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# PHYTOPLANKTON ABUNDANCE AND COMPOSITION IN THE INDIAN RIVER LAGOON 2011–2012 ANNUAL REPORT



### Phytoplankton Abundance and Composition

### In the Indian River Lagoon

### 2011 - 2012

**Annual Report 2012** 

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#### Abstract

Two major bloom events were observed in the Indian River Lagoon during the 2011-2012 study period. A bloom of picoplanktonic green algae and cyanobacteria was observed in the Southern Mosquito Lagoon, northern IRL and Banana River from July through November of 2011. Peak cell densities during the event were in excess of  $1 \times 10^6$  cells ml<sup>-1</sup>. The bloom was an extension of the bloom first observed in March of 2011, and reported in the 2010-2011 Annual Report. In 2012 a major brown tide event was observed in the Indian River Lagoon ecosystem between June and August of 2012. The bloom was the first observation of a brown tide since monthly monitoring began in 1997. The bloom was dominated by unicellular, spherical to subspherical, 2-4 µm diameter, non-flagellated golden brown cells. At the height of the bloom, chlorophyll a concentrations were 171  $\mu$ g L<sup>-1</sup> and cell densities exceeded 3 x 10<sup>6</sup> cells ml<sup>-1</sup>. Scanning Electron Microscopy showed cells with extracellular material and Transmission Electron Microscopy revealed basal bodies and a pyrenoid connected to the chloroplast via a stalk, characteristics consistent with the pelagophyte species Aureoumbra lagunensis, the species responsible for harmful algal blooms in Texas during the 1990s. Moreover, samples fluoresced in response to immunofluorescent labeling specific to an A. lagunensis antibody but not to an Aureococcus anophagefferens antibody. Microscopic examination of historical samples confirmed the presence of pelagophyte cells in the Indian River Lagoon as far back as 2005.

#### Introduction

Over the past decade concern has grown over the possible role of toxic or other harmful algae in an increasing number of aquatic animal health problems, including dolphin mortalities, the appearance of the algal neurotoxin 'saxitoxin' in puffer fish (Landsberg et al. 2006), and mass mortalities of fish and shellfish. Many of these incidents along the east coast of Florida have been centered on regions of the Indian River Lagoon subject to the least tidal mixing and longest water residence times. This region stretches from southern Mosquito Lagoon to Melboune and includes the Banana River. Recent studies have shown that this is also the region subject to the most intense and frequent algal blooms over the past decade (Phlips and Badylak 2004, Phlips et al. 2006, 2009, Badylak and Phlips 2004, Phlips et al. 2010, Phlips et al. 2011). These blooms include species of dinoflagellates suspected of toxin production in other regions of Florida and around the world (FMRI 2002, IOC 2002), most prominently the saxitoxin-producing species *Pyrodinium bahamense* (Phlips and Badylak 2004, Landsberg et al. 2006, Phlips et al. 2006), and picoplanktonic eucaryotes, including flagellated chlorophytes (Pedinophyceae sp.) and brown tide species (Pelagophyte sp.).

The objective of this study was to monitor the composition and abundance of phytoplankton in the Indian River Lagoon, with special emphasis on potentially harmful species. The long term goal of this line of research is to provide a baseline of information with which future trends in algal blooms can be evaluated and the overall integrity of the Indian River Lagoon ecosystem can be preserved through sound management practices.

#### Methods

Samples were collected from seven sites (1, 1.5, 4, 4.1, 6, and 8) in the Mosquito Lagoon and Indian River Lagoon on a monthly basis (Figure 1). Three other sites (2, 3, and 5) in the Banana River and IRL were sampled twice per month (Figure 1). The cell count, biovolume and nutrient data for these sites are provided in the Excel spreadsheets. Samples were collected with a vertical integrating tube that collects water evenly from the surface to 0.1 m from the bottom, in order to avoid misinterpretations of phytoplankton composition due to vertical stratification of organisms. Samples were split into four aliquots: cold-preserved sub-samples for picoplankton analysis, Lugols preserved sub-samples for general phytoplankton analysis, glutaraldehyde

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preserved sub-samples for long-term preservation of certain key species, and frozen sample for total nutrient analysis.

General phytoplankton composition was determined using the Utermohl method (Utermohl 1958). Lugols preserved samples were settled in 19mm I.D. diameter cylindrical chambers. Phytoplankton cells were identified and counted at 400X and 100X with a Nikon phase contrast inverted microscope. At 400X, a minimum of 100 cells of a single taxa and 30 grids were counted. If 100 cells are not counted by 30 grids, up to a maximum of 100 grids were counted until one hundred cells of a single taxa are reached. At 100X, a total bottom count was completed for taxa greater than 30  $\mu$ m. Light microscopy will be aided by other techniques to properly identify dinoflagellates and diatoms, including the squash technique (Steidinger 1979), destaining of samples with sodium thiosulfate and clearing of diatom cells with hydrogen peroxide. In addition, a backup sample preserved with glutaraldehyde in 0.1 M sodium cacodytlate buffer was also available for additional analysis of samples using scanning electron microscopy (SEM) to examine the morphology of a cell in greater detail.

Fluorescence microscopy was used to enumerate picoplanktonic cyanobacteria, e.g. *Synechococcus* (Phlips et al. 1999). Subsamples of station water were filtered onto 0.2  $\mu$ m pore nucleopore filters and mounted between a microscope slide and cover slip with immersion oil. These were stored in the freezer and counted within 72 hours using a Nikon research microscope equipped with autofluorescence (green light 530-560nm excitation and >580nm emission).

The identity of the dominant species of picoplanktonic eucaryotes during blooms in the summer of 2012 were examined using SEM and transmission electron microscopy (TEM).

General texts used most frequently to aid in taxonomic identification included Steidinger and Williams (1970), Tester and Steidinger (1979), Sournia (1986), Ricard (1987) and Tomas (1997).

Cell biovolumes were estimated by assigning combinations of geometric shapes to fit the characteristics of individual taxa (Smayda 1978). Specific phytoplankton dimensions were measured for at least 30 randomly selected cells from different sites, dates and years. In the case of species that show significant differences in cell size related to life history stage or environmental conditions, size classes were established to provide more accurate estimates of cell biovolume.

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Total nitrogen was determined using the persulfate digestion method (APHA 1989) and analyzed with a Bran-Luebbe AutoAnalyzer. Total phosphorus was determined using the persulfate digestion method (APHA 1989) and analyzed using a spectrophotometer. Chlorophyll <u>a</u> concentration was determined after extraction of the filtered samples with 90% warm ethanol (Sartory and Grobbelaar 1984), with a Hitachi U2000 dual beam spectrophotometer.

#### **Results and Discussion**

Two major bloom events were observed in the Indian River Lagoon during the 2011-2012 study period. A bloom of picoplanktonic green algae and cyanobacteria was observed in the Southern Mosquito Lagoon, northern IRL and Banana River from July through November of 2011 (Figure 2, Table 1). Peak cell densities during the event were in excess of  $1 \times 10^6$  cells ml<sup>-</sup> <sup>1</sup>. The bloom was an extension of the bloom first observed in March of 2011, and reported in the 2010-2011 Annual Report. In 2012 a major brown tide event was observed in the Indian River Lagoon ecosystem between June and August of 2012. The bloom was the first observation of a brown tide since monthly monitoring began in 1997. (Figure 2 and 3). From Eau Gallie south in the IRL phytoplankton biovolumes were relatively low throughout the contract period (Figure 2). Preliminary evidence from microscopic analyses, including light, scanning electron and transmission electron microscopy carried out at the University of Florida (Figure 4) suggest that the brown tide involved the pelagophyte species Aureoumbra lagunensis. This conclusion is further supported by preliminary immunological assays reported by Alina Corcoran of the Florida Fish and Wildlife Research Institute and 18s-RNA sequence analyses carried out by Chris Gobler at the State University of New York (Stony Brook). Aureoumbra lagunensis is the pelagophyte associated with an eight year bloom event in the Laguna Madre estuary in Texas, which resulted in serious ecological issues, including grazer inhibition, low light availability for benthic primary producers and food web alterations (Buskey and Stockwell 1993, Buskey and Hyatt 1995, Onuf 1996, Buskey et al. 1997, Liu and Busky 2000, Ward et al. 2000, Liu et al. 2001).

The Pelagophyte bloom began in the Mosquito lagoon in beginning of July 2012, before spreading to the northern IRL near Titusville in mid-July (Figure 5). Peak Pelagophyte sp. biovolumes observed during the study period were  $104 \times 10^6 \,\mu\text{m}^3 \,\text{ml}^{-1}$  (i.e.  $3.3 \times 10^6 \,\text{cells ml}^{-1}$ )

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at the Southern Mosquito Lagoon (Site 1.5) site (Table 1, Fig. 5). In the Mosquito Lagoon, the brown tide was accompanied by hypersaline conditions (i.e. salinities > 40 psu) (Figure 6), which are known to favor this highly euryhaline species (Buskey et al. 1998). The bloom also followed a major increase in rainfall in May/June, which was associated with a spike in both TN and TP concentrations (Figure 6). Bloom concentrations of Pelagophyte sp. were not observed south of Titusville or in the Banana River (Figure 2, Table 1). Observation of the 24 targeted historical samples with unidentified cells <5  $\mu$ m revealed pelagophyte cells in the Banana River in 2005, the IRL at Melbourne in 2009 and 2010, and near Vero 8 in 2009, but at low densities (i.e. < 1000 cells ml<sup>-1</sup>).

Outside of the areas subject to the brown tide event, the Top-15 biovolume observations for individual species in 2012 varied by site (Table 1). At Site 3 (Core 5) in the central Banana River the dinoflagellate Akashiwo sanguinea and the diatoms Skeletonema costatum and Chaetoceros tenuissimus reach were in the Top-15 biovolume observations. No individual species reached the Top-15 at Site 3.5 (Core 3) in 2012. At Site 4.1 (Core 4) a major Akashiwo sanguinea bloom was observed in May 2012, and three diatom species were high in the Top-15 list in 2012, i.e. Skeletonema menzelii, Leptocylindrus danicus and Chaetoceros tenuissimus. At Site 4 near Eau Gallie spherical picocyanobacteria were heavily represented in the Top-15 list in both 2011 and 2012. In 2012, a number of dinoflagellate species were in the Top-15 list, including Akashiwo sanguinea, Heterocapsa sp., Protoperidinium sp. and Cocliodinium polykrikoides. At Site 5 (Core 6) near Melbourne spherical picocyanobacteria dominated the Top-15 list in 2011, while dinoflagellates were in well represented in the Top-15 list in 2012, including Akashiwo sanguinea, Heterocapsa sp., Peridinium quinquecorne and Karlodinium veneficum. At Site 6 near Sebastian spherical picocyanobacteria and a number of diatom and dinoflagellate species populated the Top-15 list, but none reached notable biovolume levels. At Site 8 near Vero spherical picocyanobacteria and a number of diatom and dinoflagellate species populated the Top-15 list, but only one species of dinoflagellate was observe at high biovolume levels, Takayama tasmanica in July of 2011.

In addition to the basic phytoplankton data for the base monitoring sites, additional samples from bloom sites were analyzed. The results are provided in the attached data file (IRL Bloom Sample Results 2011\_2012). Most of the bloom samples from the 2011 period were

dominated by the chlorophyte Pedinophyceae sp., while most of the samples from 2012 were dominated by the brown tide species Pelagophyte sp.

Data for parameters measured in the field and the results of analyses for chlorophyll *a*, total nitrogen and total phosphorus are provided in the attached data file (IRL Physical Chemical Data 2011\_2012).

Table 1. Top-15 observations of individual phytoplankton species at each of the sampling, on a biovolume basis. DI – dinoflagellate; D – diatom; CY – cyanobacterium; CH – chlorophyte; P - pelagophyte.

			Biovolume
Date	Species	Group	$\mu m^3 ml^{-1}$
7/18/2012	Pelagophyte sp. (Aureoumbra cf)	Р	88,874,652
7/2/2012	Pelagophyte sp. (Aureoumbra cf)	Р	24,154,798
7/18/2012	Pedinophyceae sp.	СН	14,300,236
10/26/2011	Pedinophyceae sp.	СН	4,322,134
8/16/2011	Pedinophyceae sp.	CH	3,280,801
7/18/2012	Spherical picocyanobacteria spp.	CY	3,193,277
12/19/2011	Thalassiosira sp.	D	2,347,657
7/18/2012	Protoperidinium sp.	DI	2,201,361
8/16/2011	Spherical picocyanobacteria spp.	CY	1,920,768
7/12/2011	Spherical picocyanobacteria spp.	CY	1,908,763
7/2/2012	Spherical picocyanobacteria spp.	CY	1,740,696
10/26/2011	Spherical picocyanobacteria spp.	CY	1,352,541
3/20/2012	Spherical picocyanobacteria spp.	CY	1,330,132
7/2/2012	Pedinophyceae sp.	СН	1,198,997
7/18/2012	Gyrodinium cf. pingue	DI	1,090,187
	Site 1.5 (Core 1) - S. Mosquito Lagoon		
8/6/2012	Pelagophyte sp. (Aureoumbra cf)	Р	104,722,614
7/10/2012	Pelagophyte sp. (Aureoumbra cf)	Р	38,463,564
9/22/2011	Synechococcus spp.	CY	12,228,344
10/3/2011	Pedinophyceae sp.	СН	10,388,932
9/22/2011	Pedinophyceae sp.	СН	9,640,952
8/1/2011	Pedinophyceae sp.	СН	7,691,177
8/16/2011	Pedinophyceae sp.	СН	7,014,872
9/7/2011	Pedinophyceae sp.	СН	6,978,898
7/10/2012	Pedinophyceae sp.	СН	5,449,335
1/5/2012	Pedinophyceae sp.	СН	5,068,815
1/5/2012	Synechococcus spp.	CY	4,638,355
2/6/2012	Spherical picocyanobacteria spp.	CY	4,309,723
9/22/2011	Spherical picocyanobacteria spp.	CY	4,285,714
8/16/2011	Spherical picocyanobacteria spp.	CY	4,237,694
1/5/2012	Spherical picocyanobacteria spp.	CY	4,033,613

## Site 1 - C. Mosquito Lagoon

### Site 2 - Titusville

			Biovolume
Date	Species	Group	$\mu m^3 ml^{-1}$
8/22/2012	Pelagophyte sp. (Aureoumbra cf)	Р	29,414,778
8/6/2012	Pelagophyte sp. (Aureoumbra cf)	Р	27,256,698
7/18/2012	Pelagophyte sp. (Aureoumbra cf)	Р	24,492,305
10/26/2011	Pedinophyceae sp.	СН	16,226,470
7/12/2011	Pedinophyceae sp.	СН	15,711,580
8/16/2011	Pedinophyceae sp.	СН	14,684,464
10/3/2011	Pedinophyceae sp.	СН	14,359,250
9/19/2011	Pedinophyceae sp.	СН	13,864,264
9/7/2011	Pedinophyceae sp.	СН	12,619,574
7/5/2011	Pedinophyceae sp.	СН	11,705,842
8/1/2011	Pedinophyceae sp.	СН	10,295,673
4/3/2012	Akashiwo sanguinea	DI	10,126,137
7/10/2012	Pelagophyte sp. (Aureoumbra cf)	Р	9,976,112
12/5/2011	Synechococcus spp.	CY	8,096,037
4/17/2012	Akashiwo sanguinea	DI	6,821,819
	Site 3 (Core 5) - C. Banana River		
10/5/2011	Pedinophyceae sp.	СН	11,925,357
7/12/2011	Pedinophyceae sp.	СН	10,122,999
9/19/2011	Pedinophyceae sp.	СН	9,331,578
7/2/2012	Skeletonema cf. menzelii	D	7,535,130
7/2/2012	Chaetoceros tenuissimus	D	7,397,268
7/6/2011	Pedinophyceae sp.	СН	7,115,598
8/9/2012	Akashiwo sanguinea	DI	7,114,944
12/7/2011	Spherical picocyanobacteria spp.	CY	6,398,558
10/26/2011	Spherical picocyanobacteria spp.	CY	6,194,477
9/6/2011	Pedinophyceae sp.	СН	5,345,692
8/16/2011	Spherical picocyanobacteria spp.	CY	5,210,083
8/3/2011	Spherical picocyanobacteria spp.	CY	4,393,757
10/5/2011	Spherical picocyanobacteria spp.	CY	4,141,656
8/16/2011	Pedinophyceae sp.	СН	3,942,718
12/19/2011	Spherical picocyanobacteria spp.	CY	3,577,430

### Site 3.5 (Core 3) - N. Banana River

			Biovolume
Date	Species	Group	$\mu m^3 ml^{-1}$
9/7/2011	Pedinophyceae sp.	CH	16,648,628
7/18/2011	Pedinophyceae sp.	CH	15,144,928
8/16/2011	Pedinophyceae sp.	CH	14,569,349
7/6/2011	Pedinophyceae sp.	CH	14,130,469
8/3/2011	Pedinophyceae sp.	CH	13,022,479
10/5/2011	Pedinophyceae sp.	CH	12,715,116
9/22/2011	Pedinophyceae sp.	CH	10,029,468
8/3/2011	Karlodinium veneficum	DI	4,907,408
12/7/2011	Spherical picocyanobacteria spp.	CY	3,529,411
9/22/2011	Synechococcus spp.	CY	3,162,515
8/16/2011	Spherical picocyanobacteria spp.	CY	2,845,138
8/16/2011	Synechococcus spp.	CY	2,740,846
11/16/2011	Spherical picocyanobacteria spp.	CY	2,328,931
9/7/2011	Spherical picocyanobacteria spp.	CY	2,316,926
11/16/2011	Synechococcus spp.	CY	2,150,510
	Site 4 - Eau Gallie		
3/20/2012	Akashiwo sanguinea	DI	5,340,205
8/16/2011	Spherical picocyanobacteria spp.	CY	3,829,531
7/12/2011	Coscinodiscus sp.	D	3,450,388
10/26/2011	Spherical picocyanobacteria spp.	CY	2,521,008
9/19/2011	Spherical picocyanobacteria spp.	CY	2,292,917
7/12/2011	Spherical picocyanobacteria spp.	CY	2,256,902
2/21/2012	Heterocapsa sp.	DI	2,148,691
11/22/2011	Spherical picocyanobacteria spp.	CY	2,056,822
4/17/2012	Akashiwo sanguinea	DI	1,460,296
9/19/2011	Takayama sp.	DI	1,182,928
3/20/2012	Spherical picocyanobacteria spp.	CY	974,790
12/19/2011	Spherical picocyanobacteria spp.	CY	906,362
4/17/2012	Protoperidinium sp.	DI	879,962
9/19/2011	Leptocylindrus danicus	D	848,257
1/24/2012	Cochlodinium polykrikoides	DI	784,840

4/5/2012

7/18/2012

#### Biovolume $um^3 ml^{-1}$ Date **Species** Group 45,834,096 5/9/2012 Akashiwo sanguinea DI 9/20/2011 Pedinophyceae sp. CH 20,116,493 7/19/2011 Pedinophyceae sp. CH 12,173,500 8/16/2011 Pedinophyceae sp. CH 10,597,852 Pedinophyceae sp. 7/7/2011 CH 8,302,730 Pedinophyceae sp. CH 8/2/2011 5,388,860 6/11/2012 Chaetoceros tenuissimus D 5,261,437 Synechococcus spp. CY 11/15/2011 5,102,190 9/20/2011 Synechococcus spp. CY 3,542,016 2/7/2012 *Leptocylindrus danicus* D 3,367,446 *Gyrodinium spirale* 11/15/2011 DI 3,134,058 6/11/2012 Skeletonema cf. menzelii D 3,108,127 Heterocapsa sp. DI 3/15/2012 2,830,586 Spherical picocyanobacteria spp. CY 9/7/2011 2,689,075 CY 7/19/2011 Spherical picocyanobacteria spp. 2,605,042 Site 5 - Melbourne 9/19/2011 Spherical picocyanobacteria spp. CY 3,325,330 Spherical picocyanobacteria spp. CY 8/16/2011 2,821,128 DI 7/2/2012 Peridinium quinquecorne 2,607,139 10/26/2011 Spherical picocyanobacteria spp. CY 2,565,026 4/5/2012 Rhizosolenia setigera D 2,173,500 1/24/2012 Karlodinium veneficum DI 2,105,526 11/22/2011 Spherical picocyanobacteria spp. CY 1,968,787 7/12/2011 Spherical picocyanobacteria spp. CY 1,920,768 10/12/2011 Spherical picocyanobacteria spp. CY 1,906,762 11/17/2011 Spherical picocyanobacteria spp. CY 1,704,682 12/19/2011 Spherical picocyanobacteria spp. CY 1,692,677 3/14/2012 DI Heterocapsa sp. 1,625,485 7/2/2012 Centric diatom sp. D 1,574,688

#### Site 4.1 and 4.5 (Core 4) - Cocoa IRL

DI

CY

1,550,898

1,536,616

Akashiwo sanguinea

Spherical picocyanobacteria spp.

## Site 6 - Sebastian

			Biovolume
Date	Species	Group	$\mu m^3 ml^{-1}$
10/26/2011	Thalassionema nitzschioides	D	3,541,659
8/16/2011	Spherical picocyanobacteria spp.	CY	1,980,792
9/19/2011	Spherical picocyanobacteria spp.	CY	1,860,744
7/2/2012	Spherical picocyanobacteria spp.	CY	1,668,864
7/12/2011	Spherical picocyanobacteria spp.	CY	1,590,636
5/22/2012	Cerataulina pelagica	D	1,346,678
10/26/2011	Spherical picocyanobacteria spp.	CY	1,284,514
5/22/2012	Pedinophyceae sp.	СН	1,194,221
7/12/2011	Thalassionema bacillare	D	1,165,289
4/17/2012	Gyrodinium spirale	DI	932,756
8/16/2011	Protoperidinium sp.	DI	898,909
5/22/2012	Spherical picocyanobacteria spp.	CY	776,310
8/16/2011	Scrippsiella sp.	DI	744,469
11/22/2011	Spherical picocyanobacteria spp.	CY	636,254
12/19/2011	Katodinium glaucum	DI	612,818
	Site 8 - Vero		
7/12/2011	Takayama tasmanica	DI	8,002,879
9/19/2011	Scrippsiella sp.	DI	1,803,780
9/19/2011	Akashiwo sanguinea	DI	1,694,796
8/16/2011	Takayama tasmanica	DI	1,600,576
7/12/2011	Spherical picocyanobacteria spp.	CY	1,206,482
5/22/2012	Chaetoceros cf. costatus	D	1,112,131
9/19/2011	Protoperidinium sp.	DI	1,088,986
8/16/2011	Spherical picocyanobacteria spp.	CY	892,357
8/16/2011	Scrippsiella sp.	DI	742,829
5/22/2012	Pseudo-nitzschia calliantha	D	663,328
7/12/2011	Peridinium quinquecorne	DI	601,647
8/16/2011	Gyrodinium spirale	DI	584,527
9/19/2011	Spherical picocyanobacteria spp.	CY	568,227
3/20/2012	Protoperidinium sp.	DI	514,794
10/26/2011	Spherical picocyanobacteria spp.	CY	448,179



Figure 1. Sampling site locations.



Figure 2. Phytoplankton biovolume at sampling sites by major group:











Figure 2 continued





Figure 3. Photograph of brown tide in Mosquito Lagoon (provided by Kelly Young).

Figure 4. SEM and TEM images of the brown tide cell during the Indian River Lagoon bloom. Fig. 3. Cells showing the presence of a vacuole (v). Fig 4. Extracellular material on cell surface (ecl). Fig. 5. Ultrastructure of the cell with cup shaped parietal chloroplast (chl). Fig. 6. Stalk (s) connecting pyrenoid (py) with chloroplast. Fig. 7. The presence of basal bodies (bb). Fig. 8. Golgi bodies (gg) adjacent to the nucleus (nuc) with nucleolus (n) and extracellular layer (ecl) displayed with fixation using ruthenium red. Scale bars Fig.  $3 = 3\mu m$ ; Fig.  $4 = 1.2\mu m$ ; Fig.  $5 = 1\mu m$ ; Fig.  $6 = 0.5\mu m$ ; Fig.  $7 = 0.2\mu m$ ; Fig.  $8 = 0.5\mu m$ .





Figure 5. Time series of Pelagophyte sp. distribution in the Mosquito Lagoon and northern IRL and Banana River.

Figure 6. Time series of phytoplankton biovolume (top), temperature and salinity (middle), and TP and TN concentration (bottom). Abbreviations: 'Dino' is dinoflagellate, 'Diat' is diatom, 'Cyan' is cyanobacteria, 'Pelag' is Pelagophyte sp., and 'other' inlcudes all remaining taxa.



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