoplankton carbon biomass and nitrogen assimilation were all determined from subsamples of a single incubation container for each depth. This required the filtration of the entire container contents and only one value for each of the measured parameters could be made for each container that was incubated. All primary production samples were inoculated with 300–500 μCi of $H^{14}CO_3^-$ and incubated in 1 or 4 L polycarbonate containers conforming to trace metal clean protocols (Fitzwater et al., 1982; Redalje et al., 1994). Two replicate 5 mL "time zero" samples were filtered onto Whatman GF/F glass fiber filters immediately after inoculation to account for adsorption of ^{14}C onto particle surfaces (Morris et al., 1971). Incubations were then conducted in situ from sunrise to the following sunrise as described above. After the productivity arrays were recovered and the incubation bottles taken to the shipboard laboratory, two replicate 50–100 mL subsamples were removed from each bottle for the determination of primary production. The amount of ^{14}C added to each incubation bottle was determined using 0.2 mL of sample mixed into 0.5 mL of a 50% ethanol:ethanolamine mixture. The filters for primary production measurement were treated as recommended by Lean and Burnison (1979) to remove residual ^{14}C activity. Samples for primary production were analyzed using a Wallac Winspectral 1414 α/β liquid scintillation counter. Vertically integrated rates of primary production were calculated from the surface through the depth of the deepest productivity sample, generally located near the bottom of the water column. Samples were analyzed using the labeled chl *a* method of Redalje (1993) modified to include the use of the HPLC methods of Wright et al. (1991) for the determination of phytoplankton carbon specific growth rates (μ) and carbon biomass ($C_{p,0}$). With this technique, the phytoplankton carbon biomass of the initial sample ($C_{p,0}$) can be calculated in addition to that at the end of the incubation ($C_{p,t}$). An IN/US Systems, Inc. β-Ram Model 3 Radio-HPLC Detector connected inline with a Waters 600 HPLC system equipped with a 996 photodiode array detector allowed us to determine radioactivity associated each discrete pigment detected (cf. Pinckney et al., 1996). In order to compare growth rates determined with the labeled chl *a* method, we collected samples for the determinations of C specific growth rates using track autoradiography (2000 Process Cruise only; Fahnenstiel et al., 1995; McCormick et al., 1996) and by determination of diel phasing of the cell cycle of *K. brevis* and flow cytometry (Van Dolah and Leighfield, 1999). For the flow cytometry based approach, *K. brevis* cells were collected every 2–3 h over the course of a diel cycle at surface, midwater and bottom. Bloom water samples (2 L) were either fixed whole in 2% glutaraldehyde or were concentrated to 100 mL using an Amicon Cell Concentrator fitted with a 2-μm filter prior to fixation in 2% glutaraldehyde. Fixed samples were stored in the dark at 4 °C until transported to the laboratory. Zooplankton and debris were removed by filtration through a 100 μm Nitex screen. For the fixed whole water samples, *K. brevis* cells were then concentrated by filtration through a 10 μm nylon screen. The concentrated samples were centrifuged

at $800 \times g$ for 3 min to pellet the cells. Cell pellets were resuspended in 2 mL −20 °C methanol and stored overnight at −20 °C to remove pigments. Fixed, methanol permeabilized cells were stained with $10\,\mu g\,mL^{-1}$ propidium iodide (PI, Sigma, St. Louis, MO) in phosphate-buffered saline containing $10\,mg\,mL^{-1}$ Rnase (Sigma, St. Louis, MO) and 0.5% Tween-20. DNA analysis was carried out on an Epics MXL4 flow cytometer (Coulter, Miami, FL) using a 5 W argon laser with a 488 nm excitation wavelength and 635 nm emission wavelength. Cell aggregates were eliminated by gating all histograms within the linear range using a peak-area cytogram for PI fluorescence. Cell cycle distribution was analyzed using Multicycle software (Phoenix Flow Systems, San Diego, CA). In situ growth rate was determined using the following equation:

$$\mu_{min} = \ln(1 + f_{max}), \quad (2)$$

where f_{max} is the maximal fraction of cells in S + G2 + M observed during the diel cycle. Growth rates were determined using this approach on additional ECOHAB: Florida process cruises and the results from those studies have been included with the results derived from the three process cruise mentioned above.

Nitrogen assimilation rates were determined from the incorporation of ^{14}C into phytoplankton protein during the productivity experiments. Two replicate 200–400 mL samples were filtered onto Whatman GF/F filters. The filters were then placed into liquid scintillation vials that contained 3 mL of 80% ethanol. These vials were stored in a freezer until further analysis could be conducted in the laboratory. The incorporation of ^{14}C into phytoplankton protein was determined using a series of solvent extractions (Redalje and Laws, 1983). Nitrogen assimilation was calculated following the method of DiTullio (1993; cf. DiTullio and Laws, 1983, 1986; Laws et al., 1985).

In addition to samples for primary production, samples from the initial water sample were obtained for HPLC pigment analysis using the Wright et al. (1991) HPLC protocols and the Waters HPLC system described above. Samples were filtered onto 47 mm Whatman GF/F filters and stored in liquid N_2 until further analysis could be conducted in the laboratory. Once the concentrations of important photosynthetic pigments had been determined, the statistical program CHEMTAX (Mackey et al., 1996, 1997) was used to characterize the phytoplankton community structure from our HPLC data. CHEMTAX utilizes standard ratios of taxonomic marker pigments to chl *a* in order to characterize phytoplankton taxa contained within a water sample. Pigments used in this study as chemotaxonomic markers for groups/taxa included fucoxanthin and chl $c_{1/2}$ (diatoms), neoxanthin, violaxanthin, lutein, zeaxanthin, and chl *b* (chlorophytes), zeaxanthin and chl *b* (Prochlorophytes), zeaxanthin (*Trichodesmium*), chl $c_{1/2}$ and c_3, 19′-butanoyloxyfucoxanthin, fucoxanthin, 19′-hexanoyloxyfucoxanthin, gyroxanthin-diester (*K. brevis*), and chl *a* (all algae; see Millie et al., 1993, 1997; Jeffrey et al., 1999). We used an initial pigment ratio matrix that was specific for the phytoplankton community found in HAB events in our Florida study area waters (Millie, personal communication). Group absolute and relative chl *a* concentrations were then derived from suites of pigments using CHEMTAX (Mackey et al., 1996). CHEMTAX calculates the contribution of a given algal group, defined in terms of the characteristic pigment complex for each taxa of interest, to total chl *a* based on carotenoid chl a^{-1} ratios (for critical appraisals of CHEMTAX applications, see Wright et al., 1996; Mackey et al., 1998; Descy et al., 2000; Schlüter et al., 2000; Wright and van den Enden 2000). Because carotenoid chl a^{-1} ratios within phytoplankton assemblages might be expected to vary depending upon species composition, cell physiological state, PAR and other environmental characteristics, the pigment data set was divided into subsets by sampling depth (surface, mid-water column, bottom) at all stations samples during each Process Cruise prior to independent CHEMTAX calculations (c.f. Mackey et al., 1998; Schlüter et al., 2000; Wright and van den Enden 2000).

Concentrations for particulate organic carbon and nitrogen were determined from duplicate samples from each incubation depth filtered onto 25 mm 0.45 μm pore size silver filters (GE Osmonics). Samples were placed into a desiccator and stored in a freezer until analysis in the laboratory using a Carlo-Erba NA1500 nitrogen-carbon analyzer. For samples collected on the 2002 Process Cruise, an additional replicate pair of silver filters was treated with 12 N HCl fumes in a desiccator for 30 h to dissolve particulate inorganic carbon (PIC) prior to analysis with the nitrogen–carbon analyzer. These carbon concentrations could then be used to determine the importance of PIC in our samples and correct the untreated filters to account for PIC.

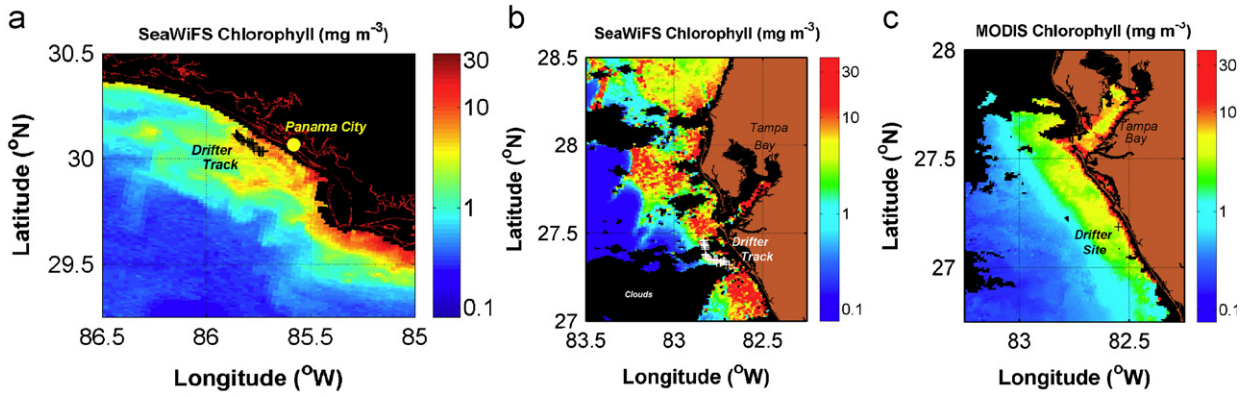

Fig. 1. The study region for each of the three Process Cruises was located within or near the edge of a *K. brevis* bloom as indicated by high chlorophyll concentrations seen in SeaWiFS (a) or MODIS Aqua (b, c) images. During the 2000 Process Cruise, the in situ primary productivity array was deployed off shore from Panama City and moved to the northwest over the course of the 3-day study; periodic position locations are marked by X on Fig. 1a. During the 2001 Process Cruise, the in situ primary productivity array was deployed off shore from Sarasota and moved to the northwest over the course of the 3-day study; periodic position locations are marked by X on Fig. 1b. During the 2002 Process Cruise, only a single in situ primary productivity array was deployed off shore from Sarasota; the X indicates the initial deployment position (c). The drifter remained near this location over the course of the 24 h incubation.

In general, we examined our data set using a variety of nonparametric statistical analyses. This approach is required here due to the small sample sizes in our data set and because they allowed us to explore interrelationships among the data without assuming anything about the nature of the data set (Sokal and Rohlf, 1995). Where applicable, we used standard parametric statistics to compare data sets. We determined the interrelationships between environmental characteristic and key physiological parameters using the nonparametric Spearman Rank Correlation test (Sokal and Rohlf, 1995). This statistical analysis was conducted for the pooled data (e.g. all three Process Cruises). In addition to the Spearman Rank Correlation test, we conducted a Kruskal–Wallis analysis of variance on ranks with multiple pairwise comparisons using the Student–Newman–Keuls test. This analysis allowed us to determine if there were significant differences between groups of data obtained in this study as well as to determine if the data were distributed normally.

3. Results

Over the course of the ECOHAB: Florida Program, we conducted process cruises during which we sampled from actively growing blooms of *K. brevis*. Samples were taken from relatively dense coastal blooms for the three Process Cruises (2000, 2001 and 2002) presented here. Satellite images obtained from the SeaWiFS (Fig. 1a, b) and MODIS Aqua (Fig. 1c) sensors indicate the locations of daily stations taken as we followed the surface tracking drifters through each of the blooms. In each case, samples were taken within or on the edges of the densest parts of the bloom. The water column structure for each of the daily primary production stations during the Process Cruises was relatively similar (Fig. 2). The shallow shelf stations were generally located in waters that were less than 15 m deep and could be characterized as relatively well-mixed water columns based on salinity and temperature profiles, although small day-to-day differences did occur.

Cell densities for samples taken just prior to sunrise varied on a daily basis within each cruise as well as between annual cruises (Fig. 3) indicated that the 2000 and 2002 Process Cruises had the lowest *K. brevis* cell densities while the 2001 bloom was most dense. Cell densities were generally similar throughout the water column on each day we sampled. Notable exceptions were 10/5/2000 and 9/4/2002 where in each case the deepest sample had the highest cell density.

Phytoplankton pigments determined using HPLC indicated the presence of a wide range of phytoplankton taxa within the daily productivity samples on each cruise (Table 1). Water column chl *a* concentrations were greatest during the 2001 Process Cruise and lowest in 2002. This same pattern was found for most of the pigments we

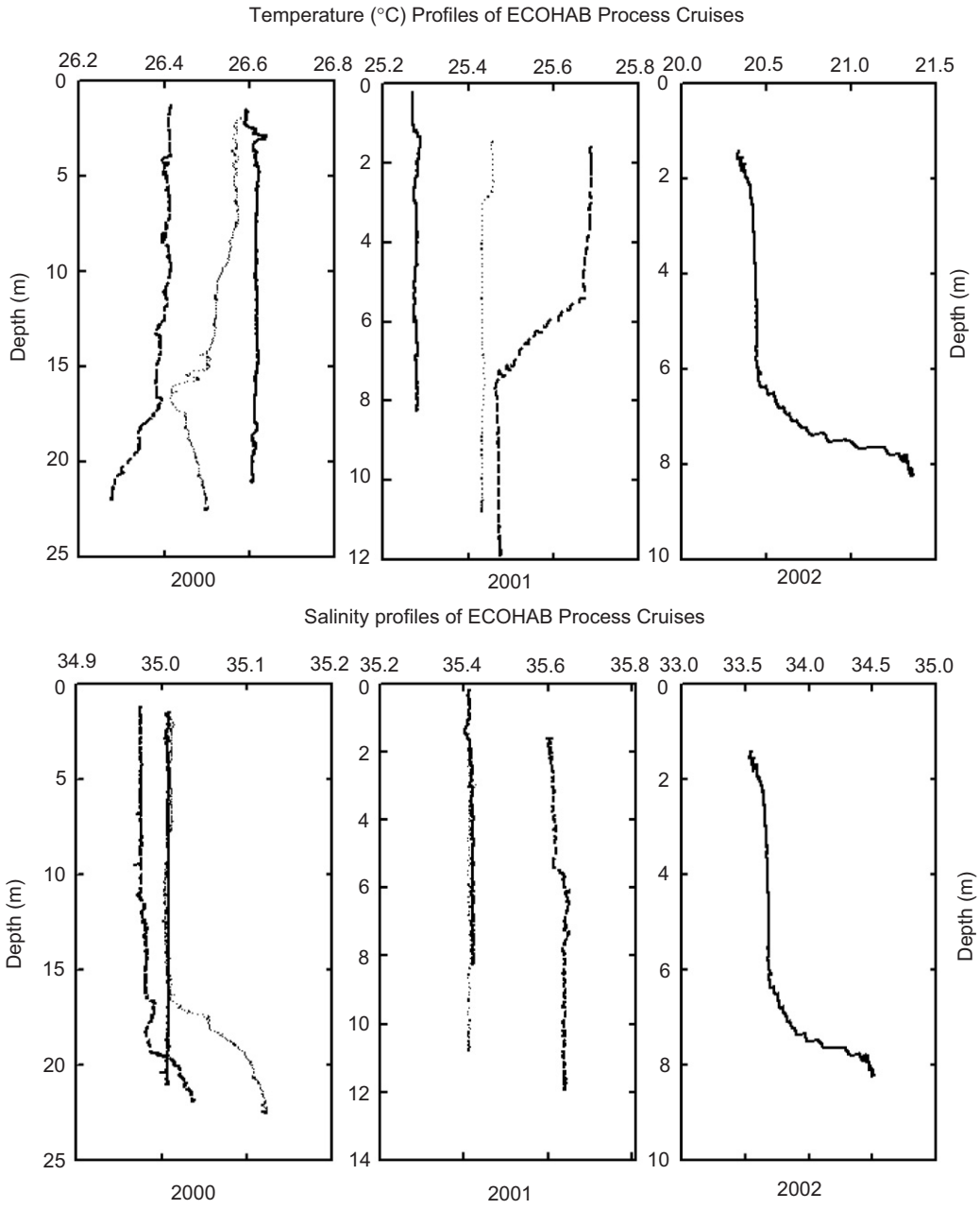

Fig. 2. Water column temperature and salinity profiles indicated that the water column was well mixed and similar for each of the days where production experiments were conducted on the Process Cruises. In each graph, data from the first day of deployment of the in situ productivity array are indicated by solid lines, the second day by dashed lines and the third day by dotted lines.

detected within the Process Cruise sample set. Gyroxanthin-diester, the characteristic carotenoid indicator pigment for *K. brevis* (Millie et al., 1997), was present in every sample except the surface sample obtained during the 2002 Process Cruise. Highest concentrations of gyroxanthin-diester were found during the 2001 Process Cruise, as was the case for chl *a*. Fucoxanthin, the primary carotenoid pigment found in both diatoms and in *K. brevis* (Jeffrey et al., 1997), was present at relatively high concentrations (a range of 0.1–1.7 $\mu g\,L^{-1}$) in all samples. Diadinoxanthin, characteristic of a wide group of phytoplankton including diatoms, β,β carotene, also characteristic of a wide range of

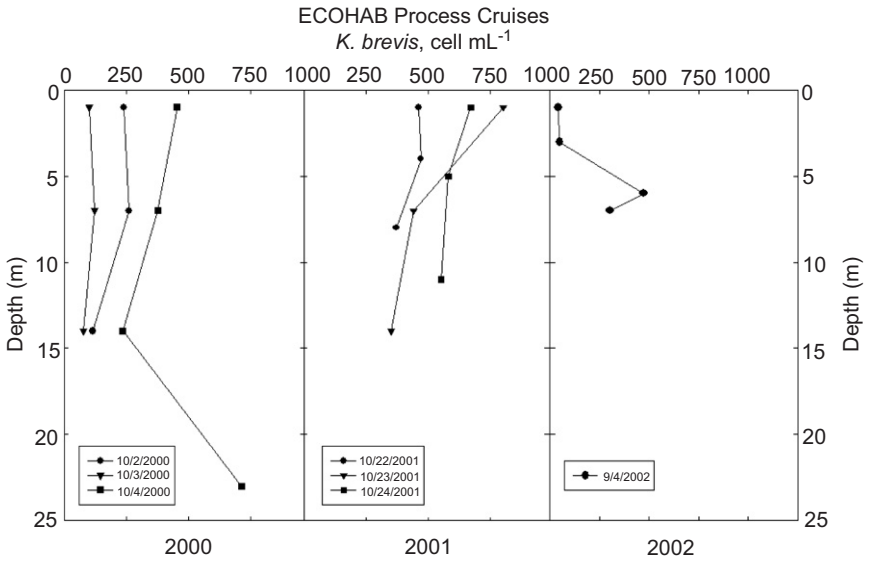

Fig. 3. Cell densities for *K. brevis* (cells mL^{-1}) determined by microscopic enumeration at sea for each of the three Process Cruises indicated that the 2001 bloom was most dense and the 2002 bloom least developed. Day-to-day differences in bloom densities were seen during each Process Cruise. Cell densities generally were uniform throughout the well-mixed water column except for the bottom samples on 10/4/2000 and 9/4/2002 that were cell density maxima for those days.

Table 1
Concentrations of the major phytoplankton pigments determined with HPLC were variable over the three Process Cruises conducted in this study

Date	Depth	Chl *a*	Chl *b*	Chl $c_{1,2}$	Chl c_3	Fuco	Gyro	Zeax	Diad	$\beta\beta$ Car	19′-but	19′-hex
10/2/00	1.0	3.80	0.05	0.05	nd	0.77	0.12	0.04	0.26	0.14	0.38	0.19
	7.0	4.06	0.07	0.32	0.18	0.85	0.13	0.04	0.30	0.09	0.44	0.22
	14.0	4.61	0.06	0.31	0.26	0.88	0.15	nd	0.32	0.13	0.33	0.23
10/3/00	1.0	0.85	0.04	nd	nd	0.17	0.01	0.05	0.04	0.05	nd	0.05
	7.0	1.86	0.05	0.23	0.12	0.40	0.04	0.07	0.13	0.11	0.07	0.12
	14.0	1.50	0.07	0.10	0.11	0.29	0.04	0.08	0.11	0.06	0.06	0.10
10/4/00	1.0	1.00	0.04	0.08	0.08	0.13	0.03	0.06	0.07	0.04	0.06	0.09
	7.0	1.65	0.03	0.13	0.11	0.20	0.05	0.05	0.13	0.04	0.11	0.20
	14.0	1.25	0.05	0.11	0.09	0.29	0.02	0.05	0.09	0.05	0.04	0.08
	23.0	3.62	0.05	0.31	0.27	0.61	0.12	0.05	0.30	0.08	0.42	0.44
10/22/01	1.0	8.04	nd	0.66	0.53	1.45	0.29	0.06	0.61	0.20	1.11	0.93
	4.4	7.67	0.03	0.66	0.54	1.28	0.27	0.06	0.62	0.12	1.04	0.91
	6.0	10.34	nd	0.93	0.74	1.69	0.37	0.06	1.02	0.15	1.47	1.27
10/23/01	1.0	3.19	nd	0.30	0.27	0.73	0.11	0.03	0.26	0.06	0.36	0.37
	7.4	3.32	0.02	0.32	0.24	0.84	0.11	0.03	0.25	0.06	0.37	0.36
	11.9	7.15	0.28	0.64	0.52	1.48	0.22	0.04	0.50	0.13	0.73	0.70
10/24/01	1.0	4.87	0.03	0.46	0.39	0.96	0.17	0.04	0.37	0.07	0.57	0.50
	4.5	5.29	nd	0.47	0.41	1.03	0.19	0.04	0.43	0.08	0.63	0.58
	6.5	6.65	0.07	0.58	0.43	1.34	0.24	0.04	0.54	0.12	0.88	0.76
9/4/02	1.5	0.90	0.04	0.09	0.06	0.28	nd	0.12	0.14	0.14	nd	0.07
	3.0	1.22	0.06	0.09	0.07	0.35	0.01	0.09	0.16	0.13	0.16	0.08
	7.0	3.98	0.09	0.28	0.25	0.81	0.08	0.10	0.37	0.20	0.14	0.09

Chlorophyll *a* (chl *a*), fucoxanthin (fuco), diadinoxanthin (diad), β,β carotene ($\beta\beta$ car) and 19′-hexanoyloxyfucoxanthin (19′-hex) were found in all samples. Gyroxanthin-diester (gyro), zeaxanthin (zeax) and chlorophyll $c_{1,2}$ were not found in only one sample. Other major pigments found included chlorophyll *b* (chl *b*), chlorophyll c_3 (chl c_3) and 19′-butanolyoxyfucoxanthin (19′-but).

taxa, and 19′-hexanoyloxyfucoxanthin, primarily found in Prymnesiophytes but also in *K. brevis*, were the only other pigments found in all samples. Zeaxanthin, primarily found in cyanobacteria, prochlorophytes and chlorophytes, was found at relatively low concentrations ($\leqslant 0.1\,\mu g\,L^{-1}$) in all samples except for the deep sample obtained on 10/2/2000.

We partitioned the chl *a* content of each sample among the major phytoplankton taxa present using CHEMTAX (Mackey et al., 1997). *K. brevis* was the major contributor to the total phytoplankton chl *a* during both the 2000 and 2001 Process cruises (Table 2a, b). However, during 2002, diatoms (46–52% of the chl *a*) and *K. brevis* (40–44% of the chl *a*) were the primary taxa present (Table 2c). The filamentous cyanobacterium *Trichodesmium erythraeum* was only present in the samples of the 2002 Process Cruise and represented only 2–9% of the total chl *a*. CHEMTAX results indicated that diatoms and chlorophytes were more important contributors to the phytoplankton community in 2000 than in 2001. *K. brevis* dominated the phytoplankton community ($\geqslant 82\%$ of the chl *a*) in 2001. Over the 3 years, the pooled group of ''other taxa'' contributed $\leqslant 3.6\%$ of the total chl *a*. These results are consistent with the suggestion that we were sampling in *K. brevis* dominated bloom situations on each of the Process Cruises, although the bloom in 2002 may represent an initial phase of bloom development due to the presence of *T. erythraeum* in the surface samples (Lenes et al., 2001; Walsh and Steidinger, 2001).

Water column integrated primary production (IPP) within the blooms studied ranged from 0.39 to $1.33\,g\,C\,m^{-2}\,d^{-1}$ (Fig. 4). The 2001 bloom had the highest levels of IPP (except for the second day—10/23/01) while IPP for each of the other blooms were comparable. This result is consistent with the observed levels of chl *a* found in each bloom. Highest chl *a* concentrations were found at the same time as the greatest values of IPP (cf. Table 1 and Fig. 4). In addition, daily integrated surface scalar PAR for the 2001 Process Cruise was 111.6, 27.5 and 93.5 mol quanta $m^{-2}\,d^{-1}$ for 10/22, 10/23/ and 10/24, respectively. The daily integrated cosine PAR for the 2002 Process Cruise experiment was 68.9 mol quanta $m^{-2}\,d^{-1}$. These values of daily integrated PAR are not strictly comparable. Daily integrated scalar PAR may be as much as 1.5 times the value for daily integrated cosine PAR (Kirk, 1983). As expected, IPP for the 2001 and 2002 cruises varied with daily irradiance ($r = 0.98$).

Phytoplankton C specific growth rates (μ, d^{-1}) were determined using three independent methods during each of the three Process Cruises presented in this study, although there were no cruises where all three approaches yielded results for the same sample; results from two methods were obtained on each of two cruises (Table 3). Cell cycle and flow cytometry based growth rates were obtained from each of the three Process Cruises presented here as well as from Process Cruises conducted in 1996, 1997, 1998 and 1999. For these earlier cruises, surface populations of *K. brevis* had a mean $\mu = 0.26 \pm 0.05$ (standard error), mid-depth populations had a mean $\mu = 0.21 \pm 0.05$ (standard error), and for the bottom samples, $\mu = 0.28 \pm 0.04$ (standard error). In 2000, cell cycle and flow cytometry based values of μ were obtained on Leg C that was conducted just prior to Leg D where μ was determined using both the labeled chl *a* technique and by track autoradiography on each of the 3 days on which studies were conducted. Values for these two methods were generally of the same order, although the labeled chl *a*-based rates were systematically greater (Table 3). During the 2001 Process Cruise, μ measurements were derived from both the cell cycle and flow cytometry method and the labeled chl *a* technique on 2 of the 3 days on which studies were conducted. The rates derived from both approaches were very comparable with values of μ ranging from 0.1 to $0.2\,d^{-1}$ (Table 3).

The labeled chl *a* technique also provides determinations of initial phytoplankton community C biomass ($C_{p,0}$, $\mu g\,L^{-1}$) that can be compared to measurements of particulate organic carbon (POC, $\mu g\,L^{-1}$) and particulate organic nitrogen (PN, $\mu g\,L^{-1}$; Fig. 5a–c). For the samples collected during the 2002 Process Cruise, duplicate silver filters were fumed to remove PIC prior to determination of POC. For the two upper depths of the water column (1.5 and 3 m), PIC was negligible ($<0.06\%$ of the total particulate carbon). However, the values for PIC at the deepest depth sampled (7 m), PIC was about 27% of the total particulate carbon. The values in Fig. 5 for the 2002 Process Cruise have been corrected for PIC. The values for the two prior cruises have not been corrected. However, if the patterns for 2000 and 2001 were similar to what we found during 2002, only the bottom depths would have contributions from PIC. Values of $C_{p,0}$ averaged only $28.6 \pm 7.2\%$ (standard error) of the

Table 2
We were able to partition the HPLC chl *a* concentration for each sample using the program CHEMTAX (Mackey et al., 1997)

Date	Depth	Total Chl *a*	% Chloro	% Diatoms	% *K. brevis*	% Other
(A) 2000 Process Cruise						
10/2/2000	1.0	3.80	3.9	22.7	72.3	1.1
	7.0	4.06	2.6	15.0	81.1	1.3
	14.0	4.61	0.2	12.9	85.8	1.1
10/3/2000	1.0	0.85	18.3	26.8	51.3	3.6
	7.0	1.86	9.8	20.7	67.7	1.8
	14.0	1.50	12.6	11.2	73.0	3.2
10/4/2000	1.0	1.00	13.1	7.8	76.3	2.8
	7.0	1.65	8.1	3.8	86.7	1.4
	14.0	1.25	9.2	19.3	68.5	3.0
	23.0	3.62	2.7	0.00	96.3	1.0
(B) 2001 Process Cruise						
10/22/2001	1.0	8.04	1.7	10.8	87.4	0.1
	4.4	7.67	1.8	4.1	93.8	0.3
	6.0	10.34	1.3	3.3	95.3	0.1
10/23/2001	1.0	3.19	1.9	15.7	82.4	0.0
	7.4	3.32	1.7	13.8	84.2	0.3
	11.9	7.15	1.4	10.7	87.7	0.2
10/24/2001	1.0	4.87	1.6	13.4	84.7	0.3
	4.5	5.29	1.6	7.1	91.3	0.0
	6.5	6.65	1.5	8.2	90.2	0.1

Date	Depth	Total Chl *a*	% Chloro	%Diatoms	% *K.brevis*	% Tricho	% Other
(C) 2002 Process Cruise							
9/4/2002	1.5	0.94	0.0	47.9	39.7	8.9	3.5
	3.0	1.24	0.8	46.5	45.2	4.4	3.1
	7.0	4.11	0.2	52.2	44.1	2.2	1.3

K. brevis was the most dominant taxon present in the samples from the 2000 Process Cruise off Panama City (1a) and the 2001 Process Cruise in the ECOHAB study region off Tampa Bay (1b) with diatoms, followed by chlorophytes and the pool of "other taxa" made up the remaining part of the phytoplankton community. In contrast, during the 2002 Process Cruise (1c), also in the ECOHAB study region off Tampa Bay, diatoms and *K. brevis* made up the major portion of the phytoplankton community with contributions from *Trichodesmium*, chlorophytes and the pool of "other taxa" making up the remaining portion.

POC for the whole data set and $17.2 \pm 3.3\%$ (standard error) of the POC for the pooled 2000 and 2001 data indicating that there was substantial detrital or other non-phytoplankton C in the samples analyzed for those 2 years. This finding is supported by the observation that the average POC/PN (mole:mole) was 29.8 ± 2.0 (standard error) for the whole data set. The POC/PN (mole:mole) for all samples from the 2000 and 2001 Process Cruises, those dominated by *K. brevis*, all exceeded a value of 30. In contrast, the average value of POC/PN (mole:mole) for the 2002 Process Cruise, equally represented by diatoms and *K. brevis* with some contributions from *T. erythraeum*, was 7.8 ± 0.4 (standard error). In these same samples, $C_{p,0}$ constituted most of the of the POC ($>72\%$), in contrast to those samples from *K. brevis* dominated blooms.

Total N assimilation ($\mu mol\, N\, L^{-1}\, d^{-1}$) determined using the incorporation of ^{14}C into phytoplankton protein was greater during the 2001 Process Cruise than for each of the other cruises (Table 4). N assimilation was lowest and most variable during the 2000 Process Cruise. We compared our observed N assimilation rates with what might be expected if phytoplankton growth were in balance and at steady state during the three blooms studied (e.g. N assimilation would be equal to μPN, where μ is that determined using the labeled chl *a* technique). We realize that this assumption is not likely to be valid. However, the values of measured N assimilation were comparable

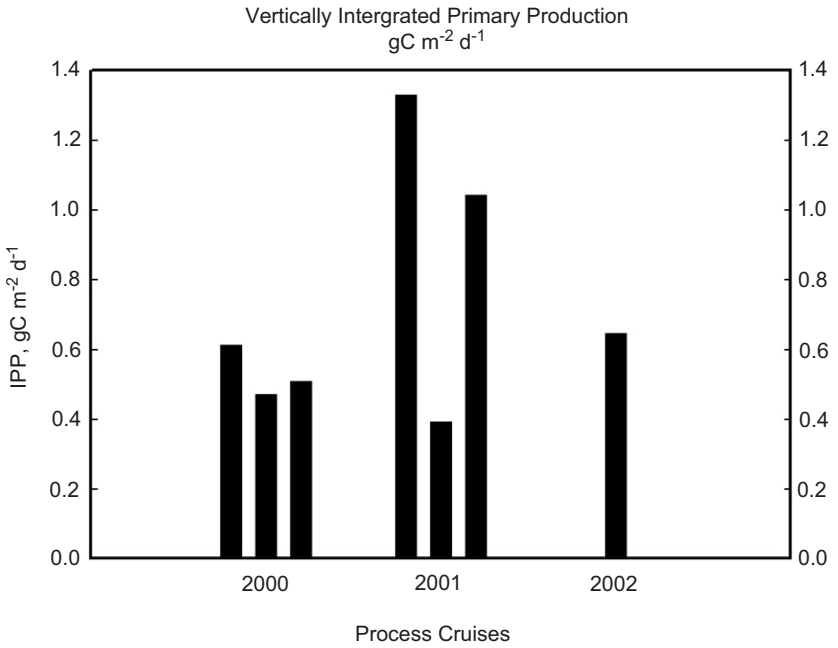

Fig. 4. Vertically integrated primary production (IPP; $g\,C\,m^{-2}\,d^{-1}$) within the blooms studied varied from 0.39 to 1.33 $g\,C\,m^{-2}\,d^{-1}$ and represented large inputs of particulate organic matter into the study region ecosystems. Values of IPP were thought to have varied due to differences in both bloom cell densities and daily incident irradiance. Each bar represents the IPP for a single 24-h incubation experiment.

to those calculated using μPN in most cases; values tended to diverge most at the bottom depths (Table 4). A Mann–Whitney rank sum test indicated that the two data sets were distributed normally and a Student's *t*-test indicated that the mean values (for ^{14}C-protein, $0.58 \pm 0.21\ \mu mol\,N\,L^{-1}\,d^{-1}$ (95% confidence limit), and for μPN, $0.73 \pm 0.12\ \mu mol\,N\,L^{-1}\,d^{-1}$ (95% confidence limi)]) for each type of N assimilation determination were not significantly different ($p < 0.05$, $n = 22$). It should be noted, however, that only one experiment was conducted during the 2002 cruise, and thus it received less weighting than either the 2000 or 2001 cruises where three experiments per cruise were conducted.

We explored interrelationships between key elements from the Process Cruise database using the Spearman Rank Order Correlation, a nonparametric statistical test (Table 5). We compared correlations between our depth specific primary production, μ from the labeled chl *a* technique, PN, POC, $C_{p,0}$ also from the labeled chl *a* technique, HPLC derived chl *a* concentrations, the % of the total chl *a* attributable to *K. brevis* (% *K. brevis*), the C/N composition ratio, the C/N assimilation ratio derived from the incorporation of ^{14}C into protein, temperature, salinity and depth ($n = 22$ for the data set). There were 35 significant correlations (at the $p < 0.1$, $p < 0.5$ and $p < 0.01$ significance levels; $n = 22$) out of 78 possible combinations (Table 5). Of these significant correlations, 25 were significant at the $p < 0.01$ level. The results of the Kruskal–Wallis analysis of variance on ranks with the Student–Newman–Keuls test supported the findings of the Spearman rank order correlation test that there are a large number of significant differences ($p < 0.001$, $n = 22$) in the database. It also indicated that our data were not normally distributed ($p < 0.001$, $n = 22$). Only three of the pairwise comparisons failed to meet the $p < 0.05$ significance criterion: POC vs. % *K. brevis*, primary production vs. salinity and PN vs. chl *a* concentration. Interestingly, both POC vs. % *K. brevis* and PN vs. chl *a* concentration were highly correlated ($p < 0.01$, $n = 22$) as seen in the Spearman rank order correlation.

4. Discussion

In this study we asked the following questions: (1) what are the levels of primary production within blooms in various stages of development; (2) what are the carbon specific growth rates (μ) and the rates of total N assimilation of *K. brevis* in these blooms;

Table 3
Carbon specific growth rates (μ, d^{-1}) were determined during each of the three Process Cruises

Process Cruise	Date	Depth	Flow cytometry	Labeled Chl *a*	Track autorad
1996 R/V Pelican	9/15/96	0.0	0.26		
		0.5	0.27		
		1.0	0.30		
		2.5	0.32		
		4.0	0.28		
		5.5	0.31		
		7.0	0.26		
1997 OSV Anderson	8/30/97	Surface	0.39		
1998 R/V Suncoaster	11/17/98	Surface	0.40		
		Mid	0.36		
		Bottom	0.34		
1999 R/V Pelican-Leg A	9/13/99	Surface	0.57		
		Mid	0.58		
		Bottom	0.53		
1999 R/V Pelican-Leg B	9/21/99	Surface	0.12		
		Mid	0.13		
		Bottom	0.16		
	9/22/99	Surface	0.12		
		Bottom	0.19		
1999 R/V Pelican-Leg C	9/27/99	Surface	0.05		
		Mid	0.06		
		Bottom	0.25		
	9/28/99	Surface	0.10		
		Mid	0.12		
		Bottom	0.22		
2000 R/V Pelican-Leg C	9/28/00	Surface	0.03		
		Bottom	0.06		
	9/29/00	Surface	0.03		
		Bottom	0.02		
2000 Pelican-Leg D	10/2/00	1.0		0.16	0.11
		7.0		0.17	0.11
		14.0		0.13	0.05
	10/3/00	1.0		0.33	
		7.0		0.25	0.11
		14.0		0.33	
	10/4/00	1.0		0.17	0.11
		7.0		0.27	0.10
		14.0		0.34	0.06
		23.0		0.09	0.02
2001 Eugenie clark	9/10/01	Surface	0.17		
		Bottom	0.15		
2001 R/V Suncoaster-Leg A	9/21/01	Surface	0.11		
		Bottom	0.10		
	9/24/01	Surface	0.14		
		Bottom	0.16		

Table 3 (*continued*)

Process Cruise	Date	Depth	Flow cytometry	Labeled Chl *a*	Track autorad
2001 Suncoaster-Leg B	10/21/01	Surface	0.19		
		Bottom	0.14		
	10/22/01	1.0	0.13	0.14	
		4.4		0.15	
		6.0	0.14	0.05	
	10/23/01	1.0	0.21	0.2	
		7.4		0.12	
		11.9	0.20	0.15	
	10/24/01	1.0		0.2	
		4.5		0.16	
		6.5		0.16	
2002 Process Cruise R/V Suncoaster	9/4/02	1.5		0.39	
		3.0		0.32	
		7.0		0.27	

Rates were determined using three independent approaches: diel phasing of the cell cycle and flow cytometry (see Materials and methods for details), track autoradiography and the labeled chl *a* technique. Over the course of the three Process Cruises, rates were generally in the range of 0.1–0.3 d^{-1}, with the different methods giving similar rates where the different methods can be compared directly.

and (3) what are the abundances of *K. brevis* in the blooms in terms of cell densities and carbon biomass and as a fraction of the observed chl *a* biomass and POC. The results that we have obtained through our research conducted over the three annual Process Cruises have enabled us to address these questions and to provide some insight on how blooms of *K. brevis* persist under these dense bloom conditions. Our results indicate that *K. brevis* blooms are highly productive and capable of productivities in excess of 1 g C m^{-2} d^{-1} and that these levels of production vary as a function of incident irradiance as well as the physiological state of *K. brevis* in the blooms. *K. brevis* demonstrated values of μ that corresponded to generation times of 2–3 days and that they were able to assimilate sufficient N from inorganic and/or organic sources to support the observed levels of growth and production. We found that for the blooms we studied, the blooms with the greatest cell densities were characterized as having *K. brevis* chl *a* concentrations representing $>80\%$ of the total community chl *a* concentrations. In addition, we found that within these *K. brevis* dominated blooms (e.g. the 2000 and 2001 Process Cruises), $C_{p,0}$ represented $<17.2\%$ of the POC. However, in a bloom where *K. brevis* was not the dominant taxon, $C_{p,0}$ was $>72\%$ of the POC. We suggest the blooms we studied in 2000 and 2001 as part of the ECOHAB: Florida program were in a different stage of development than the one studied in 2002 and could be characterized as being in a different physiological state from the 2002 bloom. As such, the results we obtained from the 2000 and 2001 Process Cruises could lead us to answer our research questions in somewhat different ways as compared to the results from the 2002 cruise, further demonstrating that the blooms were likely in different stages of development.

The observed levels of primary production for the three blooms studied here are comparable to that of many nutrient-enriched coastal areas. For example, primary production for the Mississippi River Plume (substantially enriched) have been reported in the range of 0.4 to >10 g C m^{-2} d^{-1} (cf. Lohrenz et al., 1999; Redalje et al., 1994). Production on the Louisiana Shelf adjacent to the plume was observed to be 0.1–3.0 g C m^{-2} d^{-1} (Redalje et al., 1994). The production determined here (see Fig. 4) was well within the range observed for the Louisiana Shelf and the lower range for the plume region. Radiocarbon production measurements in the West Florida Shelf waters along the 40 m isobath and to the north of where our 2001 Process Cruise was conducted (see Fig. 1b) were in the range of 1–3 g C m^{-2} d^{-1}, while both measured and modeled values further south (e.g. offshore from where our 2001 and 2002 Process Cruises were conducted; see

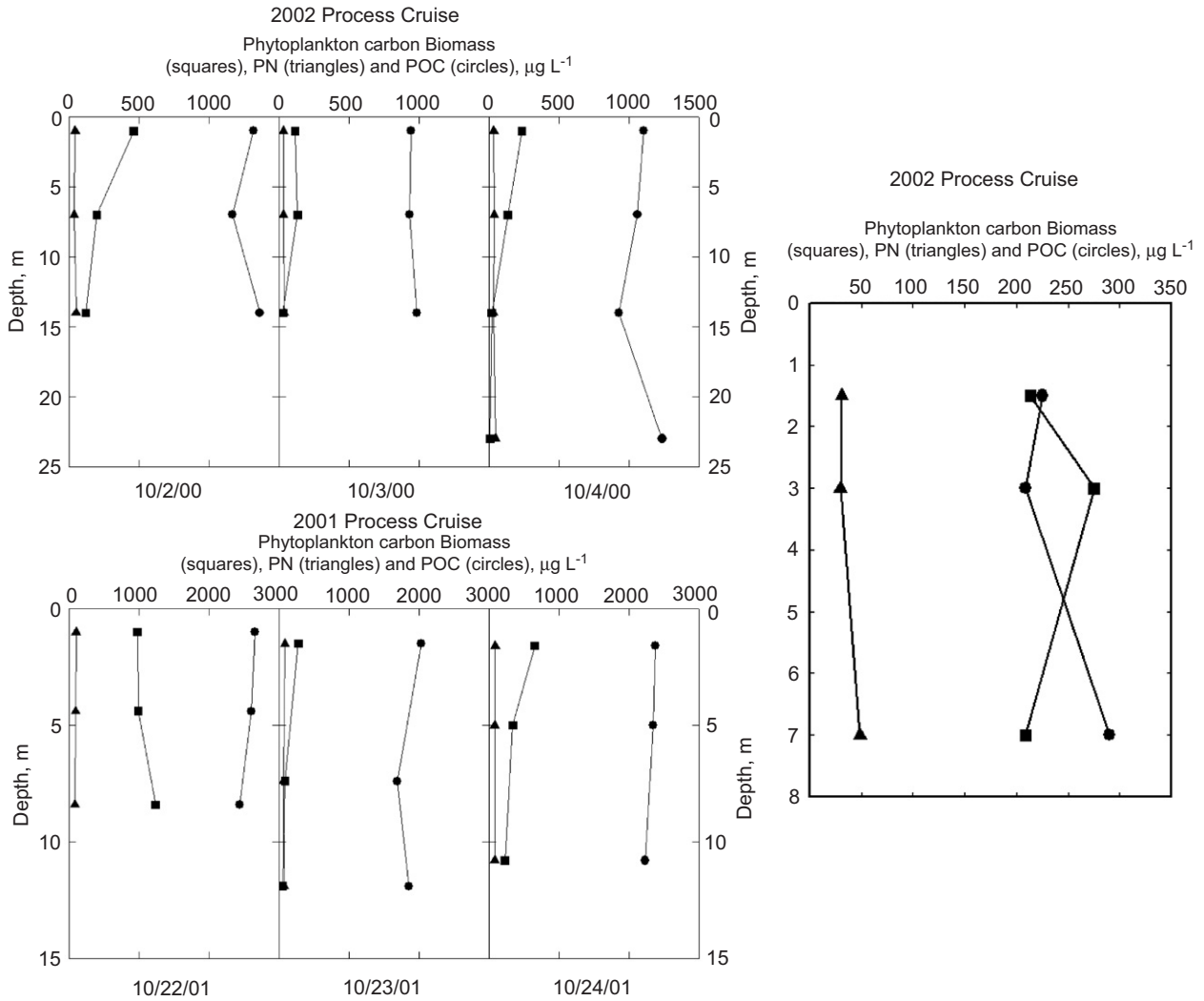

Fig. 5. Vertical profiles of phytoplankton carbon biomass ($C_{p,0}$; squares), particulate nitrogen (PN; triangles) and particulate organic carbon (POC; circles) indicated that during each of the Process Cruises that the water column was well mixed and uniform with respect to each of the three parameters plotted. The results from the 2000 and 2001 Process Cruise were similar in that $C_{p,0}$ averaged $<17.2\%$ of the POC while during the 2002 Process Cruise $C_{p,0}$ was $>72\%$ of the POC.

Fig. 1b, c) were $<0.5\,g\,C\,m^{-2}\,d^{-1}$ (data from August 1992–1993; Walsh et al., 2003). Model projections for primary production at the 30 m isobath offshore from Sarasota, near where our Process Cruises in 2001 and 2002 were conducted, suggested that production might vary from 0.02 to $0.2\,g\,C\,m^{-2}\,d^{-1}$ over the summer and fall months (Walsh et al., 2003). These model projections are clearly lower than what was measured during our study and may be a function of interannual variability and the specific details of the 1998 data set used in the model formulation. Interannual variability in production can be seen in the data collected during 1992–1993 that provided higher measured and modeled production than was the case for the 1998 data set. Their model (1998 data set; Walsh et al., 2003) provided higher estimates of primary production, values of up to $1.8\,g\,C\,m^{-2}\,d^{-1}$ for diatom-dominated blooms along the northern part of the West Florida Shelf than the model predicted for the ECOHAB study region. Our results for the 2002 Process Cruise, a phytoplankton community where diatoms and *K. brevis* were equally represented, provided a primary production rate of $0.65\,g\,C\,m^{-2}\,d^{-1}$ (see Fig. 4), a value that is about a factor of 3 less than what the Walsh et al.

Table 4
Total nitrogen assimilation ($\mu mol\,N\,L^{-1}\,d^{-1}$) was determined using the incorporation of ^{14}C into phytoplankton protein (e.g. DiTullio, 1993)

Date	Depth	N assimilation rate	μPN
10/2/00	1.0	0.64	0.51
	7.0	0.41*	0.43
	14.0	0.24	0.47
10/3/00	1.0	0.49*	0.73
	7.0	0.37	0.54
	14.0	0.15*	0.87
10/4/00	1.0	0.51	0.39
	7.0	0.31	0.70
	14.0	0.13*	0.79
	23.0	0.01*	0.30
10/22/01	1.0	0.98	1.00
	4.4	2.14*	1.02
	6.0	0.62	0.30
10/23/01	1.0	0.57	1.18
	7.4	0.18*	0.52
	11.9	0.15*	0.77
10/24/01	1.0	1.24	1.29
	4.5	0.85	0.93
	6.5	0.62	0.92
9/4/02	1.5	0.68	0.86
	3.0	0.76	0.69
	7.0	0.66	0.94

We also estimated nitrogen assimilation with the assumption that growth was balanced and that conditions were at steady state (e.g. N assimilation = μPN). Although the values derived from μPN were generally greater than those determined using the incorporation of ^{14}C into phytoplankton protein, the mean values over the course of all three Process Cruises were not significantly different ($p<0.05$). The mean $\pm$ 95% confidence limit for the incorporation of ^{14}C into phytoplankton protein was 0.58 ± 0.21 and the mean value from μPN was 0.73 ± 0.12. For those values marked by asterisks, we determined the % of ^{14}C incorporated into protein using the sum of ^{14}C recovered during the solvent extraction and protein isolation procedure (cf. Redalje, 1983). For the remaining values, we determined the % of ^{14}C incorporated into protein using the carbon assimilation values from the productivity results. We have shown the each of these approaches provided consistent results for the % of ^{14}C incorporated into protein (Redalje 1983; unpublished data).

(2003) model suggested. It is clear that as we collect more data, we can help to improve efforts to model production in *K. brevis* dominated coastal waters effectively.

The observed levels of primary production during the three blooms we examined were relatively high compared to other shelf environments (cf. Lohrenz and Verity, 2006; Lohrenz et al., 1999, 2002; Redalje et al., 1994, 2002). At this time, the fate of this photosynthetically fixed carbon is unclear. Some portion of the production might be grazed by zooplankton, although most shelf zooplankton do not appear to graze HAB forming dinoflagellates unless the HAB blooms have very high cell densities (Sutton et al., 2001; Turner et al., 1998; Walsh et al., 2003). Recent studies have suggested that *K. brevis* is not a preferred source of food for copepods due to poor nutritional quality rather than effects due to brevetoxin toxicity (Prince et al., 2006; Speekmann et al., 2006). Physical circulation processes tend to concentrate the bloom on the shelf during HAB development (Steidinger et al., 1998) until advection may become more important during bloom dissipation. Whatever the fate is for this fixed carbon, the primary production associated with the *K. brevis* blooms that occur along the West Florida Shelf represents a significant input of organic carbon to this system.

Over the course of the ECOHAB: Florida Program, there have been efforts to measure growth rates in the field in a variety of ways. It is interesting to note that each of these independent approaches has provided values for μ that are quite comparable and provide the first and most comprehensive set of field-based growth rate determinations for *K. brevis* (see Table 3). It has been known for some time that dinoflagellates are generally characterized by low growth rates (Gisselson et al., 2002; Reguera et al., 2003; Weiler, 1980; Weiler and Chisholm, 1976; Weiler and Eppley, 1979). It is clear that *K. brevis* fits within the slow growing dinoflagellate model (Heil et al., 2001; Van Dolah et al., 2001). The field-based growth rates that we measured during the three blooms studied here are also consistent with C specific growth rates for *K. brevis* in culture that are generally $0.14–0.21\,d^{-1}$ ($=0.2–0.3$ divisions d^{-1}; Magaña and Villareal, 2006; Richardson, 2000; Richardson et al., 2006). Given the cell densities for *K. brevis* dominated blooms (e.g. on the order of $0.5–1.0\times10^6$ cells L^{-1}), it is easy to appreciate the high levels of primary production reported here. For example, if we assume an average value of $3.25\times10^{-4}\,\mu g\,C\,cell^{-1}$ (determined from our track autoradiography analyses) for *K. brevis*, a cell density of 5.0×10^5 cells L^{-1} and that $\mu=0.16\,d^{-1}$ (these were the values for our surface sample during the 2000 Process Cruise on October 4), then we would expect a primary production of $26\,\mu g\,C\,L^{-1}\,d^{-1}$ for *K. brevis*. Our data also indicate that for this particular sample, *K. brevis* represented only 76.3% of the total chl *a*. If we assume a

Table 5
The nonparametric Spearman rank order correlation indicated that there were 35 out of a possible 78 comparisons between environmental and ecological parameters that were significant ($p \leqslant 0.1$) with 25 of these significant correlations highly significant ($p < 0.01$)

	μ	PN	POC	$C_{p,0}$	Chl *a*	% *K. b*	C/N_c	C/N_a	N_{assim}	Temp	Sal	Depth
Prod	0.038	0.376*	0.322	**0.915**	0.265	−0.029	−0.269	**0.794***	**0.969***	**−0.600***	0.003	**−0.802***
μ		**−0.650***	**−0.731***	−0.213	**−0.728***	**−0.768***	−0.030	0.290	0.249	0.025	**−0.717***	−0.138
PN			**0.929***	**0.512**	**0.847***	**0.723***	−0.294	0.049	0.402*	−0.313	**0.786***	−0.186
POC				**0.523**	**0.823***	**0.819***	−0.010	−0.008	0.332	−0.123	**0.856***	−0.222
$C_{p,0}$					**0.462**	0.202	−0.085	**0.673***	**0.895***	**−0.453**	0.222	**−0.743***
Chl *a*						**0.731***	−0.150	−0.049	0.327	−0.268	**0.665***	0.051
% *K. b*							0.081	−0.311	−0.011	0.007	**0.777***	0.191
C/N_c								−0.252	−0.256	**0.686***	−0.039	0.018
C/N_a									**0.699***	−0.401*	−0.270	**−0.730***
N_{assim}										**−0.624***	0.003	**−0.752***
Temp											−0.044	0.267
Sal												0.012

Correlation coefficients that were significant at the $p < 0.1$ level are indicated by a superscript asterisk, those significant at the $p < 0.05$ level are in bold, and those that are significant at the $p < 0.01$ level are indicated by both bold text and a superscript asterisk.

roughly equal production from the non-*K. brevis* organisms in the sample as for *K. brevis*, then we would have a total sample production of 34.1 $\mu g\,C\,L^{-1}\,d^{-1}$. Our measured production for this sample was 42.5 $\mu g\,C\,L^{-1}\,d^{-1}$, a value that is comparable to what we would estimate based on *K. brevis* dynamics alone.

We were also able to determine total N assimilation derived from the incorporation of ^{14}C into protein. There are very few data available of N assimilation or uptake for blooms of *K. brevis* (cf. Bronk et al., 2004). Vargo et al. (2000) suggested that ambient concentrations of NO_3^- in the coastal waters where we conducted our 2001 and 2002 Process Cruises were on the order of $<0.5\,\mu M$ while those of PO_4^- were about 0.2 μM. They suggested that estuarine and nearshore concentrations of dissolved organic nitrogen (DON) vary from 5.0 to 15.0 μM and may represent an important nutrient source for *K. brevis* blooms (Vargo et al., 2000). Walsh et al. (2003) provided similar data for nutrient concentrations obtained in 1998 and 1999 within the ECOHAB study region with values for $NO_2^- + NO_3^-$ that ranged from 0.09 to 0.16 μM in surface waters and 0.11 to 3.13 μM near the bottom. Concentrations for NH_4^+ varied from 0.06 to 0.16 μM for the surface and from 0.11 to 0.39 μM near the bottom (Walsh et al., 2003). Urea, one form of DON that *K. brevis* has been shown to use (Bronk et al., 2004), ranged from 0.09 to 0.19 μM and 0.09 to 3.13 μM for surface and near bottom samples (Walsh et al., 2003). Bronk et al. (2004) obtained samples from within the 2001 *K. brevis* bloom in the ECOHAB study region; samples were collected about 3 weeks prior to our 2001 Process Cruise. They reported low concentrations of NH_4^+ (0.04–0.15 $\mu mol\,N\,L^{-1}$) during their field study. Concentrations of DON and urea were fairly high, with values of 13.0 and 1.18–1.28 $\mu mol\,N\,L^{-1}$, respectively. Concentrations of NO_3^- were also fairly low ($<0.05\,\mu mol\,N\,L^{-1}$). These concentrations of available N suggest that both organic and inorganic forms of N were available for *K. brevis* blooms to utilize. Bronk et al. (2004) presented N uptake rates for NH_4^+, urea and glutamate based on ^{15}N additions at various concentrations of each form of N for samples taken from the surface waters. For additions of 0.05 $\mu mol\,N\,L^{-1}$, they found uptake rates of 15.5, 81.6 and 47.7 $\mu mol\,N\,L^{-1}\,h^{-1}$ for NH_4^+, urea and glutamate, respectively. For additions of 0.5 $\mu mol\,N\,L^{-1}$, uptake rates were 34.2, 35.2 and 8.9 $\mu mol\,N\,L^{-1}\,h^{-1}$ for NH_4^+, urea and glutamate, respectively (Bronk et al., 2004). It should be pointed out that N uptake and N assimilation are not the same process. Organisms may take up various forms of both organic and inorganic N and store these materials in internal pools prior to synthesis of proteins and other N containing components (cf. Dortch et al., 1982; Vincent, 1992). Thus, N uptake rates would always be expected to exceed rates of N assimilation. Our total N assimilation rates represent the sum of assimilation of all forms of available N and were on the order of 0.57–1.24 $\mu mol\,N\,L^{-1}\,d^{-1}$ for surface samples incubated in situ for 24 h (Table 4), values that were significantly less than those reported for uptake by Bronk et al. (2004).

If we assume that steady state and balanced growth occurred within our incubations, then our N assimilation rates should be approximately equivalent to μPN for the same sample and depth. We have demonstrated that our N assimilation rates and values of μPN compare well in most instances for the two shallower depths, but often diverge near the bottom (Table 4). Indeed, for the complete data set, our N assimilation rates and μPN were not significantly different ($p > 0.05$, $n = 22$; Table 4) suggesting that the ^{14}C incorporation into protein approach was valid for *K. brevis* and the data were representative of total N assimilation for the three blooms studied.

The approach that we used is quite different from that used by Bronk et al. (2004) in that our samples were incubated in situ for 24 h and their samples were incubated on deck in incubators for 1 h. In addition, our approach does not involve any additions of N while their approach involved ^{15}N tracer techniques. Although the experiments were conducted in the same extended *K. brevis* bloom within the ECOHAB study area, the Bronk et al. (2004) studies were conducted about 3 weeks prior to our cruise. It would be useful and instructive to conduct each type of experiment at the same time to explore how comparable the results would be for an actively growing *K. brevis* bloom. Comparisons between methods have been made using both phytoplankton cultures (Redalje and Koike, unpublished data) and samples collected from the north Pacific Central Gyre (Harrison et al., 1992; Knauer et al., 1990). Where heterotrophic processes were relatively less important, the ^{14}C protein-based approach provided results that were comparable to those derived from the use of ^{15}N (Harrison et al., 1992; Knauer et al., 1990; Laws et al., 1985). Redalje and Koike (unpublished data), using the same cultures that were used for the NH_4^+ uptake studies of Koike et al. (1983), found that at steady state, the N uptake rates for two species of marine microflagellates grown in NH_4^+-limited continuous culture (*Pelagomonas calceolata* [clone MC1, CCMP#1214] and *Tetraselmis* sp. [clone MC2, CCMP#961, *T. chui* type]) based on the uptake of $^{15}NH_4^+$ and on the incorporation of ^{14}C into protein were the same as that from steady-state kinetics (e.g. μPN) after incubations of 24 h. Thus, we feel that our approach to N dynamics within *K. brevis* blooms is potentially very useful in exploring how this HAB species was able to obtain critical resources and maintain such high cell densities for extended periods. Recently, it was found that *K. brevis* has demonstrated alkaline phosphatase activity under low phosphate conditions such as those found for our process cruise studies (Craney et al., 2004). Hu et al. (2006) have suggested that submarine groundwater discharges may be significant for coastal harmful algal blooms and represent an important pathway for the introduction of nutrients at a rate sufficient to support large blooms such as that we observed during the 2001 Process Cruise. These mechanisms for nutrient access may be very important for bloom maintenance when ambient nutrients are found to be low in concentration and help large bloom persist over the late summer and fall harmful bloom season (Hu et al., 2006). However, groundwater inputs, in particular, are difficult to assess in a comprehensive manner.

We often use chl *a* concentrations determined during shipboard surveys or from remote sensing techniques to delineate the size of HABs. It is often assumed that within an HAB, the bloom forming species represents the dominant taxon present. Our results suggest that the 2000 and 2001 blooms were, indeed, dominated by *K. brevis* (see Table 2a, b) in terms of the portion of the total chl *a* that could be attributed to *K. brevis*. This was not the case for the bloom we studied in 2002 where diatoms and *K. brevis* contributed the most to total chl *a*; diatoms were slightly more important than *K. brevis* in that bloom, particularly at the surface (Table 2c). Thus, we have confidence that our rate measurements were representative of *K. brevis* for the 2000 and 2001 Process Cruise studies, but that values from the 2002 Process Cruise studies represent the combined dynamics of the diatoms present and *K. brevis*.

One of the more interesting results we obtained during the three Process Cruises was that for blooms that were dominated by *K. brevis*, the phytoplankton C ($C_{p,0}$) represented only a small portion of the total POC within the water column (see Fig. 5a, b). Thus, other organic material and detritus (e.g. not associated with intact phytoplankton cells) made up the major portion of the POC in those blooms. This result may serve to complicate efforts to detect and distinguish *K. brevis* blooms using bio-optical or remote sensing approaches. The non-algal POC may absorb and/or scatter light in ways that are not consistent with patterns associated with *K. brevis* cells. The other components of this POC might be detritus, bacteria and/or zooplankton; our data do not allow us to differ-

entiate this POC other than from that associated with phytoplankton (e.g. $C_{p,0}$). In contrast, it appears that when *K. brevis* is not the dominant organism within a bloom (e.g. 2002 Process Cruise) phytoplankton carbon represents a more expected portion of the shelf POC pool (cf. Redalje, 1983). If we look at the values for POC/PN (only mean ratios were given, but POC and PN are shown in Fig. 5a–c), we see that at all depths during both the 2000 and 2001 Process Cruises the POC/PN (mole:mole) ratios are all >30. This suggests that the pool of non-algal POC was C rich. We did not correct the POC samples from 2000 and 2001 for the presence of PIC. However, the PIC for the two shallower depths during the 2002 Process Cruise was negligible while that for the deepest sample was about 27% of the total particulate carbon. If we assume that PIC contributed similarly during the 2000 and 2001 studies, it is difficult to reconcile the high POC/PN ratios we found as being the result of PIC in the more shallow samples. Thus, we suggest that there was a pool of C rich and N poor particulate material suspended in the water column during the *K. brevis* blooms that was not present when the bloom was composed of both diatoms and *K. brevis* (e.g. during the 2002 Process Cruise). This C-rich material may well be detritus and/or zooplankton. However, we cannot verify this speculation. We can only verify that the C-rich material was present in the 2000 and 2001 blooms and not present during the 2002 bloom.

The proportion of the total chl *a* that was attributed to *K. brevis* was positively correlated with POC, PN and total chl *a* concentration and negatively correlated with both μ and salinity (see Table 5). Blooms of *K. brevis* are often concentrated along the shoreline due to physical forcing and are thus often in waters with lower salinities (Steidinger et al., 2000; Stumpf et al., 2000; Walsh et al., 2003). It has also been suggested that DON contained in lower salinity estuarine waters may help to support the growth of coastal *K. brevis* blooms (Walsh et al., 2003). Each of these observations would support the idea that *K. brevis* would be most dominant in blooms that are near shore in lower salinity waters.

We had anticipated that each of the blooms studied would have been in a different stage of development. It is clear that the 2002 bloom was in an early stage of development with *Trichodesmium* present at the surface and *K. brevis* more abundant at depth, fitting the classical pattern of early stage blooms suggested by Steidinger et al. (1998), Walsh and Steidinger (2001), Lenes et al. (2001) and Walsh et al. (2003). Our data as well as the observations of Bronk et al. (2004) and Heil et al. (2004) suggest that the 2001 bloom was in an active growth phase (cf. Steidinger et al., 2000). That bloom had higher values of N assimilation, production, chl *a* concentration and $C_{p,0}$ than what was observed during the 2000 bloom. In addition, *K. brevis* in the 2001 bloom was consistently the most important component of the total chl *a*; this was not the case for the 2000 bloom. We suggest that the 2001 bloom was in a better physiological state than the 2000 bloom because of the differences in these physiological rate and biomass parameters. We know from personal observations that the 2001 bloom continued to persist for a month or more after the 2001 Process Cruise had ended. In contrast, the *K. brevis* cell densities for the leg just prior to our 2000 Process Cruise were generally 1.5–2 times greater than what we found during our cruise leg and that the bloom dissipated soon after the cruise (data not shown). This observation of declining cell densities along with the somewhat low physiological rates and biomass tend to support the suggestion that the 2000 bloom was in a dissipation phase or perhaps just beginning to dissipate. However, our results for the 2000 and 2001 blooms are not sufficiently different to conclude that the 2000 bloom was in a dissipation phase.

5. Conclusions

In conclusion, we found that blooms of *K. brevis* on the West Florida Shelf that occurred during Fall 2000, 2001 and 2002 were highly productive with productivities comparable to many nutrient-enriched coastal regions. We also found that *K. brevis* in these blooms grew at rates corresponding to 2–3 day generation times. For actively growing blooms, *K. brevis* could account for $>80\%$ of the observed chl *a* while in early stage blooms, this proportion might be more on the order of 45%. In *K. brevis* dominated blooms, phytoplankton carbon biomass represented a relatively small portion of the POC. This observation is in contrast to early stage blooms, with much lower chl *a* contributions from *K. brevis*, where phytoplankton C was the largest portion of the POC. We have suggested that the 2000 and 2001 blooms studied here were in a different stage of HAB development from the 2002

bloom, which appeared to be in a bloom initiation stage.

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