Comprehensive Monitoring of Florida Shellfish Hatchery/Nursery Operations

to Improve Seed Health

Objective 4 – Phytoplankton Quality and Quantity

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Edward Phlips and Susan Badylak Fisheries and Aquatic Sciences Program, SFFGS, IFAS, University of Florida

Introduction

The objective of this component of the study is to determine the composition and biomass of phytoplankton communities in five coastal regions of Florida associated with bivalve mariculture activities. The focus of the effort is on the trophic state of the regions and potential threats for the health of bivalves represented by the presence of harmful algal species (Lundholm et al. 2009, Lassus et al. 2016).

Methods

General phytoplankton composition was determined using the Utermöhl method (Utermöhl, 1958). Samples preserved in Lugol's were settled in 19-mm diameter cylindrical chambers. Phytoplankton cells were identified and counted at 400× and 100× with a Leica phase contrast inverted microscope. At 400×, a minimum of 100 cells of a single taxon and 5 grids were counted. At 100×, a total bottom count was completed for taxa >20-30 μ m in size. Fluorescence microscopy was used to enumerate picoplanktonic cyanobacteria (e.g., *Synechococcus* spp. and spherical picocyanobacteria spp.) at 1000x magnification (Phlips et al., 1999). Subsamples of seawater were filtered onto 0.2- μ m Nucleopore filters and mounted between a microscope slide and cover slip with immersion oil.

Cell biovolumes (μ m⁻³ cell⁻¹) were estimated by assigning combinations of geometric shapes to fit the characteristics of individual taxa (Smayda, 1978; Sun and Liu, 2003). Specific phytoplankton dimensions were measured for at least 30 randomly selected cells. Species which vary substantially in size, such as many diatom species, were placed into size categories. Phytoplankton biomass as carbon values (μ g carbon L⁻¹) was estimated by using conversion

factors for different taxonomic groups applied to biovolume estimates, i.e., 0.065 x biovolume ($\mu m^{-3} ml^{-1} x 10^{-6}$) of diatoms, 0.16 x biovolume of dinoflagellates and 0.22 x biovolume of cyanobacteria and other phytoplankton taxa (Strathmann, 1967; Ahlgren, 1983; Sicko-Goad et al., 1984; Verity et al., 1992; Work et al., 2005).

Results and Discussion

Mean total phytoplankton biomass over the study period at the five sampling sites ranged from 178 μ g carbon L⁻¹ at Site T383 to 461 μ g carbon L⁻¹ at Site C578 (Table 1). These mean values fall within the range of mean values observed in the lower Caloosahatchee estuary (Phlips et al. 2023) and lower Tampa Bay on the west coast of Florida (Badylak et al. 2007), but are higher than values observed in the open water region of the Cape Canaveral shelf environment of the off the east coast of Florida (i.e. 122 μ g carbon L⁻¹) (Tate et al. 2020). The mean values are considerably lower than in the northern Indian River Lagoon, where mean values were over 3000 μ g carbon L⁻¹ for the period from 2011 to 2020 (Phlips et al. 2021). Peak biomass levels observed during the study ranged between 1000 to 1400 μ g carbon L⁻¹ at Sites C578, J737, O613, and W329 (Figure 1). The highest value at Sites J737 was 600 μ g carbon L⁻¹. By comparison, peak biomass levels in the northern Indian River Lagoon reached levels over 20,000 μ g carbon L⁻¹. The highest biomass levels at Sites C578, J737, and W329 were observed during the Spring/early Summer of 2022. At Site O613, peak biomass levels were similar in both 2021 and 2022.

The range of mean biomass values observed in this study are roughly equivalent to 3-8 μ g chlorophyll *a* L⁻¹, based on relationships observed in a previous study of the Caloosahatchee estuary (Mathews et al. 2015, Phlips personal communications). The highest values observed in the study, i.e., between 1000 and 1400 μ g carbon L⁻¹, would be in the range of 20-25 μ g chlorophyll *a* L⁻¹. From the perspective of trophic state indices for coastal marine systems, the mean biomass range would be indicative of oligotrophic to lower mesotrophic conditions (ICWA 2021), which would generally be considered good water quality conditions from the perspective of general phytoplankton biomass levels, in terms of overall ecosystem function (TCWA 2021).

In order to examine differences in the structure of phytoplankton communities at the five sampling sites, biomass time-series were sub-divided into four major groups, i.e., dinoflagellates, diatoms, cyanobacteria and all "other" taxa (Fig. 1). Over the study period, the distribution of

mean biomass of the four groupings differed between sites. At C578, mean diatom and "other' group biomass was greater than mean dinoflagellate and cyanobacteria biomass (Table 1). At J737 and W329, mean diatom biomass was higher than the other three groupings. At O613, mean dinoflagellate biomass was higher than the other three groupings. At T383, mean cyanobacteria and "other' group biomass was greater than mean dinoflagellate and diatom biomass.

The four groupings of the phytoplankton biomass provide the basis for evaluating potential threats to bivalve and ecosystem health represented by key phytoplankton taxa at the five sampling sites. The threats can be viewed from two perspectives, 1) The Top-50 biomass observations of individual taxa at the five sampling sites over the study period, as a measure of taxa that reach significant levels of biomass at each site (Table 2), and 2) The presence of potentially harmful species that were not necessarily observed at high levels of biomass, but represent the existence of a potential future threat (Table 3).

Site C578

At Site C578, the most commonly observed species on the Top-50 list were spherical picoplanktonic cyanobacteria, undefined nanoplanktonic eukaryotes, and cryptophytes. The results of this study do not provide specific evidence that taxa within these groups contain species harmful to bivalves in the study regions (Table 2). The only Harmful Algal Bloom (HAB) species (Lundholm et al. 2009, Lassus et al. 2016) that were observed at significant levels of biomass in the Top-50 list were the dinoflagellates *Karlodinium veneficum*, and *Peridinium quadridentatum*.

Karlodinium veneficum has been observed to produce the toxin karlotoxin in a number of coastal ecosystems around the world (Lassus et al. 2016, Pace et al. 2012). Karlotoxin is an ichthyotoxin (i.e. harmful to fish) that produces strong hemolytic activity (Bachvaroff, et al. 2009, Goshorn et al. 2004, Műller et al. 2019, Neilsen 1993, Nielsen and Stromgren 1991). The toxin has been linked to incidents of fish mortalities (Abbott and Ballentine 1957, Deeds et al. 2002, Deeds et al. 2004, Landsberg 2002, Place et al. 2012), and has also been shown to have lethal, or adverse sublethal effects on a wide range of marine invertebrates, including mussels and scallops (Daugbjerg et al. 2000, Landsberg 2002, Lassus et al. 2016). *K. veneficum* was observed at peak cell densities of 755,000 cells L^{-1} at C578 (Table 2). However, the abundances

of *K. veneficum* observed in this study were well below peak values associated with serious harmful bloom events observed in other ecosystems, e.g. 10^7 - 10^8 cells L⁻¹ (Place et al. 2012).

The other HAB dinoflagellate species on the Top-50 list multiple times is *Peridinium quadridentatum* (Table 2). *P. quadridentatum* is not known to be toxic, but has been associated with ecosystem disruptions during intense bloom conditions (Alkawri et al. 2016,Gárate-Lizárraga and Muñetón-Gómez 2008, Trigueros and Orive 2000), such as the formation of hypoxia conditions. The highest concentrations of *P. quadridentatum* observed at Site C578 was 363,000 cells L⁻¹. The latter cell concentrations is well below that associated with major disruptive blooms in other ecosystems, such as the 14.3 x 10^6 cells L⁻¹ observed during a mass fish mortality event in the Red Sea (Alkawri et al. 2016).

Among the HAB species that may affect bivalve issues, but were not present in the Top-50 list for Site C578, the most prominent taxa were in the genus *Prorocentrum* (Table 3). A number of species in this genus have been associated with both direct impacts on bivalve health and accumulation of Okadaic Acid (aka, DSP, Diarrhetic Shellfish Poison), a toxin that can impact human health (Landsberg 2002, Lassus et al. 2016). Among diatom taxa, *Pseudonitzschia* species were also observed at C578 at relatively low abundances (Table 3). Many species in this genus are capable of producing domoic acid (amnesiac shellfish poison), which represents a neurotoxic risk for human health through consumption of contaminated bivalves (Landsberg 2002, Lassus et al. 2016). In addition, A number of taxa of *Chaetoceros* were also observed at C578. Many *Chaetoceros* species have spins, some of which have been identified as problematic for fish at high densities in terms of physical damage to gills (Haigh 2010, Horner et al. 1991). Implications for bivalve species are not well defined.

HAB species from additional taxonomic groups were observed at C578 at relatively low abundances (Table 3), the haptophyte *Chrysochromulina* and the raphidophyte *Chattonella*, both of which are known to have species capable of producing ichthyotoxic substances that may also affect bivalve health (Lassus et al. 2016).

Site J737

At Site J737, the most commonly observed species on the Top-50 list were picoplanktonic cyanobacteria (spherical taxa and *Synechococcus* spp.), the diatom *Skeletonema costatum*, and undefined nanoplanktonic eukaryotes(Table 2). The results of this study do not

provide specific evidence that taxa within these groups contain species harmful to bivalves in the study regions (Table 2). The only HAB species that were observed at moderate levels of biomass in the Top-50 list were the dinoflagellates *Prorocentrum minimum* and *Peridinium quadridentatum*. As discussed above, many species of *Prorocentrum* have been linked to the production of the toxin okadaic acid (DSP) and have been linked to issues with bivalve health (Landsberg 2002, Lassus et al. 2016). As discussed for Site C578, *P. quadridentatum* is not known to be toxic, but has been associated with ecosystem disruptive conditions during intense bloom conditions (Alkawri et al. 2016, Gárate-Lizárraga and Muñetón-Gómez 2008, Trigueros and Orive 2000), such as the formation of hypoxia conditions.

Among the HAB species that may affect bivalve issues, but were not present in the Top-50 list for Site J737, the most prominent species were two dinoflagellates *Prorocentrum texanum* and *K. veneficum* (Table 3). *P. texanum* has been associated with both direct impacts on bivalve health and accumulation of Okadaic Acid (aka, DSP, Diarrhetic Shellfish Poison), which can impact human health (Landsberg 2002, Lassus et al. 2016). *K veneficum* has been observed to produce the toxin karlotoxin in a number of coastal ecosystems around the world (Lassus et al. 2016, Pace et al. 2012), and has been shown to have lethal, or adverse sublethal effects on a wide range of marine invertebrates, including mussels and scallops (Daugbjerg et al. 2000, Landsberg 2002, Lassus et al. 2016).

Among diatom taxa, *Pseudo-nitzschia* species were also observed at J737, as noted for C578 (Table 3). Many species in this genus are capable of producing domoic acid (amnesiac shellfish poison), which represents a neurotoxic risk for human health through consumption of contaminated bivalves (Landsberg 2002, Lassus et al. 2016). In addition, A number of taxa of *Chaetoceros* were also observed at J737. Many *Chaetoceros* species have spins, some of which have been identified as problematic for fish at high densities in terms of physical damage to gills (Haigh 2010, Horner et al. 1991). Implications for bivalve species are not well defined.

HAB species from other taxonomic groups were observed at J737 at low levels (Table 3), including the cyanobacterium *Trichodesmium erythraeum*, haptophyte *Chrysochromulina* and the raphidophyte *Chattonella*, all of which are known to have species capable of producing ichthyotoxic substances that may also affect issues involving bivalves (Lassus et al. 2016).

Site O613

At Site O613, the most commonly observed species on the Top-50 list were spherical picoplanktonic cyanobacteria, the HAB dinoflagellate *Akashiwo sanguinea*, the mixotrophic dinoflagellate *Gyrodinium spirale*, and cryptophytes (Table 2). Site O613 was the only site where *A. sanguinea* was observed (Table 3). The results of this study do not provide specific evidence that taxa within these groups are harmful to bivalves in the study regions (Table 2). Three other HAB species in the Top-50 list were observed at moderate levels of biomass, including the dinoflagellates *Peridinium quadridentatum*, *Karlodinium veneficum*, and *Prorocentrum rhathymum*.

A. sanguinea is cosmopolitan in distribution, and has been observed to form blooms in coastal ecosystems around the world (Badylak et al. 2014a, Hallegraeff 2003, Horner et al. 1997, Lassus et al. 2016), including the Gulf of Mexico and the Atlantic coast of Florida (Quinlan and Phlips 2007, Badylak et al. 2014a, Hart et al. 2015, Mathews et al. 2016, Phlips et al. 2010, 2012, 2021, 2023,). *A. sanguinea* plays a major role in the ecology of many marine environments, including coastal ecosystems with variable salinities, where its euryhaline character makes it competitive (Badylak et al. 2014, Matsubara et al. 2007). While *A. sanguinea* has not been reported to be toxic, blooms of the species have been associated with mass mortalities of invertebrates and fish in various regions of the world (Bricelj et al. 1992, Cardwell et al. 1979, Harper and Gullen 1989, Kahru et al. 2004, Landsberg 2002, Lassus et al. 2016, Schumway 1990). One of the harmful impacts of intense *A. sanguinea* blooms is the potential for the development of hypoxic conditions (Hallegraeff 2003). *A. sanguinea* is also known to produce large quantities extracellular carbohydrate polymer (Badylak et al. 2014b), that can be ecosystem disruptive, including impacts on benthic and pelagic grazer populations (Galimany et al. 2020, Gobler et al. 2013, Smayda 2008, Sunda et al. 2006).

As discussed earlier, *P. quadridentatum* is not known to be toxic, but has been associated with ecosystem disruptive conditions during intense bloom conditions (Alkawri et al. 2016,Gárate-Lizárraga and Muñetón-Gómez 2008, Trigueros and Orive 2000), such as the formation of hypoxia conditions. By contrast, *K. veneficum* has been observed to produce the toxin karlotoxin in a number of coastal ecosystems around the world (Lassus et al. 2016, Pace et al. 2012), and has been shown to have lethal and adverse or sublethal effects on a wide range of marine invertebrates, including mussels and scallops (Daugbjerg et al. 2000, Landsberg 2002,

Lassus et al. 2016). Similarly, *P. rhathymum* has been linked to the production of the toxin okadaic acid (aka, DSP, Diarrhetic Shellfish Poison), which has also been linked to issues with bivalve health (Landsberg 2002, Lassus et al. 2016).

Among the HAB species that may affect bivalve issues, but were not present in the Top-50 list for Site O613, the most prominent taxa were the dinoflagellates *Prorocentrum texanum* and *Heterocapsa* (Table 3). *P. texanum* has been associated with both direct impacts on bivalve health and accumulation of Okadaic Acid (aka, DSP, Diarrhetic Shellfish Poison) that can impact human health in association with consumption of contaminated shellfish (Landsberg 2002, Lassus et al. 2016). A number of species in the genus *Heterocapsa* have been associated with the production of hemolytic toxins in coastal ecosystems around the world (Lassus et al. 2016).

Among diatom taxa, *Pseudo-nitzschia* species were also observed at O613 (Table 3). As discussed above, many species in this genus are capable of producing domoic acid (amnesiac shellfish poison), which represents a neurotoxic risk for human health through consumption of contaminated bivalves (Landsberg 2002, Lassus et al. 2016). In addition, A number of taxa of *Chaetoceros* were also observed at O613. Many *Chaetoceros* species have spins, some of which have been identified as problematic for fish at high densities in terms of physical damage to gills (Haigh 2010, Horner et al. 1997). Implications for bivalve species are not well defined.

The HAB haptophyte *Chrysochromulina* species was observed at O613 at low levels (Table 3). *Chrysochromulina* has been shown to production ichthyotoxic substances that may affect issues involving bivalves (Lassus et al. 2016).

Two non-HAB phytoplankton taxa were observed at significant levels of biomass at Site O613, the diatom *Skeletonema costatum* and the dinoflagellate *Tripos hircus* (Fig. 1). A high percent contribution of diatoms to biomass is generally considered a positive feature of coastal food webs (Wasmund et al. 2017), with the possible exception of certain HAB species, such as *Pseudo-nitzschia* species that produce the neurotoxin domoic acid (ASP-Amnesiac Shellfish Poison) (Badylak et al. 2006, Bates et al. 2018) (Table 3), which threaten bivalve production systems in terms of human and aquatic animal health issues (Bates et al. 2018, Landsberg 2002).

Site T383

At Site T383, the most commonly observed species on the Top-50 list were spherical picoplanktonic cyanobacteria, undefined nanoplanktonic eukaryotes, and cryptophytes. The

results of this study do not provide specific evidence that taxa within these groups contain species harmful to bivalves in the study regions (Table 2). The only HAB specie that was observed at moderate levels of biomass in the Top-50 list was the dinoflagellate *Karlodinium veneficum* (Table 2, Fig. 1). As noted for the other sampling sites, *K. veneficum* has been observed to produce the toxin karlotoxin in a number of coastal ecosystems around the world (Lassus et al. 2016, Pace et al. 2012), and has been shown to have lethal and adverse or sublethal effects on a wide range of marine invertebrates, including mussels and scallops (Daugbjerg et al. 2000, Landsberg 2002, Lassus et al. 2016). Two other HAB species were on the Top-50 list at abundances and biomass, *Pseudo-nitzschia* sp. and *Peridinium quadridentatum*. Of the two, the latter is more noteworthy because of the potential bioaccumulation of the toxin domoic acid in bivalves, which is a human and animal health risk (see previous discussion sections for additional detail). Overall, Site T383, had lower biomass peaks than the other four sites in the study (Tables 2, Fig. 1).

Among the HAB species that may affect bivalve issues, but were not present in the Top-50 list for Site T383, the most prominent taxa were the dinoflagellates *P. texanum, P. minimum* and *Heterocapsa* (Table 3). Both *Prorocentrum* species have been associated with direct impacts on bivalve health and accumulation of Okadaic Acid (aka, DSP, Diarrhetic Shellfish Poison) that can impact human health (Landsberg 2002, Lassus et al. 2016). A number of species in the genus *Heterocapsa* have been associated with the production of hemolytic toxins in coastal ecosystems around the world (Lassus et al. 2016).

Among diatom taxa, *Pseudo-nitzschia* species were also observed at T383 (Table 3). As discussed above, many species in this genus are capable of producing domoic acid (amnesiac shellfish poison), which represents a neurotoxic risk for human health through consumption of contaminated shellfish (Landsberg 2002, Lassus et al. 2016). In addition, A number of taxa of *Chaetoceros* were also observed at T383. Many *Chaetoceros* species have spins, some of which have been identified as problematic for fish at high densities in terms of physical damage to gills (Haigh 2010, Horner et al. 1991). Implications for bivalve species are not well defined.

The HAB haptophyte *Chrysochromulina* species was observed at O613 at low levels (Table 3). *Chrysochromulina* has been shown to production ichthyotoxic substances that may affect issues involving bivalves (Lassus et al. 2016).

Site W329

At Site W329, the most commonly observed species on the Top-50 list were spherical picoplanktonic cyanobacteria, undefined nanoplanktonic eukaryotes, and a number of diatom taxa, including *Skeletonema costatum*, *Pleurosigma/Gyrosigma* sp., *Amphora/Entomoneis* sp. and *Odontella sinensis* (Table 2, Fig. 1). The results of this study do not provide specific evidence that taxa within these groups contain species harmful to bivalves in the study regions (Table 2). The only HAB specie that was observed at moderate levels of biomass in the Top-50 list was the dinoflagellate *Prorocentrum minimum* (Table 2, Fig. 1). As mentioned in discussions of previous sites, a number of species in this genus have been associated with both direct impacts on bivalve health and accumulation of Okadaic Acid (aka, DSP, Diarrhetic Shellfish Poison) that can impact human health (Landsberg 2002, Lassus et al. 2016).

Among the HAB species that may affect bivalve issues, but were not present in the Top-50 list for Site W329, the most prominent species were *Prorocentrum* and *K. veneficum* (Table 3). As detailed for other sites, many *Prorocentrum* species have been associated with both direct impacts on bivalve health and accumulation of Okadaic Acid (aka, DSP, Diarrhetic Shellfish Poison) that can impact human health (Landsberg 2002, Lassus et al. 2016). *K veneficum* has been observed to produce the toxin karlotoxin in a number of coastal ecosystems around the world (Lassus et al. 2016, Pace et al. 2012), and has been shown to have lethal, or adverse sublethal effects on a wide range of marine invertebrates, including mussels and scallops (Daugbjerg et al. 2000, Landsberg 2002, Lassus et al. 2016).

Among diatom taxa, *Pseudo-nitzschia* species were also observed at W329 (Table 3). As detailed above, many species in this genus are capable of producing domoic acid (amnesiac shellfish poison), which represents a neurotoxic risk for human health through consumption of contaminated bivalves (Landsberg 2002, Lassus et al. 2016). In addition, A number of taxa of *Chaetoceros* were also observed at J737. Many *Chaetoceros* species have spins, some of which have been identified as problematic for fish at high densities in terms of physical damage to gills (Haigh 2010, Horner et al. 1997). Implications of bivalve species are not well defined.

The HAB cyanobacterium species *Trichodesmium erythraeum* was also observed at W329 at low levels (Table 3). It has been shown to be capable of producing ichthyotoxic substances that may also affect issues involving bivalve (Lassus et al. 2016).

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Fig. 1. Time series of phytoplankton biomass at the five sampling sites. Biomass levels are divided into four major groups: dinoflagellates (red), diatoms (yellow), cyanobacteria (blue), and all "other" taxa (green). Letters associated with peaks in biomass refer to the dominant taxa.



Am, *Amphora*; Cp, *Cerataulina pelagica*; CR, cryptophytes; E, Euglena; Kv, *Karlodinium veneficum*; N, nanoeukaryote; PC, picocyanobacteria; PD, pennate diatom; Pq, *Peridinium quadridentatum;* Sc, *Skeletonema costatum*.

Figure 1 continued



Am, Amphora; Cp, Cerataulina pelagica; Df, Dactyliosolen fragilissimus; E, Euglena; L, Leptocylindrus; N, nanoeukaryote; PC, picocyanobacteria; PG, Pleurosigma-Gyrosigma; Pq, Peridinium quadridentatum, Sc, Skeletonema costatum

Fig. 1 continued



Ak, Akashiwo sanguinea; Gs, Gyrodinium spirale; Kv, Karlodinium veneficum; L, Leptocylindrus; N, nanoeukaryote; PC, picocyanobacteria; PD, pennate diatom; Pq, Peridinium quadridentatum, Sc, Skeletonema costatum; Th, Tripos hircus.

Fig. 1 continued



CR, cryptophytes; Kv, Karlodinium veneficum; N, nanoeukaryote; PC, Picocyanobacteria

Figure 1 continued



Am, *Amphora*; Cp, *Cerataulina pelagica*; N, nanoeukaryote; Os, *Odentella sinensis*; PC, picocyanobacteria; PG, *Pleurosigma-Gyrosigma*; Pm, *Prorocentrum minimum*; Pr, *Protoperidinium*; Sc, *Skeletonema costatum*

Site	Dino	(std.)	Diatom	(std.)	Cyano	(std.)	Other	(std.)	Total	(std.)
C578	60	(97)	160	(214)	70	(71)	171	(191)	461	(327)
	13%		35%		15%		37%			
J737	33	(81)	146	(136)	50	(45)	65	(64)	295	(213)
	11%		50%		17%		22%			
0613	219	(243)	60	(155)	73	(76)	98	(67)	450	(290)
	49%		13%		16%		22%			
T383	22	(37)	37	(43)	53	(59)	67	(60)	178	(127)
	12%		21%		30%		38%			
W329	52	(89)	195	(220)	66	(70)	92	(50)	404	(247)
	13%		48%		16%		23%			

Table 1. Mean biomass (μ g carbon L⁻¹) by phytoplankton group (Dino – dinoflagellates; Diatoms, Cyano – cyanobacteria, Other taxa) and total. Standard deviations are in parentheses. Percent contribution of each group for each site is shown below the mean values.

Table 2. Top-50 biomass observations of individual taxa for each of the five sites. Columns show frequency of occurrence in the Top-50, range of biomass values for the entries in the Top-40 and the highest cell density observed. Taxa in red are listed as harmful algal bloom (HAB) species by the IOC (Lassus et al. 2016).

	Site C578									
			Biomass	Max.						
		Frequency	Range	Density						
Species	Group	in Top-50	µg C L-1	10 ³ Cells L ⁻¹						
Spherical Picocyanobacteria	Cyanobacteria	10	65-225	1279154						
Nanoplankton (6µ-10µ) UD	Eukaryote	6	75-857	3809						
Nannoplankton (2µ-5µ) UD	Eukaryote	6	67-255	81923						
Cryptophyte spp.	Cryptophyte	5	56-131	932						
Amphora/Entomoneis sp.	Diatom	4	61-204	1721						
<i>Eutreptia</i> sp.	Euglena	3	59-117	363						
Karlodinium veneficum	Dinoflagellate	3	94-195	755						
Peridinium quadridentatum	Dinoflagellate	2	193-385	363						
Skeletonema costatum	Diatom	2	107-285	5985						
Pennate diatom sp.	Diatom	2	131-167	21587						
<i>Euglena</i> spp. (<30µ length)	Euglena	2	116	363						
Odontella sinensis	Diatom	1	857	107						
Cerataulina pelagica	Diatom	1	195	1088						
Scrippsiella trochoidea	Dinoflagellate	1	93	91						
Gymnoid spp. (< 15µ)	Dinoflagellate	1	77	2358						
Synechococcus spp.	Cyanobacteria	1	71	204276						
	Overall biomass range: 56 - 857 μg C L ⁻¹									
Red text - HAB issues with l	Red text - HAB issues with bivalves; Blue text - HAB sp.; Brown - Possible HAB									

	Site J737									
		Frequency	Biomass Range	Max. Density						
Species	Group	in Top-50	µg C L ⁻¹	10 ³ Cells L ⁻¹						
Spherical Picocyanobacteria	Cyanobacteria	9	42-113	642009						
Nannoplankton (2µ-5µ) UD	Eukaryote	8	45-89	28661						
Skeletonema costatum	Diatom	6	41-407	8526						
Synechococcus spp.	Cyanobacteria	6	47-67	194548						
Peridinium quadridentatum	Dinoflagellate	3	193-385	363						
Pleurosigma/Gyrosigma sp.	Diatom	3	81-323	725						
Cerataulina pelagica	Diatom	2	55-263	181						
<i>Eutreptia</i> sp.	Euglena	2	59-235	726						
Prorocentrum minimum	Dinoflagellate	2	46-93	363						
Thalassiosira sp.	Diatom	2	73-89	5260						
Nanoplankton (6µ-10µ) UD	Eukaryote	2	64-75	1270						
Amphora/Entomoneis sp.	Diatom	2	43-47	399						
Leptocylindrus danicus	Diatom	1	79	363						
Leptocylindrus minimus	Diatom	1	75	5079						
Dactyliosolen fragilissimus	Diatom	1	50	464						
	Overall biomass range: 41 - 407 μg C L ⁻¹									
Red text - HAB issues with b	vivalves; Blue text -	HAB sp.; Br	own - Possi	ble HAB						

	Site O613			
		Frequency	Biomass Range	Max. Density
Species	Group	in Top-50	μg C L-1	10 ³ Cells L ⁻¹
Spherical Picocyanobacteria	Cyanobacteria	9	51-215	1219182
Akashiwo sanguinea	Dinoflagellate	6	53-345	40
Gyrodinium spirale	Dinoflagellate	5	66-227	23
Cryptophyte spp.	Cryptophyte	4	67-84	9068
Eutreptia sp.	Euglena	3	59-60	182
Peridinium quadridentatum	Dinoflagellate	2	193-964	907
Skeletonema costatum	Diatom	2	65-346	7256
Tripos hircus	Dinoflagellate	2	89-342	73
Gyrodinium sp.	Dinoflagellate	2	58-115	181
Nannoplankton (2µ-5µ) UD	Eukaryote	2	70-94	30228
Nanoplankton (6µ-10µ) UD	Eukaryote	2	63-64	1088
Karlodinium veneficum	Dinoflagellate	2	47-51	363
Leptocylindrus danicus	Diatom	1	278	1270
Scrippsiella trochoidea	Dinoflagellate	1	187	181
Protoperidinium bipes	Dinoflagellate	1	88	181
Prorocentrum rhathymum	Dinoflagellate	1	87	24
Pyramimonas sp.	Chlorophyte	1	69	1632
Synechococcus spp.	Cyanobacteria	1	68	197793
Rhizosolenia setigera	Diatom	1	65	181
Picoplanktonic eukaryote	Eukaryote	1	53	109424
Cyclotella sp.	Diatom	1	49	2902
	Overall b	iomass range:	47 - 964 μ	g C L ⁻¹
Red text - HAB issues with	bivalves: Blue text -	HAB sp.: Br	own - Possi	ble HAB

	Site T383										
			Biomass	Max.							
		Frequency	Range	Density							
Species	Group	in Top-50	μg C L-1	10 ³ Cells L ⁻¹							
Spherical Picocyanobacteria	Cyanobacteria	15	22-291	1653624							
Cryptophyte spp.	Cryptophyte	7	32-99	10701							
Nannoplankton (2µ-5µ) UD	Eukaryote	6	41-140	44987							
Nanoplankton (6µ-10µ) UD	Eukaryote	5	21-107	1814							
Karlodinium veneficum	Dinoflagellate	4	25-140	544							
Coscinodiscus sp.	Diatom	3	41-94	4							
Amphora/Entomoneis sp.	Diatom	2	21-43	363							
Synechococcus spp.	Cyanobacteria	2	25	72956							
Cyclotella sp.	Diatom	1	42	725							
Pseudo-nitzschia sp.	Diatom	1	30	2176							
Peridinium quadridentatum	Dinoflagellate	1	28	26							
Gymnoid spp. (< 15µ)	Dinoflagellate	1	24	725							
Thalassionema bacillare	Diatom	1	23	181							
Heterocapsa niei	Dinoflagellate	1	16	181							
	Overall biomass range: 16 - 291 μg C L ⁻¹										
Red text - HAB issues with b	ivalves; Blue text -	HAB sp.; Bro	own - Possi	ble HAB							

	Site W329										
		Frequency	Biomass Range	Max. Density							
Species	Group	in Top-50	µg C L ⁻¹	10 ³ Cells L ⁻¹							
Spherical Picocyanobacteria	Cyanobacteria	8	66-285	1619614							
Nannoplankton (2µ-5µ) UD	Eukaryote	6	65-148	47527							
Skeletonema costatum	Diatom	5	64-132	9250							
Pleurosigma/Gyrosigma sp.	Diatom	4	81-243	544							
Amphora/Entomoneis sp.	Diatom	4	80-193	1632							
Odontella sinensis	Diatom	3	80-400	50							
Leptocylindrus danicus	Diatom	3	79-119	544							
Nanoplankton (6µ-10µ) UD	Eukaryote	3	64-96	1632							
Cerataulina pelagica	Diatom	2	263-526	363							
Prorocentrum minimum	Dinoflagellate	2	139-417	1632							
Protoperidinium sp.	Dinoflagellate	2	97-139	259							
Guinardia striata	Diatom	2	70-94	70							
Synechococcus spp.	Cyanobacteria	2	62-66	189684							
Peridinium quadridentatum	Dinoflagellate	1	193	181							
Protoperidinium leonis	Dinoflagellate	1	150	24							
Protoperidinium steinii	Dinoflagellate	1	122	181							
Cryptophyte spp.	Cryptophyte	1	75	8161							
	Overall biomass range: 62 - 526 μg C L ⁻¹										
Red text - HAB issues with b	vivalves; Blue text -	HAB sp.; Bro	own - Possi	ble HAB							

ncludes number of times observed, biomass range for each taxa, highest cell density, the toxin or known HAB taxa. Taxa in purple have physical features that can be associated with HAB effects. should be considered potential threats. It should be noted that any HAB species that reaches very high biomass levels can be associated with the potential for the development of hypoxia (Fang et Table 3. Complete list of HAB species observed during the study at the five sampling sites. List confirmed HAB species known to be associated with bivalve issues. The taxa in blue are other Taxa in black are not sufficiently defined to allow for evaluation of specific HAB threats, but HAB factor associated with each taxa and the effects of the HAB factor. Taxa in red are al. 2019)

		Reference	20, 21	20, 21	20, 21	20, 21	9, 13, 16	3,6	10	10, 11, 15, 22	10, 11, 15, 22	10, 11, 15, 22	20, 21	20, 21	14	2	1, 5, 12	10	10	20, 21	10, 11, 15, 22	10
		Effects	Diarrhetic Shellfish Poison	Diarrhetic Shellfish Poison	Diarrhetic Shellfish Poison	Diarrhetic Shellfish Poison	Hemolysis	Neurotoxins	Neurotoxins	Hemolysis, physical disruption	Hemolysis, physical disruption	Hemolysis, physical disruption	Diarrhetic Shellfish Poison	Diarrhetic Shellfish Poison	Physical Disruption/Low O ₂	Physical Disruption/Low O ₂	Neurotoxin, Ichthyotoxic	Neurotoxins	Neurotoxins	DSP	Hemolysis, physical disruption	Neurotoxins
		Toxin and/or other HAB factor	Okadaic acid/hepatotoxin	Okadaic acid/hepatotoxin	Okadaic acid/hepatotoxin	Okadaic acid/hepatotoxin	Cytotoxins	e.g. Saxitoxin	e.g. Saxitoxin	Hemolytic toxin, mucos	Hemolytic toxin, mucos	Hemolytic toxin, mucos	Okadaic acid/hepatotoxin	Okadaic acid/hepatotoxin	High biomass	High biomass, mucos	Karlotoxin	e.g. Saxitoxin	e.g. Saxitoxin	Okadaic acid/hepatotoxin	Hemolytic toxin, mucos	e.g. Saxitoxin
		Where Observed	C578, J737, O613, T383, W329	C578, J737, T383, W329	0613	O613, T383, W329	C578, O613	C578, O613	W329	0613	0613	T383	C578	0613	C578, J737, O613, T383, W329	0613	C578, J737, O613, T383, W329	C578, J737, O613, T383, W329	C578, J737, O613, T383, W329	J737 O613	J737, W329	O613
Highest	Density	10 ³ Cells/ml	0.2	1632	24	1	52	0.1	182	180	90.7	181	30	0.4	907	40	755	2358	583	34	181	0.2
Biomass	Range	µg С.L ⁻¹	0.7-7	0.3-417	0.7-87	0.7-5	0.7-31	0.1 - 0.7	57	27	26	16	6	1.4	0.2-964	2-345	9-195	0.3-77	0.1-49	0.3-48	1.6-5	0.1
	#	Observ.	24	12	10	S	7	7	1	1	1	1	1	1	22	17	18	99	56	14	7	1
		Dinoflagellate Species	Prorocentrum texanum var cuspidatum	Prorocentrum minimum	Prorocentrum rhathymum	Prorocentrum sp.	Karenia mikimoto	Alexandrium sp.	Takayama sp. (<30µ)	Heterocapsa pygmaea	Heterocapsa triqueta	Heterocapsa niei	Prorocentrum balticum	Prorocentrum lima	Peridinium quadridentatum	Akashiwo sanguinea	Karlodinium veneficum	Gymnoid spp. (< 15μ)	Gynmoid spp. (15-25µ)	Gymnoid s pp.(50-75µ)	Heterocapsa sp.	<i>Karenia sp.</i> (<30μ length)

1993, 13. Roberts et al. 1983, 14. Rodriguez-Gomez et al. 2019, 15. Saburova et al. 2022, 16. Trainer et al. 2010, 17. I. Adolf et al. 2007, 2. Badylak et al. 2017, 3. Bass et al. 1983, 4. Buck et al. 1992, 5. Haigh 2010, 6. Hansen et al. Furner et al. 1984, 18. Underdal 1981, 19. Villac et al. 1993, 20. Wickfors and Smolowitz 1993, 21. Wickfors and 1992, 7. Hallegraeff 1991, 8. Horner et al 1991, 9. Jenkinson and Arzul 1998, 10. Lassus et al. 2016, 12. Nielsen Smolowitz 1995, 22. Wu et al. 2022

		eference	16, 19	16, 19	8	8	8	8	8	8	8		0		18		0
		Effects R	Amnesiac Shellfish Poison 3,	Amnesiac Shellfish Poison 3,	Physical Disruption 5,	Physical Disruption 5,	Physical Disruption 5,	Physical Disruption 5,	Physical Disruption 5,	Physical Disruption 5,	Physical Disruption 5,		Neurotoxin (PSP), hepatotoxin 10		Hemolysis 7,	Neurotoxin/Ichthyotoxic 10	Physical Disruption/Low O, 10
	and the second se	Toxin and/or other HAB factor	Domoic acid	Domoic acid	Spines	Spines	Spines	Spines	Spines	Spines	Spines		Frichotoxin, microcystin, ciguatoxin		Ichthyotoxin	NSP/High biomass	High biomass, mucos
		Where Observed	C578, J737, O613, T383, W329	0613	C578, J737, O613, T383, W329	C578, J737, O613, T383, W329	C578, O613, T383	0613	J737, T383	0613	0613		J737, W329		C578, J737, O613, T383	C578, J737	J737
Highest	Density	Cells/ml	2176.4	725.6	362.73	11609.6	7.6	544.2	181.4	1088.4	181.4		0.4		207.2	27.3	725.6
Biomass	Range		<0.1-30	v.	<0.1-21	0.1-24	<0.1-1	1.2-3.6	0.2-1	42	18		2-4		0.1-1.5	0.6-1.7	5
	#	Observ.	30	7	17	14	3	2	2	1	1		4		s	2	1
	and the second sec	Diatom Species	Pseudo-nitzschia (delicat. complex)	Pseudo-nitzschia cf. turgidula	Chaetoceros (10µ cell diameter)	Chaetoceros (5µ cell diameter)	Chaetoceros (20µ cell diameter)	Chaetoceros subtillis	Chaetoceros tenuissimus	Chaetoceros diadema	Chaetoceros costatus	Cvanohacteria Snecies	Trichodesnium erythraeum	Other Species	Chrysochromulina sp.	Chattonella sp.	Aureoumbra lagunensis

continued	
Table 3	

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Physical Disruption/Low O₂

High biomass, mucos

J737

725.6

5

Aureoumbra lagunensis