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Characterization of common phytoplankton on the Louisiana shelf

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ABSTRACT

Phytoplankton and accompanying environmental data (temperature, salinity, secchi depth, stratification, and inorganic nutrients) were analyzed from 672 surface water samples (0 to 1.5 m depth) collected from 95 stations located on the Louisiana shelf between April 1990 and August 2011. Phytoplankton were identified to the lowest practical taxonomic unit from glutaraldehyde-preserved samples using epifluorescent microscopy and reported as cells L^{-1}. Twenty-six phytoplankton taxa (primarily diatoms) that were > 8 μm in size, identified to genus-level resolution and ranked in the top 20 in at least one of three separate categories (average abundance; frequency of occurrence; and bloom frequency) were used in subsequent analyses. Temperature, stratification, and secchi depth constituted the environmental variable combination best related to the phytoplankton community composition patterns across the 672 samples ($r = 0.288$; $p < 0.01$) according to BEST analysis (PRIMER 7). The environmental optima of the 26 taxa were calculated using the weighted-averaging algorithm in the C2 program and then used to group the taxa into common phytoplankton clusters (i.e., niches) using PRIMER 7 CLUSTER. The phytoplankton clustered into three groups: Group A (summer assemblage), Group B (winter assemblage), and Group C (spring bloom assemblage). The results demonstrate that the composition of the phytoplankton community is most related to seasonality and physical variables, whereas nutrients appear to play a larger role in driving overall phytoplankton biomass. This study provides a platform to examine phytoplankton responses to future environmental perturbations in the region.

1. Introduction

The Mississippi River is the sixth largest river in the world in terms of freshwater discharge (Milliman and Meade, 1983), supplying 80% of the dissolved inorganic nitrogen to the northern Gulf of Mexico (Xue et al., 2013), a product of draining over 40% of the conterminous United States, particularly the intensive agricultural lands of the Midwest. The Atchafalaya River carries 30% of the river flow for the last ~140 miles from south central Louisiana to the Gulf of Mexico, approximately 120 miles to the west of the birdsfoot delta. The shelf waters of Louisiana are highly productive (~300 g C m^{-2} yr^{-1}; Sklar and Turner, 1981; Lohrenz et al., 1990), and frequently exhibit elevated concentrations of chlorophyll (>10 mg m^{-3}; Rabalais et al., 1998, Walker and Rabalais, 2006). Nutrient inputs from the Mississippi River drive this primary production (Riley, 1937; Biggs and Sanchez, 1997; Lohrenz et al., 2008; D'Sa, 2014), which in turn supports a productive fishery, including the second largest U.S. fishery by weight (mainly Gulf menhaden, *Brevoortia patronus*), and the fifth largest by value ($300–400 million per year; due primarily to the harvest of penaeid shrimps; de Mutsert et al., 2008). This high phytoplankton productivity has had negative consequences, however, including chronic hypoxia of bottom waters on the shelf (Rabalais and Turner, 2019) and frequent harmful algal blooms (HABs), particularly of the diatom, *Pseudo-nitzschia* (Parsons et al., 2013; Bargu et al., 2016). Additionally, the high nitrate inputs of the Mississippi River can lead to secondary nutrient limitation, particularly silica (Dortch et al., 2001) and phosphate (Sylvan et al., 2006; Quigg et al., 2011; Turner and Rabalais, 2013), the former of which causes a trophic cascade in the reduction in diatom biomass and a subsequent reduction in copepod biomass (Turner et al., 1998). Fluctuations in phytoplankton biomass and composition, therefore, can have multiple outcomes meriting the

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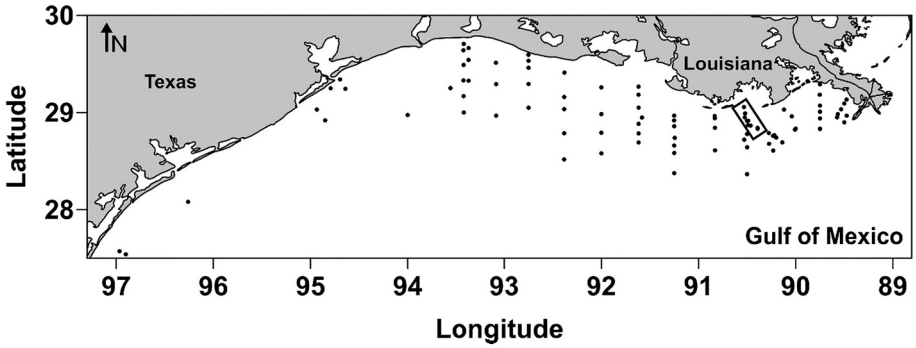

Fig. 1. Map of the 95 stations sampled on the Louisiana – Texas Shelf from 1990 to 2011 used in this study. The rectangle depicts stations C1-C9 of the C-transect, from which the majority of samples analyzed in this study were collected (Table S1).

need to study the population dynamics of this community influenced by the Mississippi River.

Numerous studies have focused on the influence of Mississippi River discharges on primary production on the Louisiana shelf, particularly in regards to nutrients, light attenuation and salinity (Sklar and Turner, 1981; Lohrenz et al., 1997; Lehrter et al., 2009; Quigg et al., 2011). For example, Sklar and Turner (1981) reported that primary productivity peaked when river flow was high and was lowest when river flow was at a minimum. Lohrenz et al. (1997) found that primary productivity was significantly correlated with nitrate-nitrite (NO_x) concentrations. Light was found to be limiting closer to the river delta (Lohrenz et al., 1999; D'Sa and Miller, 2003; Lehrter et al., 2009), creating a confounding scenario where light may be limiting at lower salinities and nutrients may be limiting at higher salinities. Data support this scenario as highest productivity rates are often found at intermediate (~25) salinities (Lohrenz et al., 1990; Quigg et al., 2011; Guo et al., 2012). Mixing and advection are also important drivers of primary production, particularly as the plume waters mix with higher salinity, sub-surface waters on the shelf (Lohrenz et al., 1999). The importance of such physical processes is further evidenced by seasonal wind and current patterns. Generally, the Louisiana coastal current (and river plume) flow westward along the shelf (Wiseman et al., 1997). During the summer, however, southeast winds cause the coastal current to reverse and flow eastwards (Ohlmann and Niiler, 2005) and plume waters are retained on the shelf (Guo et al., 2012). This retention can increase residence times and result in higher primary production rates in the summer when temperatures (and metabolic rates) are also higher (Redalje et al., 1994).

In addition to over-arching studies of phytoplankton responses to river discharges and other drivers, other studies have looked deeper into phytoplankton dynamics by examining community-level responses to river inputs and physical processes on the shelf. Spatial variability, for example, was documented by Chakraborty et al. (2017) who observed that diatoms dominated waters closer to shore, whereas cyanobacteria and prochlorophytes dominated offshore. Haptophyte abundance also increased as one moved offshore. Similarly, Liu et al. (2021) found that microphytoplankton (>20 μm) dominated estuarine and nearshore waters whereas picoplankton (<2 μm) dominated offshore. Temporally, Zhao and Quigg (2015) observed that diatoms dominated in April and cyanobacteria dominated in August. Temperature and nutrient availability were thought to be the factors driving this difference. Green et al. (2008) modeled primary production in the Mississippi River plume and found that small phytoplankton (cyanobacteria, flagellates and dinoflagellates) accounted for 80% of the primary production at salinities >15, whereas the removal (sedimentation) of phytoplankton biomass was primarily a function of diatom sinking, grazing and mortality.

Taxonomy-based studies have generally lagged behind those utilizing productivity measurements, although ecologists acknowledge that phytoplankton species composition can significantly impact ecosystem structure and function (Wood and Leatham, 1992). On the Louisiana shelf, two such examples include harmful algal blooms and sedimentation. For example, *Pseudo-nitzschia* spp. tend to bloom in shelf waters in early spring (Bargu et al., 2016) before zooplankton grazing becomes well established (Dagg, 1995). River-borne nutrients are thought to fuel these blooms (Dortch et al., 1997; Parsons et al., 2002). While sinking diatoms have been shown to be an important removal process of phytoplankton from the water column (Dortch et al., 2001; Green et al., 2008), some diatom taxa contribute more to sinking (e.g., *Skeletonema costatum* and *Thalassiosira rotula*) than others (e.g., *Rhizosolenia fragilissima* and *Cerataulina pelagica*; Fahnenstiel et al., 1995), demonstrating the need to better understand which taxa are contributing to sedimentation (either via directly sinking or through zooplankton egestion).

In order for a phytoplankton taxon to bloom, it must outcompete other taxa for resources or better resist grazing pressures from herbivores. Resource competition is a central dogma of ecological theory, including Hardin's (1960) principle of competitive exclusion. In general, phytoplankton blooms are rare events; taxa typically co-exist in a water body, all competing for the same resources. This observation led Hutchinson (1961) to propose the "Paradox of the Plankton", which points out that such coexistence is counter-intuitive and counter to Hardin's (1960) principle of competitive exclusion (Reynolds, 2006). Subsequent studies have explored factors that might help explain phytoplankton coexistence, including top-down controls (e.g., Paine, 1966; Brun et al., 2015), functional groups (Litchman et al., 2007), and niche differentiation (Irwin et al., 2012). Hutchinson himself offered that spatiotemporal variability in factors that drive the outcome of competition is responsible for the encountered diversity of the phytoplankton community (Hutchinson, 1961). Here, we focus our efforts on defining and differentiating the environmental conditions most favorable for the common phytoplankton found in Louisiana shelf waters. Ultimately, this effort will provide a platform to examine how the phytoplankton community will respond to changing environmental conditions, including perturbations (e.g., climate change, Mississippi River droughts or floods, future oil spills). We compiled data on surface sampling of phytoplankton and associated environmental data on the Louisiana shelf. These data were analyzed to determine the phytoplankton taxa common in the shelf waters and the environmental conditions within which these taxa most commonly occurred.

2. Methods

2.1. Sample collection and preparation

Surface water samples (0 to 1.5 m depth) were collected at 95 stations on the Louisiana shelf (Fig. 1) from April 16, 1990 to August 23, 2011. Station information and characteristics are provided in Table S1. Eighty-six of these stations were in Louisiana waters proper, whereas nine stations were to the west in Texas waters. Most of these stations (59) were sampled only once over this time period, whereas 11 stations

were sampled over 10 times. Most samples (472) were collected on the "C-transect", the primary focus of hypoxia studies in the region (Rabalais and Turner, 2019). Ranges of sampling dates, and the maximum/minimum values of the environmental parameters are also provided in Table S1. Water samples were prepared for microscopy according to Dortch et al. (1997). Water samples (typically 125 mL HDPE bottles) were preserved in 0.5% glutaraldehyde and refrigerated for at least 1 h (up to several months) prior to filtering and mounting. Aliquots were subsequently size-fractionated through 25 mm diameter polycarbonate 0.2, 3, and 8 μm pore-sized filters (0.1 to 25 mL was filtered, depending on particle density in sample), the latter two fractions of which were stained with 0.03% proflavine hemisulfate. Filters were then mounted onto a microscope slide using immersion oil in preparation for microscopic analysis and stored in the freezer (−20 °C) until examination. Slides were generally counted within months of collection, although many later samples (particularly from 2008 to 2010) were not filtered nor examined until 2013–2015 due to funding constraints. A comparison of counts on slides made on samples filtered and mounted within 1 year of collection versus samples filtered and mounted >2 years after collection demonstrated that larger cells were still well-preserved and easily identifiable. There was, however, a noticeable loss of fluorescence in smaller (<10 μm) flagellates and cyanobacteria that made their identification and enumeration more difficult (W. Morrison, pers. obs.). The use of these data is therefore limited. As presented below in the results, however, the affected taxa were not included in the subsequent multivariate analyses and this limitation had no bearing on the findings of this study.

Slides were examined on an Olympus BH2-RFCA epifluorescence microscope equipped with blue and green excitation light, as well as transmitted light if necessary. All cells were identified to the lowest practical taxonomic unit and enumerated. While seven taxonomists were responsible for phytoplankton analyses between 1990 and 2011, W. Morrison (2000 to 2015) and M. Parsons (1996 to 1999) conducted the majority of the counts used in analysis. Consistency in identifications and counts were ensured by having departing taxonomists train incoming taxonomists and by reference to an extensive identification logbook complete with descriptions and photographs maintained since 1990. In cases where identifications could not be made to the genus-level (e.g., small (< 10 μm diameter) *Cyclotella* species versus other small centric species), a broader classification was adopted (e.g., centric diatom <10 μm diameter) to ensure consistency. The abundance of phytoplankton cells (cells L^{-1}) was calculated based on the number of fields counted per filter and volume of water filtered. Encountered phytoplankton were also grouped and summed at higher taxonomic groupings (e.g., diatoms, dinoflagellates, etc.) for overall description and comparison with other studies in the region.

2.2. Environmental variables

The environmental variables used in analysis included temperature, salinity, inorganic nutrients (nitrate+nitrite (NO_x), ammonium, silicate, and phosphate), secchi depth, and seawater density. Temperature, salinity, and density were measured at each site using a Seabird 911 + CTD system, a Hydrolab Surveyor 3, or a YSI 6820. A stratification index was calculated by subtracting surface water density values from bottom water density values (hereafter called stratification). The concentrations of inorganic nutrients were determined using either a Technicon Auto-Analyzer II or an Alpkem RFA/2 Rapid Flow Analyzer and were reported in μM units. Dissolved inorganic nitrogen (DIN) was computed as a sum of NO_x and ammonium and was reported in μM units. An f-ratio (ratio of new nitrogen to DIN) was computed as NO_x /DIN. Several nutrient rations (N:P, Si:N, and Si:P) were calculated by dividing DIN by phosphate, silicate by DIN, and silicate by phosphate, respectively. Secchi depth (m) was measured using a standard secchi disk. Seasonality was calculated by sine-transforming the day of the year (DOY; 1 through 365) as follows: seasonality = absolute value (Sine(π·(DOY + 11/365)). Values approaching "1" equate to summer (day 172; June 21), whereas values approaching zero equate to winter (day 354; December 21). Overall, a total of 15 environmental variables were measured and/or calculated (temperature, salinity, stratification, NO_x, ammonium, silicate, phosphate, DIN, f-ratio, N:P, Si:N, Si:P, secchi depth, DOY, and seasonality).

2.3. Data analysis

Phytoplankton taxa encountered in the 672 samples were ranked according to three separate criteria: 1) overall average abundance (cells L^{-1}); 2) overall frequency of occurrence (% of samples); and 3) bloom frequency (% of samples of all sampling events selected where cell abundances were $\geq 10^6$ cells L^{-1}). The 20 taxa (identified at least to genus level) with the highest values in each of these categories were selected for further analysis based on these species being common (and representative) to Louisiana coastal waters and/or bloom-forming taxa in the region.

A Bray-Curtis resemblance matrix was derived from the phytoplankton abundance data (log-transformed as ln(cells L^{-1} + 1)) in PRIMER 7 (Clarke and Gorley, 2015). The environmental data were normalized by subtracting the parameter mean from each variable and dividing this value by the standard deviation of that variable. Normalization is recommended to transform the environmental data onto a common scale so that each variable will contribute equally to subsequent analyses (Clarke and Gorley, 2015). A BEST (Bio-Env + Stepwise) analysis was then conducted to determine the best match between sample patterns derived from the phytoplankton data versus the environmental data. Only 99 permutations were done to test for significance due to the large number of samples analyzed (672) which resulted in long computation times (>12 h). Therefore, significance could only be determined down to a p-value ≤1%. Three-dimensional plots (Sigma Plot 13) were generated to examine how the most influential parameters related to each other across samples. Correlation analysis (Pearson and Spearman) was conducted using SPSS 26 to determine if any of the environmental variables were strongly correlated with each other ($r > 0.8$; $p < 0.0001$) which could indicate collinearity and cause redundancy in the analysis. The results were interpreted with this consideration in mind.

Environmental optima (i.e., the parameter value for each environmental variable associated with the highest cell abundance) and tolerance values (i.e., the range of each environmental variable over which the cells occur) for each of the representative phytoplankton taxa were determined using weighted averaging calculations in the program C2 1.7.6 (Juggins, 2014). The optima values were then normalized and used to calculate a new resemblance matrix in PRIMER using a Spearman rank correlation approach. This method was used to capture how well the environmental optima correlated among the taxa. The resulting resemblance matrix was then processed using CLUSTER with group averaging and a SIMPROF analysis (999 permutations) to determine if the resulting cluster groups of phytoplankton taxa were significantly different from each other based on the environmental optima. A SIMPER analysis was then used to examine how the resultant cluster groups differed in terms of environmental optima. Analysis of variance (ANOVA) with post-hoc Tukey Honestly Significant Difference multiple comparisons was also conducted on the environmental optima data to test if there were significant differences ($\alpha = 0.05$) in optima values among the resultant cluster groups using SPSS 26. Lastly, the SIMPER results were compared to the earlier BEST results to determine how the groupings of the phytoplankton taxa by their environmental optima equate with the relationship of the environmental variables on the phytoplankton community as a whole.

Table 1
Overall relative abundance and average absolute abundance (cells L^{-1}) of phytoplankton groups across all 672 samples analyzed in this study.

Group	Relative abundance	Average absolute abundance
Cyanobacteria	88.9%	1.18×10^{10}
Diatom	8.8%	1.17×10^{9}
Phytoflagellate	1.5%	1.93×10^{8}
Dinoflagellate	0.5%	6.27×10^{7}
Cryptomonad	0.2%	3.31×10^{7}
Ciliate	< 0.1%	2.70×10^{6}
Silicoflagellate	< 0.1%	1.74×10^{6}
Coccolithophorid	< 0.1%	1.61×10^{6}
Euglenoid	< 0.1%	8.65×10^{5}
Chlorophyte	< 0.1%	3.72×10^{5}

3. Results

3.1. Sample selection

There were 672 samples that had complete phytoplankton and environmental data (i.e., no missing values; a requirement of PRIMER) and were used in subsequent analysis. The phytoplankton community was dominated numerically by cyanobacteria (89% relative abundance; primarily unicellular taxa such as *Synechococcus*) and diatoms (8.8% relative abundance) (Table 1). Twenty-six phytoplankton taxa met the criteria of being both: 1) identified at least to genus-level; and 2) ranked in the top-twenty taxa in at least one of the three abundance categories (overall average abundance, overall frequency of occurrence, and/or bloom frequency; Table 2). Nineteen taxa were diatoms, four were dinoflagellates (*Heterocapsa rotundata*, *Prorocentrum scutellum/compressum*; *Scrippsiella* spp.; *Torodinium* spp.), two were cyanobacteria (*Anabaena* spp. and *Trichodesmium* spp.), and one was a ciliate (*Mesodinium rubrum*). *Skeletonema costatum* (sensu lato) had the highest overall average abundance (>1×10^{6} cells L^{-1}), followed by *Pseudo-nitzschia* spp., and *Trichodesmium* spp., respectively. *Pseudo-nitzschia* spp. was the most commonly occurring taxa, being present in 81% of the samples examined. *Dactyliosolen fragilissimus* and *Thalassionema nitzschioides* were the next most common, occurring in 74% and 73% of samples, respectively. *Pseudo-nitzschia* spp. was the most common bloom-former (13% of samples), with *Skeletonema costatum* and *Dactyliosolen fragilissimus* being the next most frequent bloomers (at 11% and 6%, respectively).

3.2. Data analysis

Several parameters exhibited collinearity, resulting in the omission of some of them in further analyses (BEST, CLUSTER, SIMPROF, SIMPER, and ANOVA). For example, NO_x^-, DIN, and N:P were significantly correlated ($r > 0.8$; $p < 0.0001$), and NO_x^- was arbitrarily chosen for inclusion in the above analyses. Similarly, temperature and seasonality were significantly correlated ($r > 0.8$; $p < 0.0001$), and temperature was arbitrarily used in the above analyses.

The BEST analysis results indicated that the environmental variable combination that best matched the phytoplankton patterns (based on correlation and parsimony considerations) across the 672 samples was temperature, stratification, and secchi depth ($r = 0.288$; $p < 0.01$). The correlation value went up slightly ($r = 0.289$) when salinity and the f-ratio parameters were also included. Generally speaking, the samples expressing the highest temperatures and salinities also had the deepest secchi depths (Fig. 2a), indicative of summer, low river flow or offshore stations. Stratification tended to be highest at lower salinities and higher temperatures (Fig. 2b), indicative of summer, high river flow or nearshore stations. Interestingly, the deepest secchi depths were associated with mid-range stratification values and high temperature (Fig. 2c), possibly reflecting a lack of a lower-salinity, upper water layer related to river inputs (and hence weaker stratification).

While the environmental optima occupy a narrow range of values across the 26 taxa for some parameters (e.g., < 2-fold for seasonality, salinity and temperature), the range was much greater for other parameters (e.g., up to >17-fold for NO_x and Si:N). These results suggest that the environmental optima were more closely grouped across taxa for physical parameters versus nutrient-based parameters (Table 3). The tolerances exhibit a similar pattern, in which physical parameters account for the three lowest average tolerance ranges (temperature, salinity, and seasonality), whereas nutrient-based parameters occupy

Table 2
List of the 26 phytoplankton taxa that could be identified to genus-level and ranked in the top 20 in at least one of three categories (average abundance, % occurrence, and % occurrence blooming ($\geq 10^6$ cells L^{-1})). A higher-level classification is also provided (Phytoplankton Group). The species abbreviations used in Figs. 3 and 4 are provided, as are the groupings determined by the cluster analysis.

Species	Phytoplankton group	Species abbreviation	Cluster group	Average abundance	% occurrence	% $\geq 10^6$ cells L^{-1}
Anabaena spp.	Cyanobacteria	Ab	A	3.47×10^{4}	10%	1%
Asterionellopsis glacialis	Diatom	Ag	C	6.77×10^{4}	34%	1%
Cerataulina pelagica	Diatom	Cp	A	4.83×10^{4}	38%	1%
Chaetoceros affinis	Diatom	Ca	A	4.79×10^{4}	25%	0%
Chaetoceros compressus	Diatom	Cc	B	3.38×10^{4}	23%	1%
Chaetoceros didymus	Diatom	Cd	B	1.17×10^{4}	26%	0%
Chaetoceros socialis	Diatom	Cs	B	1.94×10^{5}	19%	4%
Chaetoceros subtilis var. *abnormis* f. *simplex*	Diatom	Ch	C	2.19×10^{4}	8%	0%
Dactyliosolen fragilissimus	Diatom	Df	A	3.08×10^{5}	74%	6%
Guinardia delicatula	Diatom	Gd	C	3.55×10^{4}	38%	1%
Guinardia striata	Diatom	Gs	B	1.25×10^{4}	51%	0%
Gyrosigma/Pleurosigma spp.	Diatom	GP	B	1.27×10^{3}	46%	0%
Heterocapsa rotundata	Dinoflagellate	Hr	C	3.47×10^{4}	21%	1%
Leptocylindrus minimus	Diatom	Lm	B	1.48×10^{5}	34%	3%
Leptocylindrus spp.	Diatom	Ls	C	1.06×10^{4}	11%	0%
Mesodinium rubrum	Ciliate	Mr	B	3.88×10^{3}	41%	0%
Proboscia alata	Diatom	Pa	A	1.63×10^{4}	48%	0%
Prorocentrum scutellum/compressum	Dinoflagellate	Ps	A	7.79×10^{3}	69%	0%
Pseudo-nitzschia spp.	Diatom	Pn	A	5.98×10^{5}	81%	13%
Rhizosolenia setigera	Diatom	Rs	A	7.21×10^{3}	57%	0%
Scrippsiella spp.	Dinoflagellate	Ss	C	7.49×10^{3}	35%	0%
Skeletonema costatum	Diatom	Sc	A	1.09×10^{6}	59%	11%
Skeletonema potamos	Diatom	Sp	C	2.68×10^{4}	8%	0%
Thalassionema nitzschioides	Diatom	Tn	A	2.61×10^{4}	73%	0%
Torodinium spp.	Dinoflagellate	To	A	1.75×10^{3}	46%	0%
Trichodesmium spp.	Cyanobacteria	Tr	A	1.39×10^{5}	17%	1%

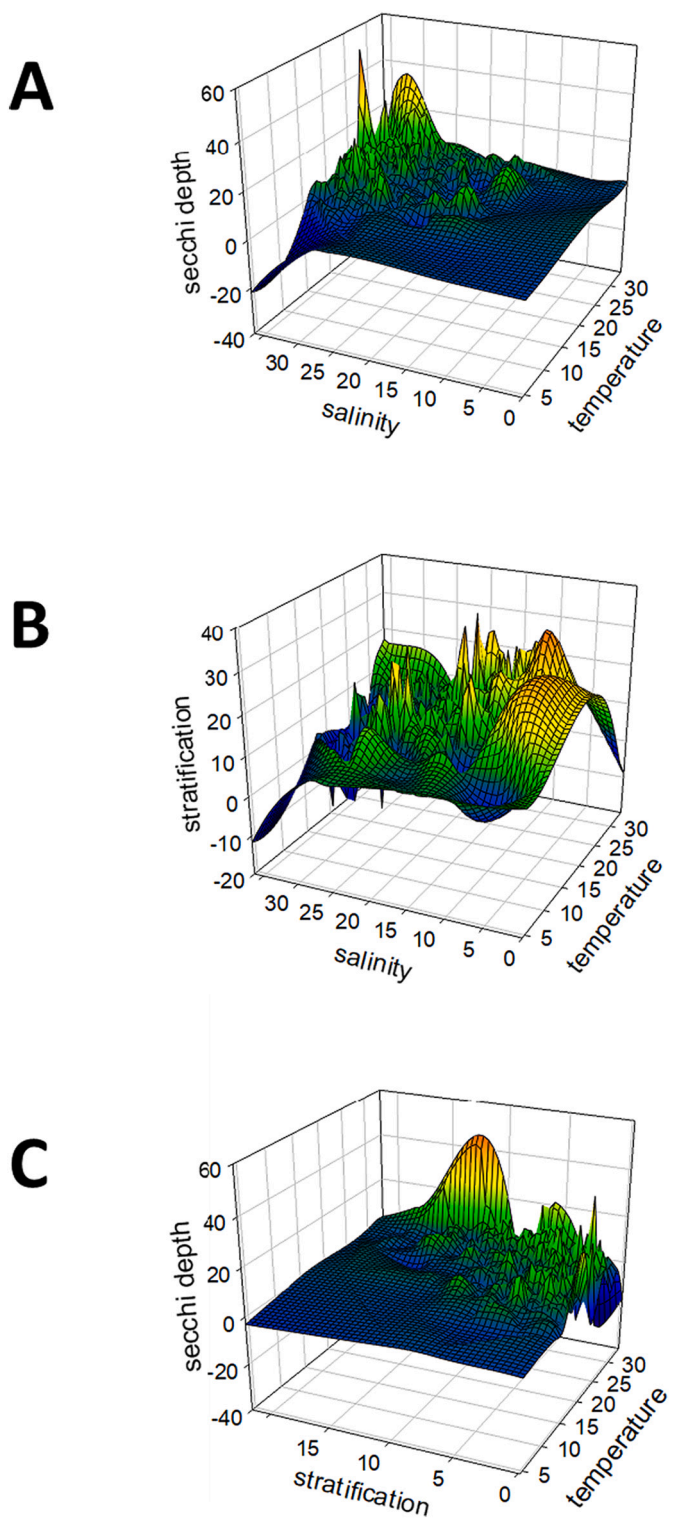

Fig. 2. Three-dimensional plots of the five variables that were most related to phytoplankton assemblage variability in the 672 samples analyzed in this study. A. secchi depth (m), salinity, and temperature (°C); B. stratification, salinity, and temperature; and C. secchi depth, stratification, and temperature.

the three highest averages (phosphate, NO_x, and Si:N; Table 4). *Proboscia alata* had the highest tally of rankings for highest (4) and lowest (4) optima; generally, high optima were recorded for physical parameters (e.g., temperature, salinity, and seasonality), whereas low optima were for nutrient-based parameters (e.g., DIN, NO_x and N:P). *Proboscia alata* also tallied the most ranked tolerance values (5); two wide and three narrow tolerance ranges. *Chaetoceros affinis* also had five ranked tolerance values: one wide and four narrow. Narrow tolerances were generally exhibited for the physical parameters (except temperature), whereas wide tolerance ranges were exhibited for nutrient-based parameters (except phosphate).

Other notable aspects of the optima and tolerance data are that the optima values of *Pseudo-nitzschia* spp. (the most prominent HAB species in Louisiana shelf waters) for Si:N and silica were among the three lowest optima values for each category (Table 3). Interestingly, optima and tolerances were negatively correlated for 9 out of 15 environmental parameters (Table 5), where four nutrient-based parameters (ammonium, phosphate, N:P, and Si:N) and two physical-based parameters (secchi and temperature) did not exhibit a significant relationship. The three cluster groups displayed significant differences in the environmental optima for all but five parameters (stratification, ammonium, phosphate, secchi, and Si:N) (Table 6).

The phytoplankton taxa were classified into three groups according to the CLUSTER and SIMPROF results (Figs. 3 & 4; Tables 2 & 6). The weights of the environmental parameters provided by the SIMPROF analysis indicate how the three groups could be distinguished from each other (Table 6). Group A was characterized by higher optima for temperature, seasonality, stratification, and ammonium than the other groups (Fig. 4; Table 6). Group B was characterized by lower optima for temperature, seasonality, NO_x, and ammonium, and higher optima for salinity and Si:N. Group C was distinguished with higher optima for f-ratio, NO_x, Si:P, and silica, and lower optima for salinity and secchi depth.

The taxa within each group displayed similar optima for some, but not all of the six environmental parameters that distinguished each of the groups (Fig. 4). For example, taxa within Groups A and B had similar optima for seasonality, but Group C was more variable. Group A was best defined by seasonality and Si:P. Group B taxa had similar optima for f-ratio, seasonality, and NO_x. Group C taxa optima were most variable overall, but exhibited the narrowest range for f-ratio.

4. Discussion

The 26 taxa examined in this study are considered to be cosmopolitan species, commonly occurring in coastal waters around the globe. In fact, all of the taxa could be collectively accounted for in as few as five studies ranging from the Adriatic Sea (Caroppo et al., 1999) to the Gulf of Thailand (Boonyapiwat, 1999), San Francisco Bay (Cloern and Dufford, 2005), Chesapeake Bay (Marshall et al., 2006), and New Zealand (Chang, 1988). These taxa have also been reported as common members of the phytoplankton in the northern Gulf of Mexico in previous studies (e.g., Bontempi, 1995; Al-Abdulkader, 1996; Schaeffer et al., 2012; Chakraborty and Lohrenz, 2015, and Bargu et al., 2016).

Although these 26 taxa are cosmopolitan, they have different environmental optima and tolerance levels (Tables 3 and 4), and can be grouped into three separate categories (Fig. 3) with distinct characteristics (Table 6). Group A is composed of taxa with optima typical of summer months: higher temperatures, seasonality values typical of June and July, highly stratified waters, and higher ammonium concentrations that would be expected of post-spring bloom conditions (i.e., summer) when recycled nitrogen would be more abundant (Dortch and Whitledge, 1992). Group B is characterized as a winter group, with lower optima for temperature, seasonality values typical of December and January, lower NO_x and ammonium (i.e., lower river inputs in winter; Turner and Rabalais, 1991) and higher optima for salinity and Si:N (reflecting generally lower river flow and nitrogen inputs then). Group C appears to be a higher flow (and/or nearshore) group with evidence of higher nitrate levels (including the f-ratio) and higher Si:P and SiO_3, expected with higher freshwater inputs.

Overall, the results of this analysis demonstrate that the common phytoplankton present in coastal Louisiana waters appear to be responding to seasonal changes (particularly distinguished for summer

versus winter months) and higher river flow (particularly during the spring and early summer, resulting in higher nutrients, lower salinity, and lower light penetration). These results are at least partially corroborated by the BEST analysis relating the 26 taxa back to the raw environmental values (rather than the optima), in which temperature, stratification, and secchi depth were found to be most related to phytoplankton assemblage variability. Temperature directly relates to seasonal differences, whereas stratification is a function of temperature (particularly in summer) and river flow (establishment of the pycnocline). Secchi depth is a function of season (less light intensity in winter months) and river flow (less light penetration in presence of turbid river discharges). Therefore, the analysis presented herein suggests that the phytoplankton that reside on the Louisiana shelf primarily respond to the changing seasons (particularly temperature) and related river flow (Fig. 2). These findings are in agreement with past studies. The influence of the river has been documented (e.g., Sklar and Turner, 1981; Lohrenz et al., 1997), as have the importance of temperature (e.g., Lohrenz et al., 1994; Dagg et al., 2007; Zhao and Quigg, 2015) and light conditions (e.g., Lohrenz et al., 1990; Lehrter et al., 2009; Quigg et al., 2011) in driving primary production on the shelf.

Putting these results in context, the changing phytoplankton assemblage across the course of a year represents a continuum, in which some taxa become more abundant as conditions become optimal for growth while others become less abundant as those same conditions become less tolerable (Reynolds, 2006). In this study, this scenario may proceed with the resident phytoplankton community (coastal and oceanic) being influenced by temperature and then being exposed to fresher, more nutrient-rich waters of the Mississippi River, which contain a different phytoplankton assemblage. As the river water mass mixes with the coastal (and/or oceanic) water mass, the accompanying phytoplankton must acclimate (tolerate) or perish; freshwater species must acclimate to higher salinities and oceanic species must acclimate to lower salinities (Quigg et al., 2011). The resultant phytoplankton assemblage, therefore, represents an amalgamation of species, potentially including freshwater forms (e.g., *Skeletonema potamos* and *Anabaena* spp.), oceanic species (e.g., *Trichodesmium* spp.), and typical coastal species (e.g., *Skeletonema costatum*). As Keddy (1992) states, the resultant assemblages examined in each sample represent a sub-set of the community that "survived" the twin filters of dispersal and habitat suitability. Additionally, the resultant community will vary over time as the water masses mix, due to seasonal influences (i.e., temperature effect).

While the phytoplankton may exist across a continuum in the scenario described here, it is not an example of coexistence, because conditions (and phytoplankton assemblages) change via the mixing of water masses (Sommer et al., 1993). Instead of random outcomes in response to these mixing processes, the phytoplankton appear to follow relatively predictable outcomes: e.g., spring diatom blooms dominated by *Pseudo-nitzschia* or *Skeletonema* (Dortch et al., 1997) when river flow and temperature changes (seasons) align (i.e., spring flood of the Mississippi River typically peaks in April; Turner and Rabalais, 1991). Therefore, the outcomes indicate the existence of (pseudo)stable ecological groups as described in this study.

Many other studies have identified distinctive phytoplankton groupings (e.g., Reynolds, 2006; Litchman et al., 2007, 2012). Additionally, the importance of physical variables (temperature, light, water

Table 3
Environmental optima of the 26 representative taxa. strat = stratification; DOY = day of year; DIN = dissolved inorganic nitrogen (μM); NO_x^- = nitrate + nitrite (μM); N:P = DIN to phosphate ratio; PO_4^{+3} = phosphate (μM); ppt = salinity; Si:N = silica to DIN ratio; SiO_3 = silica (μM); Si:P = silica to phosphate ratio; and temp = temperature (C). High and low optima values are shaded; low are italicized and high are bolded. The optima were calculated using a weighted-averaging technique for each taxa and environmental parameter across all sampling events (672). The optima, therefore, are independent of other optima within a taxon as well as between taxa.

Species	strat	DOY	seasonality	DIN	f-ratio	NH_4^+	NO_x^-	N:P	PO_4^{-3}	ppt	secchi	Si:N	SiO_3	Si:P	temp
Anabaena spp.	*2.2*	173.3	0.9	10.0	0.6	2.0	8.0	33.7	0.7	20.8	1.6	4.9	19.5	59.1	28.5
Asterionellopsis glacialis	6.7	121.4	0.8	10.5	0.8	1.2	9.3	30.9	0.4	24.7	2.0	1.5	11.7	35.1	21.2
Cerataulina pelagica	6.1	133.7	0.8	8.2	0.6	1.6	6.5	18.6	0.6	24.3	2.2	3.1	12.8	32.7	23.3
Chaetoceros affinis	9.1	153.9	0.9	5.0	0.8	1.0	4.0	24.3	*0.2*	25.0	3.3	1.4	6.5	31.3	27.3
Chaetoceros compressus	5.1	141.0	0.7	7.1	0.7	1.4	5.7	27.7	0.4	26.7	3.6	2.8	8.0	28.3	23.5
Chaetoceros didymus	4.6	86.5	0.6	7.3	0.8	1.1	6.2	39.8	0.3	27.5	2.7	1.4	*4.5*	22.4	20.4
Chaetoceros socialis	4.2	170.1	0.7	6.0	0.6	1.3	4.7	25.8	0.3	26.8	2.2	1.3	7.0	40.5	21.4
Chaetoceros subtilis var. *abnormis* f. *simplex*	5.9	179.5	0.8	19.6	0.8	4.2	15.4	47.7	0.6	21.7	1.5	3.5	26.7	72.7	26.1
Dactyliosolen fragilissimus	6.8	139.0	0.9	8.4	0.6	1.7	6.7	25.6	0.5	23.0	2.0	3.5	8.3	25.3	23.5
Guinardia delicatula	6.4	138.2	0.8	15.8	0.8	1.9	13.9	73.8	0.3	23.8	2.6	2.4	19.0	**97.8**	22.7
Guinardia striata	4.4	188.7	0.7	6.3	0.6	1.8	4.5	24.8	0.6	27.3	3.1	3.4	8.2	31.2	24.9
Gyrosigma/Pleurosigma spp.	3.1	137.4	0.7	7.2	0.6	1.8	5.3	22.4	0.8	27.2	2.1	2.6	11.1	29.3	22.2

Heterocapsa rotundata	6.2	112.7	0.7	19.8	0.8	2.0	17.9	36.1	**0.9**	*19.7*	*1.4*	3.4	33.2	59.1	21.5
Leptocylindrus minimus	4.7	103.6	0.8	7.6	0.7	1.4	6.2	27.4	0.8	25.6	1.8	3.1	13.5	53.4	*19.8*
Leptocylindrus spp.	7.4	143.9	0.9	19.0	0.7	2.2	16.7	56.8	0.5	25.3	2.3	1.8	12.9	42.3	23.3
Mesodinium rubrum	6.4	163.3	0.8	7.3	0.8	1.0	6.2	24.7	0.6	27.0	2.7	**14.0**	15.7	36.3	22.1
Proboscia alata	6.0	195.0	**1.0**	*2.8*	0.4	1.7	*1.1*	*16.3*	0.3	**32.2**	**6.0**	2.0	**38.0**	*14.9*	28.7
Prorocentrum scutellum/compressum	6.6	142.2	0.9	10.6	0.7	2.2	8.4	34.3	0.8	23.1	2.6	2.2	11.6	33.1	24.0
Pseudo-nitzschia spp.	5.8	129.7	0.8	8.5	0.6	2.3	6.3	39.2	0.5	25.8	3.0	1.4	6.0	25.7	22.4
Rhizosolenia setigera	5.3	186.1	0.8	6.0	0.6	2.0	4.0	22.8	0.4	26.0	2.9	3.2	6.2	22.7	26.6
Scrippsiella spp.	5.0	148.2	0.8	17.4	0.7	3.3	14.1	**90.9**	0.5	24.1	3.4	2.3	15.4	65.9	24.0
Skeletonema costatum	**9.7**	156.0	0.9	**23.4**	0.8	4.2	**19.3**	77.6	0.6	20.5	1.7	*0.8*	10.4	24.1	24.7
Skeletonema potamos	3.6	*61.7*	*0.5*	14.6	**0.9**	*0.9*	13.7	38.2	0.8	24.3	1.9	2.2	27.4	47.2	20.0
Thalassionema nitzschioides	7.2	197.9	0.9	12.6	0.6	3.6	8.8	37.7	0.6	23.3	2.4	4.1	10.6	30.6	27.5
Torodinium spp.	8.1	159.9	0.8	16.8	0.7	2.1	14.6	67.0	0.4	24.3	3.5	1.8	4.9	20.8	24.8
Trichodesmium spp.	5.3	**199.6**	0.9	6.6	*0.2*	**4.9**	1.7	22.4	0.3	22.7	5.3	2.0	11.1	37.0	**28.9**
Average	5.9	146.5	0.8	11.1	0.7	2.0	9.1	38.6	0.5	24.8	2.6	3.0	14.0	39.3	23.8
Minimum	2.2	61.7	0.5	2.8	0.4	0.9	1.1	16.3	0.2	19.7	1.4	0.8	4.5	14.9	19.8
Maximum	9.7	197.9	1.0	23.4	0.9	4.2	19.3	90.9	0.9	32.2	6.0	14.0	38.0	97.8	28.7

column stability) in driving phytoplankton succession in coastal waters is well-established (e.g., Margalef, 1978; Smayda, 1980; Lohrenz et al., 1999; D'Sa, 2014). Our results concur; variations in temperature, stratification, and secchi depth (light) were most related to changes in the phytoplankton composition. The role of nutrients, however, was more nuanced and likely a secondary driver affecting phytoplankton composition. This finding is not to say that nutrients are not an important driver for overall phytoplankton productivity (i.e., biomass and production; Lohrenz et al., 1990, Scavia et al., 2003, Quigg et al., 2011), but rather that the composition of the phytoplankton may be more dictated by physical processes; i.e., the 26 taxa used in this study generally responded to nutrient conditions in a similar way (hence, partially explaining why these 26 taxa are "common").

The majority of the taxa examined in our study are diatoms, which are known to respond rapidly to nutrient pulses (Cloern and Dufford, 2005), as expected when riverine waters mix with the coastal shelf waters. High nutrient conditions often coincide with low light conditions, creating a confounding scenario for analysis (Lohrenz et al., 1990). As Litchman et al. (2007) pointed out, many phytoplankton species make compromises related to nutrient utilization; e.g., tolerating lower light conditions in order to access higher nutrient concentrations. Nitrogen and silica were found to be important parameters distinguishing the three phytoplankton groups (as well as Si:P), and previous studies have demonstrated the importance of these nutrients and nutrient limitation in influencing phytoplankton dynamics on the Louisiana shelf (Turner et al., 1998; Lohrenz et al., 1999; Dagg et al., 2007; Quigg et al., 2011). Such limitation may well be significant in dictating when particular phytoplankton bloom (such as *Pseudo-nitzschia* under low Si:N conditions (Dortch et al., 1997); reflected in the low Si:N optima for this species; Table 3). Therefore, while nutrients were not found to overly affect the composition of phytoplankton as analyzed in this study, they are undoubtedly important in specific cases.

Irwin et al. (2012) also reported that species with optima (mean niches) on the periphery of ranges (i.e., high and low ends of the scale) tended to have narrower tolerances. We report a similar phenomenon in this study, where taxa with low optima tended to have larger tolerance ranges and vice versa (Table 5). These findings may indicate that taxa operating at the lower environmental ranges (i.e., lower nutrients, temperature and salinity) are more adaptable to variable conditions versus the more "steno" taxa at the upper ranges.

The results of this study indicate that under typical (average) conditions, the phytoplankton community is anticipated to change in a

Table 4
Environmental tolerances of the 26 representative taxa. strat = stratification; DOY = day of year; DIN = dissolved inorganic nitrogen (μM); NO_x^- = nitrate + nitrite (μM); N:P = DIN to phosphate ratio; PO_4^{+3} = phosphate (μM); ppt = salinity; Si:N = silica to DIN ratio; SiO_3 = silica (μM); Si:P = silica to phosphate ratio; and temp = temperature (C). High and low optima values are shaded; low are italicized and high are bolded.

Species	strat	DOY	seasonality	DIN	f-ratio	NH_4^+	NO_x^-	N:P	PO_4^{-3}	ppt	secchi	Si:N	SiO_3	Si:P	temp
Anabaena spp.	**159%**	23%	13%	146%	50%	76%	177%	231%	164%	29%	96%	137%	63%	103%	12%
Asterionellopsis glacialis	63%	56%	31%	91%	34%	149%	101%	84%	134%	20%	72%	171%	92%	98%	14%
Cerataulina pelagica	63%	41%	17%	124%	48%	138%	145%	131%	99%	18%	71%	125%	95%	99%	21%
Chaetoceros affinis	*42%*	34%	20%	120%	28%	**248%**	119%	120%	118%	*13%*	*43%*	*103%*	100%	103%	18%
Chaetoceros compressus	75%	62%	35%	85%	36%	91%	93%	105%	90%	15%	79%	153%	105%	145%	19%
Chaetoceros didymus	82%	**96%**	**60%**	146%	29%	172%	153%	179%	129%	19%	73%	124%	164%	141%	17%
Chaetoceros socialis	84%	58%	50%	158%	50%	95%	194%	116%	117%	18%	68%	189%	**178%**	**230%**	11%
Chaetoceros subtilis var. *abnormis* f. *simplex*	76%	38%	21%	87%	*22%*	111%	88%	104%	*85%*	21%	**162%**	144%	64%	100%	16%
Dactyliosolen fragilissimus	69%	35%	12%	159%	55%	164%	190%	153%	143%	24%	82%	449%	129%	149%	17%
Guinardia delicatula	75%	45%	19%	86%	30%	120%	99%	*77%*	143%	17%	69%	130%	*50%*	88%	15%
Guinardia striata	82%	47%	40%	123%	46%	160%	154%	140%	187%	15%	70%	240%	95%	142%	17%
Gyrosigma/Pleurosigma spp.	103%	66%	41%	104%	43%	129%	131%	144%	175%	17%	100%	134%	117%	122%	20%
Heterocapsa rotundata	73%	72%	47%	81%	31%	96%	85%	132%	114%	**39%**	107%	168%	60%	107%	**37%**
Leptocylindrus minimus	78%	47%	20%	125%	40%	114%	143%	113%	**273%**	15%	67%	135%	95%	111%	14%
Leptocylindrus spp.	62%	30%	11%	110%	39%	99%	122%	110%	93%	19%	62%	166%	94%	93%	14%
Mesodinium rubrum	83%	53%	37%	180%	28%	184%	196%	226%	98%	28%	89%	105%	85%	125%	22%
Proboscia alata	62%	*13%*	*5%*	**186%**	60%	230%	304%	**251%**	92%	14%	51%	122%	70%	129%	*7%*
Prorocentrum scutellum/compressum	72%	41%	18%	111%	40%	112%	127%	128%	211%	21%	69%	166%	101%	144%	18%
Pseudo-nitzschia spp.	65%	43%	18%	118%	47%	196%	149%	144%	235%	19%	67%	240%	137%	169%	13%
Rhizosolenia setigera	76%	36%	24%	172%	55%	201%	234%	140%	162%	15%	75%	**457%**	133%	132%	17%

Scrippsiella spp.	97%	57%	35%	117%	49%	140%	137%	136%	171%	28%	95%	384%	95%	106%	25%
Skeletonema costatum	58%	38%	19%	80%	28%	138%	89%	107%	126%	25%	66%	185%	118%	124%	19%
Skeletonema potamos	59%	85%	57%	*64%*	25%	91%	*67%*	114%	131%	27%	85%	104%	86%	72%	17%
Thalassionema nitzschioides	73%	29%	21%	113%	58%	123%	139%	139%	152%	23%	93%	381%	95%	159%	15%
Torodinium spp.	71%	51%	32%	107%	41%	107%	119%	95%	171%	28%	101%	182%	136%	178%	17%
Trichodesmium spp.	65%	22%	12%	125%	**160%**	*62%*	**475%**	97%	218%	25%	61%	135%	86%	*69%*	16%
Average	76%	47%	27%	120%	45%	136%	155%	135%	147%	21%	80%	193%	102%	125%	17%
Minimum	42%	13%	5%	64%	22%	62%	67%	77%	85%	13%	43%	103%	50%	69%	7%
Maximum	159%	96%	60%	186%	160%	248%	475%	251%	273%	39%	162%	457%	178%	230%	37%

Table 5
Regression results of ranked optima versus ranked tolerances across taxa for all parameters. DOY = day of year; DIN = dissolved inorganic nitrogen (μM); NH_4^+ = ammonium (μM); NOx- = nitrate + nitrite (μM); N:P = DIN to phosphate ratio; PO_4^{+3} = phosphate (μM); Si:N = silica to DIN ratio; SiO_3 = silica (μM); Si:P = silica to phosphate ratio.

Parameter	R^2	p-Value	Slope
Stratification	0.401	0.001	−0.634
DOY	0.425	0.0001	−0.652
Seasonality	0.78	0.0001	−0.883
DIN	0.456	0.0001	−0.675
f-ratio	0.868	0.0001	−0.932
NH_4^+	0.054	0.252	–
NO_x^-	0.508	0.0001	−0.713
N:P	0.130	0.070	–
PO_4^{+3}	0.041	0.321	–
Salinity	0.423	0.0001	−0.651
Secchi	0.126	0.076	–
Si:N	0.006	0.701	–
SiO_3	0.791	0.0001	−0.889
Si:P	0.371	0.001	−0.609
Temperature	0.010	0.629	–

predictable manner over the course of the year when undisturbed. The winter assemblage (Group B) is expected to transition into the spring bloom assemblage (Group C) and then to Group A (the summer assemblage). The magnitudes (biomass) of the phytoplankton cells will be a function of riverine inputs of nutrients (Sklar and Turner, 1981; Lohrenz et al., 1990; Scavia et al., 2003) and variations (anomalies) from the norm will be due to perturbations (e.g., floods, droughts, weather, currents, nutrient limitation). Additionally, there will be longer-term trends related to climatic variability (e.g., lower river flow, increasing sea surface temperatures) that will influence the phytoplankton dynamic (Justić et al., 2005). The results of this study, therefore, provide a blueprint from which such perturbations and trends can be evaluated.

This is a more general approach than that used by Parsons et al. (2015) to study impacts of the Macondo oil spill, who compared years most similar to 2010 in terms of physical and chemical parameters, and examined how the phytoplankton composition differed between those years and 2010. This study rather focuses on general trends from which major changes in the phytoplankton community can be gauged. As other studies have shown, phytoplankton do not respond unimodally to exposure to crude oil residue and dispersant; some species are stimulated, others are tolerant, and the remainder are inhibited (Quigg et al., 2021). The trajectory of the phytoplankton response will also vary, depending on the initiation point (i.e., community composition and time of year; Ozhan et al., 2014). Therefore, there is not a "one size fits all" solution to assess phytoplankton responses to potential oiling, and a variety of tools and approaches may be needed to provide an accurate evaluation of the impacts of oiling. A specific spatio-temporal analysis of potential oiling impacts on phytoplankton on the Louisiana shelf in 2010, including data utilized in this study, was also completed (Brandt et al., 2021). Briefly, they found that the oiling effects on phytoplankton composition were secondary to physical drivers (such as temperature and salinity) and seasonality; factors shown to be important in this study as well.

Many advancements can be made from this study. This study focused on 26 common taxa, but there are notable omissions due to the taxonomic criteria utilized (i.e., a minimum of genus-level resolution) as well as limitations in the use of epifluorescent microscopy, the sample preservative used (glutaraldehyde), and the length of time some samples sat prior to analysis. Many smaller taxa (e.g., phytoflagellates) could not be identified any further as flagella were often absent (due to glutaraldehyde preservation) and diagnostic morphological characteristics could not be adequately examined using epifluorescent microscopy. Also, picoplankton were not included, again due to the lack of visual diagnostic queues. While the majority of the picoplankton encountered in these samples were likely *Synechococcus* species (under cyanobacteria; Table 1), the microscopic counts did not distinguish among the picoplankton enumerated (e.g., prochlorophytes were also likely contained within this group). Similarly, small eukaryotic cells were also omitted, and likely included various phytoflagellates and members of Mamiellales. Qian et al. (2003) reported that prymnesiophytes, prokaryotes, pelagophytes and diatoms were the four major phytoplankton groups encountered in their HPLC-based study east of the Mississippi River. Wysocki et al. (2006) also reported that prymnesiophytes could be abundant in the region. We, however, did not distinguish prymnesiophytes and pelagophytes from other taxa in our study, although our results do concur in the abundance of prokaryotes and diatoms

Table 6
SIMPER and ANOVA Tukey results distinguishing the three phytoplankton groups classified in the CLUSTER analysis. The environmental parameters are defined in Table 2. The values listed under each Group heading represent the average of the normalized value for each parameter. The numbers listed under each group comparison (e.g., A vs B) are the weights of the environmental parameters that account for 70% of the cumulative differences between each group based on the SIMPER analysis. The Tukey groupings indicate if the normalized parameters are significantly different among the three groups (as denoted by the lowercase letters, where "a" is higher and "b" is lower).

Parameter	Group A	Group B	Group C	A vs B	A vs C	B vs C	Tukey A	Tukey B	Tukey C
Stratification	0.39	−0.70	0.03	4	–	–	a	a	a
f-ratio	−0.50	0.09	0.76	–	3	–	b	ab	a
NH_4^+	0.31	−0.65	0.12	7	–	–	a	a	a
NO_x^-	−0.27	−0.64	1.09	–	2	2	b	b	a
PO_4^{-3}	−0.22	0.10	0.28	6	–	6	a	a	a
Salinity	−0.18	0.82	−0.52	5	–	5	ab	a	b
Secchi	0.33	−0.06	−0.50	–	7	–	a	a	a
Si:N	−0.16	0.47	−0.20	1	–	1	a	a	a
Si:P	−0.50	−0.25	1.10	–	1	3	b	b	a
SiO_3	−0.19	−0.47	0.81	–	4	4	ab	b	a
DOY	0.71	−0.84	−0.39	3	5	–	a	b	b
Temperature	0.69	−0.71	−0.48	2	6	–	a	b	b

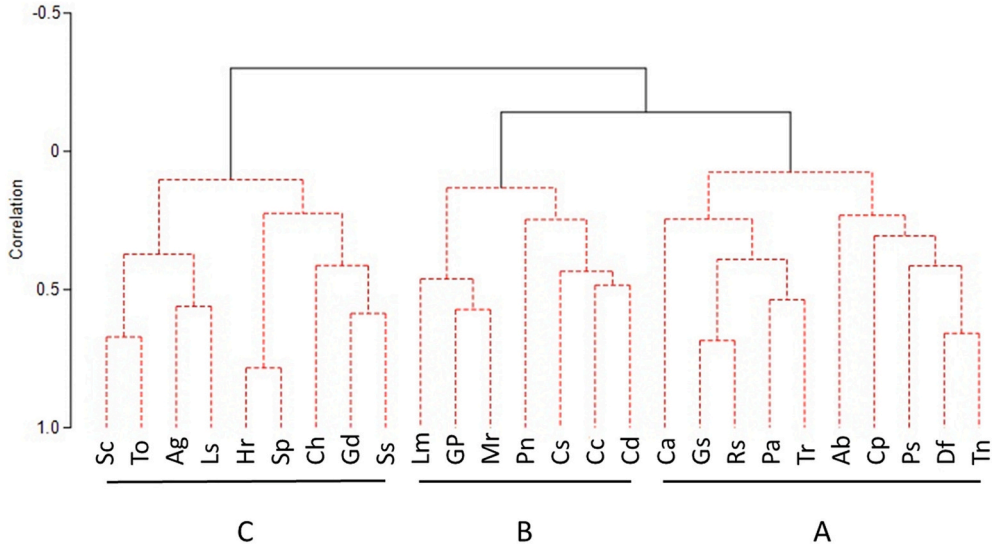

Fig. 3. A Dendrogram depicts the three phytoplankton groups determined by the CLUSTER routine in PRIMER7. The red dashed lines indicate the significant separations between the three groups as determined by the SIMPROF analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Table 1). Chakraborty and Lohrenz (2015) conducted a similar HPLC-based study on the Louisiana shelf in the same region as our study and found that diatoms, cryptophytes, cyanobacteria and chlorophytes dominated. While we identified chlorophytes and cryptophytes in our microscope-based study, they were generally in low relative abundance (< 0.1 and 0.2% abundance, respectively). Chakraborty and Lohrenz (2015) also reported that haptophytes (prymnesiophytes) were abundant in many samples, whereas Schaeffer et al. (2012) did not report the presence of any prymnesiophytes in their study. The low relative abundance of this group in our study suggests that 1) our methods (preservation, identification and enumeration) were not adequate for this group of phytoplankton; or 2) prymnesiophytes were not extremely abundant in our samples. It should be noted that it can be difficult to achieve agreement on phytoplankton composition among different methodologies, however, even when samples are split and analyzed simultaneously (See et al., 2005).

Many centric diatoms were also not included as individual cells could not be identified to genus or species level with the epifluorescent methodologies utilized in this study (e.g., *Coscinodiscus*, *Thalassiosira*, and *Cyclotella* spp.). Additionally, a "bottom up" philosophy was applied to this study in which grazing considerations were not included. But grazing is known to influence phytoplankton composition (Cloern and Dufford, 2005) and is an important process on the Louisiana shelf (Dagg, 1995; Turner et al., 1998), particularly for phytoplankton cells <20 μm (Fahnenstiel et al., 1995).

The majority of samples analyzed in this study were from the C-transect (Table S1, Fig. 1). As such, the responses observed in the phytoplankton likely reflect temporal (seasonal) changes rather than spatial variation. Phytoplankton are known to vary on a spatial scale in the northern Gulf of Mexico (e.g., Dagg et al., 2007; Quigg et al., 2011) and our study is limited in this regard (e.g., we did not capture open ocean conditions; the "far field" of Dagg et al., 2007). Different water masses do move across and along the shelf, however. While the coastal boundary current (and the Mississippi River plume) move predominantly to the west throughout the year (Wiseman et al., 1997), southeastern winds in the summer can reverse the flow towards the east and oligotrophic offshore waters can move inshore (Chen et al., 2000). The summer phytoplankton (Group A) may be the result of such water movements. Future studies can expand the scope to capture more of the spatial variability in the field (e.g., expanding on previous work such as that done by Williams et al., 2015).

A closer examination of extreme scenarios (floods, droughts, nutrient limitation, oil spills) can further reveal phytoplankton community dynamics, particularly sensitivity to the perturbations (i.e., how strong of a

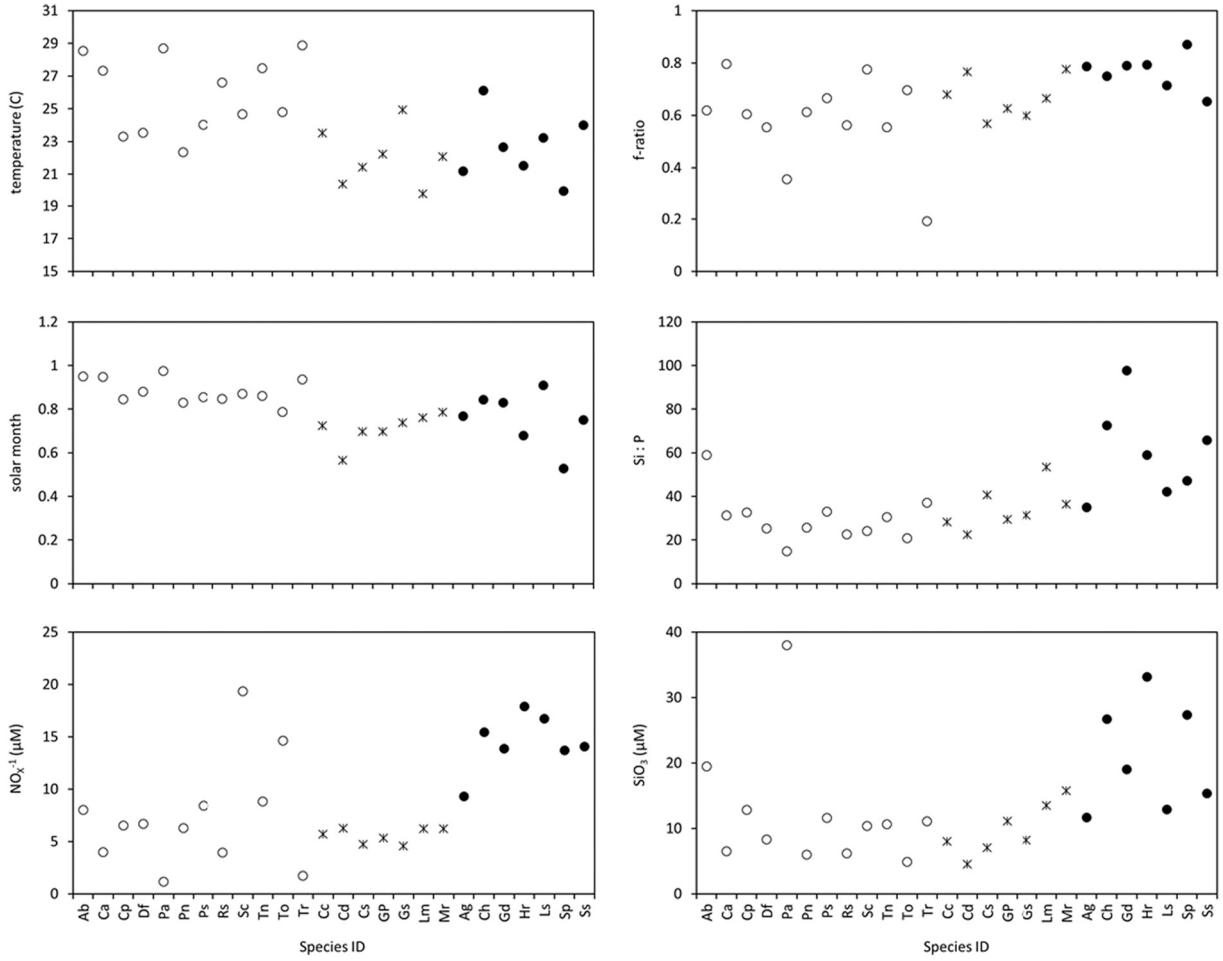

Fig. 4. The optima for the six environmental parameters that best distinguish the three phytoplankton groups from one another according to the SIMPER analysis. The phytoplankton taxa are listed on the x-axis (abbreviations are defined in Table 1). Open circles represent Group A; x's are Group B, and closed circles are Group C.

response is elicited) and resiliency (i.e., how long does it take for the phytoplankton to return to a "normal" state?). Future studies should therefore focus on a more complete sample of the phytoplankton community (using molecular techniques or imaging), grazing considerations, and examination of extreme scenarios (perturbations) to better ascertain phytoplankton sensitivity and resilience on the Louisiana shelf.

In conclusion, however, this study presents a basic blueprint for studying phytoplankton dynamics on the Louisiana shelf. It demonstrates the importance of physical processes and seasonality, and distinguishes specific taxa in terms of high and low environmental optima. Additionally, the negative relationships that exist between optima and tolerance values may be indicative of adaptability of certain taxa to perturbations. The examination of phytoplankton responses to future perturbations using the criteria developed herein will provide the opportunity to evaluate their applicability and usefulness in the assessment of the impacts of such perturbations.

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CRediT authorship contribution statement

All authors (Parsons, Brandt, Turner, Morrison, and Rabalais) have seen and approved the final version of the manuscript being submitted. We warrant that the article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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