Temporal and spatial variability in *Pseudo-nitzschia* spp. in Alabama coastal waters: A “hot spot” linked to submarine groundwater discharge?

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**A B S T R A C T**

The potentially toxic diatom *Pseudo-nitzschia* is common in the northern Gulf of Mexico. Seven sites along the Alabama Gulf Coast have been monitored weekly to bi-weekly for *Pseudo-nitzschia* spp., which were detected in 489 of 829 samples (59%) taken between November 2003 and July 2008. Mean population density peaked at 19.6 ± 3.2 °C but bloom densities (>10^6 cells L^-1) occurred at 20–32 °C. Mean population density peaked at a salinity of 30.1 ± 3.2, with blooms occurring between salinities of 26 and 32. Peaks in abundance occurred in April–May, with secondary peaks in fall. A cluster analysis of the relative frequency distributions of abundance by site showed that Little Lagoon Pass had a strong dissimilarity compared to other sites, due to a higher frequency of bloom densities and a lower frequency of absences. Salinities at Little Lagoon Pass were higher and less variable than at other sites. *Pseudo-nitzschia* spp. were absent more frequently from sites at the mouths of Perdido and Mobile Bays, where salinity was lower and more variable. Freshwater transport from Baldwin County, which lies between these bays, has previously been shown to be primarily through submarine groundwater discharge into the Gulf of Mexico. Groundwater in Baldwin County has high nitrate concentrations and discharge is most likely to occur adjacent to Little Lagoon. Blooms of *Pseudo-nitzschia* spp. at Little Lagoon Pass in spring were highly correlated with discharge from the Styx River, a proxy for groundwater discharge. Little Lagoon Pass may therefore be a hot-spot for blooms of *Pseudo-nitzschia* spp., because local maxima in discharge result in nutrient availability without significant reductions in salinity.

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1. **Introduction**

Members of the potentially toxic diatom genus *Pseudo-nitzschia* (Hasle, 1994) frequently form blooms along temperate coastlines throughout the world. Toxic species within this genus can produce domoic acid (DA), a neurotoxin that has been implicated in human poisonings and deaths following the consumption of contaminated shellfish (Rates et al., 1998). Mortalities and poisoning by DA have been observed in birds and marine mammals through other vectors such as planktonic fish (Work et al., 1993; Scholin et al., 2000). Domoic acid has been shown to accumulate in a variety of other organisms such as copepods, krill, and squids and be passed on to their predators (Bargu et al., 2002, Lefebvre et al., 2002). *Pseudo-nitzschia* blooms can also have large economic impacts due to the restriction of the shellfish industry during toxic blooms (Horner et al., 1997).

As a result of the ecological and economic risks posed by *Pseudo-nitzschia* spp., past studies have attempted to characterize the suite of environmental conditions associated with *Pseudo-nitzschia* spp., which were absent more frequently from sites at the mouths of Perdido and Mobile Bays, where salinity was lower and more variable. Freshwater transport from Baldwin County, which lies between these bays, has previously been shown to be primarily through submarine groundwater discharge into the Gulf of Mexico. Groundwater in Baldwin County has high nitrate concentrations and discharge is most likely to occur adjacent to Little Lagoon. Blooms of *Pseudo-nitzschia* spp. at Little Lagoon Pass in spring were highly correlated with discharge from the Styx River, a proxy for groundwater discharge. Little Lagoon Pass may therefore be a hot-spot for blooms of *Pseudo-nitzschia* spp., because local maxima in discharge result in nutrient availability without significant reductions in salinity.

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initiation and toxicity of their blooms. Diatoms as a group are characterized by high intrinsic growth rates (reviewed by MacIntyre et al., 2002), high nutrient uptake rates and capacities (Lomas and Glibert, 2000), and robust non-photochemical quenching mechanisms (Lavaud et al., 2007) that make them competitive in turbulent water columns with variable nutrient and light conditions. *Pseudo-nitzschia* blooms have been associated with upwelling conditions (Trainer et al., 2000, 2002) and large coastal runoff or discharge events (Smith et al., 1990), both of which are characterized by pulses of new nutrients. *Pseudo-nitzschia* pungens has also been shown to be more competitive than other diatoms under low Si:N conditions, presumably due to its light silicification (Sommer, 1994).

In addition to being abundant in areas such as southeastern Canada, the Pacific coast of North America, Japan, and Scotland, toxic blooms of *Pseudo-nitzschia* spp. have also been observed in the northern Gulf of Mexico (Pan et al., 2001). In the northern Gulf, *Pseudo-nitzschia* spp. are a common component of diatom assemblages, being present year-round and often in high densities relative to more temperate locations (Dortch et al., 1997). *Pseudo-nitzschia* spp. abundance has increased in the northern Gulf of Mexico over the past 60 years, presumably in response to increased nutrient loading (Parsons et al., 2002).
A link between coastal eutrophication and the increasing abundance of species that form harmful algal blooms (HABs) is certainly not unique to *Pseudo-nitzschia* (Anderson et al., 2002). However, groundwater has only rarely been demonstrated as a source of new nutrients that may fuel HABs (e.g. Jones and Bachmann, 1974; Dillon and Rigler, 1975; Valiela et al., 1992; LaRoche et al., 1997; Lapointe et al., 1999; Hu et al., 2006; Lee and Kim, 2007), although submarine groundwater discharge (SGD) can represent a large component of the nutrient load and can induce shifts in phytoplankton community structure or toxicity through changes in nutrient ratios (Paerl, 1997). Nutrient inputs from SGD are distinct from surface runoff or river discharge due to their high degree of temporal and spatial heterogeneity (Corbett et al., 1999) and the difficulty of their measurement. Due to the long residence time of groundwater, the nutrients within SGD may be the results of decades-old nutrient additions to coastal aquifers, thus posing potential difficulties for management of HABs in relation to groundwater inputs.

The Alabama coast of the northern Gulf of Mexico has high abundances of *Pseudo-nitzschia* spp., and high nutrient inputs due to both surface water discharge and SGD. We present an analysis of 829 samples collected from this area in which *Pseudo-nitzschia* densities were measured to test for spatial and temporal patterns in *Pseudo-nitzschia* abundance and correlations with environmental factors. We argue that seasonal variability is driven largely by temperature and that geographic and inter-annual variability can best be described by variations in SGD. We infer that SGD plays an important role in the population dynamics of *Pseudo-nitzschia* spp. in the study area.

2. Methods

2.1. Study area

Coastal Alabama is dominated by Mobile Bay, an estuary that drains the state of Alabama and parts of Mississippi, Tennessee, and Georgia, forming the fourth-largest drainage basin of estuaries in the USA (Bricker et al., 2007). Mobile Bay is separated from the Gulf of Mexico to the east of the bay mouth by the Fort Morgan Peninsula (Fig. 1) and to the west by Dauphin Island. Coastal Alabama has two sets of surface-water inputs, from Mobile Bay and from Perdido Bay to the east. The dominant alongshore transport is east–west, so that sites in Baldwin County, which lies between these two water bodies, are in the plume of the Perdido River. Because the average freshwater delivery to Mobile Bay is 36 times higher than to Perdido Bay (USEPA, 1999), waters to the west of Mobile Bay are dominated by the Mobile Bay plume.

Coastal Baldwin County (the area south of the US Geological Survey Perdido River gauging station) is a single hydrological unit that lacks major rivers (Dowling et al., 2004). This area is the recharge zone for the surficial aquifer, which is about 50–60 m deep, and the rivers that drain it are the surface expression of the aquifer (Dowling et al., 2004). Daily discharge data from the four USGS gauging stations in Baldwin County were highly correlated over the duration of this study (November 2003–June 2008, inclusive). Pair-wise correlation coefficients (Pearson's $R$) for log-transformed data averaged 0.79 (range 0.69–0.92).

Installation of a groundwater monitoring station (Fig. 1) in late 2006 shows that the aquifer water level (i.e. distance below surface) is correlated with discharge, rising during periods of precipitation and increased river discharge and falling during periods of drought, as would be expected when rivers are an expression of the surficial aquifer. Because groundwater data do not exist for most of the period in this study, we use discharge from the Styx River as an index of groundwater discharge. Nitrate levels in the surficial aquifer are relatively high: concentrations ranged from 3 to 269 (mean 59 ± 76) μmol L$^{-1}$ (Dowling et al., 2004) and many sites had a high probability (>67%) of having concentrations above 63 μmol L$^{-1}$ (Nolan et al., 2002). Transport of nitrate to the northern Gulf of Mexico by submarine groundwater discharge along the Fort Morgan Peninsula is estimated at 50% of the transport through surface waters by Mobile Bay (Dowling et al., 2004). It is unlikely that this would not affect microalgae in nearshore waters.

2.2. Sampling

The presence and abundance of potentially pathogenic or toxic micro-organisms in Alabama's coastal water is monitored by the State Departments of Environmental Management and Public Health and the Baldwin County Health Department under the aegis of the federal BEACH (Beaches Environmental Assessment and Coastal Health) act. Monitoring for enterococcal coliform bacteria and potentially toxic algae started at five recreational beaches along the Alabama coastline in 1999 and was subsequently expanded to include a further six sites on the coast and in Mobile Bay. Monitoring for *Pseudo-nitzschia* spp. started in November 2003. Eight BEACH sites (Fig. 1) are sampled routinely, weekly in the spring-fall (approximately April–September) and biweekly for the rest of the year. Water samples are collected at the shore-line, preserved with acid Lugol's solution and potential HABs are identified to genus (*Pseudo-nitzschia* spp. and raphidophytes) or species (dinoflagellates) and counted using an inverted microscope with a Nunc Lab-Tek II chamber slide system (Thermo Fisher Scientific, Rochester, NY). A database of all species identified is maintained by Alabama Department of Public Health. Supporting measurements of water temperature and salinity were collected for 600 and 629 of the 829 samples analyzed in this study, respectively. Sampling was interrupted in 2004–2005 when Alabama Point and Gulf Shores Pavilion became inaccessible in the aftermath of Hurricane Ivan; and in 2005 when Dauphin Island Public Beach was inaccessible in the aftermath of Hurricane Katrina.

2.3. Statistical analyses

The BEACH sampling schedule is *ad hoc*, with sampling that is not synoptic between sites, which is unbalanced within or between
months, and with response sampling during blooms that covers both the regular and additional sites at higher frequency. The data were therefore gated to include only routine weekly or bi-weekly sampling at eight sites (Fig. 1) that had the highest and most consistent temporal coverage. One was in the Perdido Bay complex (Orange Beach, OB): four were along the Fort Morgan Peninsula (AL, GSP, GSPB and LLP) and two were on Dauphin Island (DIEE and DIPB). Cell densities were transformed as log(n + 1), grouped into 0.5-unit bins (which correspond to 3.16× ranges of raw data) and ranked, either by site or by month. Frequency distributions were normalized to total sample number to account for differences in the sampling intensity between sites (110 ≤ n ≤ 131) and between months (31 ≤ n ≤ 131). The data can be viewed as cumulative frequency distributions (cf. Bricker et al., 2005). Temporal and spatial trends in the site- or month-based relative frequency distributions were tested through matrices of pair-wise Bray–Curtis similarity coefficients, using the non-parametric routines in PRIMER-E (Clarke and Warwicke, 2001). Overall similarity between sites or months was compared using cluster analysis of these similarity coefficients and visualized in multi-dimensional scaling (MDS) plots.

3. Results

3.1. Temperature, salinity, and hydrographic features

The temperature at three of the sampling sites throughout the study period is shown in Fig. 2A. Temperature clearly follows a seasonal pattern as expected in coastal, temperate waters, with no clear differences between sites. The average temperature for all samples was 24.8 ± 5.6 °C with a maximum of 34 and a minimum of 10 °C. Salinity differed between sites and through time (Fig. 2B). Sites near the mouth of Mobile Bay (i.e. DIEE) and Perdido River (i.e. OB) had highly variable salinity, presumably due to their proximity to areas of high freshwater discharge. Little Lagoon Pass (LLP) was the only site with relatively stable salinity, with a mean salinity of 31.5 ± 2.4.

The Tensaw and Styx rivers, which empty into Mobile and Perdido Bay, respectively, differ greatly in the magnitude of discharge, with a c. 6-fold difference in peak discharge and c. 50-fold difference in mean discharge over the study period (Fig. 2C and D). Discharge from the two rivers was correlated (R = 0.61 for log-transformed data) between 2003 and 2008. The Tensaw showed periods of high discharge (>125 × 10^8 m^3 day^-1) in late winter–spring (January–May) in each year of the study. Relatively high discharge was also observed between fall 2004, and spring 2005. Similar patterns overall were observed for the Styx; however, it had higher discharge in April 2005, and April 2008, relative to its average discharge. Two extensive periods of drought were observed in the discharge of both rivers. A hydrologic drought was observed in the late spring to early fall of 2006 (June–October) and was particularly apparent in the Tensaw. A more extensive drought period was observed from April 2007 to January 2008.

3.2. Spatial and temporal distribution of Pseudo-nitzschia spp.

Pseudo-nitzschia spp. were observed at all study sites and were present in the study area throughout each year of the study. Bloom densities (>10^6 cells L^-1) occurred at each of the sites along the Fort Morgan Peninsula at least once. Pseudo-nitzschia spp. were detected in 489 of the 829 samples (59%) analyzed for this study. Temporal and spatial distributions of Pseudo-nitzschia spp. are shown in Fig. 3. One clear feature of these data is that sub-bloom densities (>10^5 cells L^-1) occur at LLP more often than at the other sites. It is clear that absences and low densities of Pseudo-nitzschia spp. are more common at OB. These patterns are shown more clearly in cumulative distribution plots of Pseudo-nitzschia spp. density at each site (Fig. 4A). The plot was constructed by sorting data by abundance for each site in half-decade-sized bins and normalizing to total sample number in a relative frequency plot, and then summing with increasing bin-size. This allows one to see the relative absence of Pseudo-nitzschia spp. (the y-intercept) and relative frequency of high-density populations (the slope).

Differences in the structure of the population data by site (based on analysis of the relative frequency distributions) is shown graphically in a MDS plot (Fig. 4B). Each pair of sites was compared using Curtis–Bray similarity coefficients and the overall similarity was defined by cluster analysis (Fig. 4B). Similarity analysis (the SIMPER test in PRIMER-E) showed that similarity between all sites except DIEE, LLP and OB was 91.5%. This cluster and DIEE differed by 15.2%. The remaining two sites differed from the dominant cluster by 20.8% (LLP) and 40.8% (OB). Similarity between sites was driven primarily by the absence of Pseudo-nitzschia spp. rather than
high densities or the overall distribution at certain sites. *Pseudo-nitzschia* spp. were absent from 23% of samples from LLP, 76% of samples from OB and 34–46% of samples from the other sites.

Temporal patterns in the density of *Pseudo-nitzschia* spp. are difficult to discern from Fig. 3. One noticeable event is the presence of *Pseudo-nitzschia* spp. at densities >10^6 cells L^{-1} at four sites in May 2005. Densities during this bloom ranged from 1.2 \times 10^6 cell L^{-1} at the two sites at Gulf Shores (GSP and GSPB) to 3.3 \times 10^6 cells L^{-1} at LLP. This event was the first toxic bloom of *Pseudo-nitzschia* spp. recorded in coastal Alabama (H.L. MacIntyre et al. unpublished). Bloom densities were also found at LLP in September 2005, and April 2008, and at AP in June 2007, but were restricted to single sites and single samples on those occasions. In general, peak densities occur in the spring (March–May) and fall (September–November) months, while absences occur more frequently in the summer months (June–August).

Differences in the structure of the population data (i.e. the relative frequency of samples in any size-bin) is shown graphically by month in a polar plot of cumulative frequency distribution (Fig. 4C) and similarity between months is shown in a multi-dimensional scaling plot (Fig. 4D). Cluster analysis of the relative frequency distributions by month shows two distinct groupings. One contained the months in which *Pseudo-nitzschia* spp. were absent in only 21–33% of samples (March–May, September–October), which were similar at 80.8%, and the other (similar at 76.9%) contained the remaining months. Of these, August, in which 60% of samples contained no detectable *Pseudo-nitzschia* spp., was an outlier. The two major clusters had a dissimilarity of 32.3%. Note
that the two clusters were not driven by unequal sample sizes resulting from the weekly vs bi-weekly sampling: the first had sample sizes ranging from 37 to 127 and the second had sample sizes ranging from 31 to 131.

3.3. Pseudo-nitzschia spp. abundance in relation to temperature, salinity and hydrography

Fig. 5A shows the relationships between Pseudo-nitzschia spp. densities and temperature. Blooms of Pseudo-nitzschia spp. were observed at temperature ranges of 20–24 °C and 28–32 °C, shown by the horizontal black bars on the graph. Because of the uneven distribution of samples with respect to temperature (Fig. 5B), blooms accounted for 5/150 (3.3%) of samples in the 20–24 °C range but only 2/334 (0.6%) of samples in the 28–32 °C range. Mean concentrations of Pseudo-nitzschia spp. were highest at intermediate temperature (Fig. 5A). The data could be fit to a non-linear relationship proposed to describe the optimum in the relationship between photosynthetic rate and temperature (Blanchard et al., 1996). To avoid bias through undersampling, only the means from bins in the frequency distribution that represented more than 2% of the total population (12–34 °C) were included in the fit. The fit between geometric mean cell density (i.e. the back-transform of the mean of log-transformed data) and temperature for the range 12–34 °C was highly significant (p < 0.001) although the coefficient of determination was relatively low (R² = 0.43). The maximum in mean population density occurred at 19.6 ± 3.2 °C.

Pseudo-nitzschia spp. were abundant over a broad range of salinity (Fig. 5C). Bloom densities were found between salinities of 26 and 32. Because of the highly skewed distribution of salinity (Fig. 5D), blooms accounted for 1/41 (2.4%) to 2/153 (1.3%) of samples at these salinities. As with temperature, the fit between the geometric mean abundance of Pseudo-nitzschia spp. and salinity was highly significant (p < 0.001), although the coefficient of determination was low (R² = 0.40). The fit was limited to the range in which data-points represented 2% of more of the total population (salinities of 18–36). The maximum in mean population density occurred at a salinity of 30.1 ± 3.2.

The highest abundances of Pseudo-nitzschia spp. were found at sites along the Fort Morgan Peninsula, in most cases following peaks in river discharge. Because of the east–west along-shore current in this region, the abundances were compared to discharge from the Styx River rather than the Tensaw, which discharges to the west of these sites. The most extensive bloom observed in the area, the toxic bloom in April–May 2005, was preceded by the largest discharge event during the study period (Fig. 2D). Other occasions where a bloom of Pseudo-nitzschia spp. was found at a single site, followed periods of high discharge in September 2005 and April 2008. Periods of sustained drought occurred in the summer months of 2006 and 2007, when Pseudo-nitzschia spp. abundance was at its lowest.

The blooms (>10⁶ cells L⁻¹) at sites along the Fort Morgan Peninsula in 2005 and 2008 occurred between mid-April and mid-May (Fig. 3). Annual variability in abundance at the four sites along the peninsula (AP, GSP, GSPB and LLP) during this period, April 15–May 15 and discharge from the Styx River are shown in Fig. 6. A lag period was used to account for the delay between the recharge of the aquifer in Baldwin County and discharge to the Gulf of Mexico and also to account for the response time of the microalgal community. The same trends can be seen in both data sets. Because
of the temporal mismatch in the data and the uneven sample sizes, it is difficult to make a rigorous statistical comparison between discharge and abundance. However, mean abundance of *Pseudo-nitzschia* spp. was significantly correlated (*p* < 0.05, Pearson’s *R* = 0.92; *n* = 5) with mean discharge by year. The correlation was largely driven by data from LLP. When data were separated by site according to the cluster analysis (Fig. 3), the correlation was highly significant for LLP (0.97 < *R* < 0.99, *p* < 0.05) for discharge data offset by 1–3 weeks but not for the cluster containing the other three sites (Table 1). There was no significant correlation between discharge and *Pseudo-nitzschia* spp. densities at OB. (The comparison was not performed for the two sites on Dauphin Island because of the dominance of the Mobile Bay plume.)

### 4. Discussion

We have shown that *Pseudo-nitzschia* spp. are common members of the microalgal community in near-shore waters of the Gulf of Mexico off Alabama, just as they are in other regions of the northern Gulf of Mexico, such as Louisiana (Dortch et al., 1997). Representatives of the genus were present in 59% of 829 samples collected over a nearly 5-year period. The focus of the BEACH sampling effort in Alabama is to monitor the presence and abundance of fecal indicator organisms (enterococcal bacteria) and potentially toxic microalgae with little supporting data on environmental conditions. Consequently, while there are consistent spatial and temporal trends in the abundance of *Pseudo-nitzschia* spp., there is a paucity of data that can be used to infer the causal factors. These would include bottom-up factors such as temperature, salinity and the availability of nutrients and light; and top-down factors such as physical transport and grazing pressure. There are supporting temperature and salinity data from 88% and 81% of the cell counts respectively and continuous records of local river discharge from USGS. We consider their relationships to the abundance data below.

#### 4.1. Temperature effects

When all data were aggregated, it was apparent that *Pseudo-nitzschia* spp. are present throughout the range of reported temperatures (10–34°C). The highest numbers were found in a relatively broad range, 22–28°C, which is slightly higher than the temperature at which the maximum mean density was found (19.6 ± 3.2°C). In another study from the Gulf of Mexico off the coast of Louisiana and Texas, representatives of the genus were abundant over a relatively wide temperature range of 24.4 ± 4.9°C (Dortch et al., 1997). When considering this broad temperature range, it should be noted that multiple species within the genus *Pseudo-nitzschia* co-occur in the northern Gulf of Mexico (Dortch et al., 1997; Fryxell et al., 1990; Parsons et al., 1999) and that there seem to be different temperature responses among these species (Fryxell et al., 1990; Dickey et al., 1992).

The occurrence of bloom densities at the upper portion of this range may be due to the temperature requirements for growth and photosynthesis comparable to those noted for *Pseudo-nitzschia multiseries*. Prior studies have shown a strong temperature-dependence of light-saturated photosynthetic rate and growth in *Pseudo-nitzschia multiseries* (Pan et al., 1993; Lewis et al., 1993; reviewed in Bates et al., 1998), which was formerly identified as

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**Table 1**

<table>
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<th>Site(s) offset (weeks)</th>
<th>OB</th>
<th>AP, GSP, GSPB</th>
<th>LLP</th>
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<td>2</td>
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</tr>
<tr>
<td>3</td>
<td>0.86† 0.77 (NS) 0.99*</td>
<td></td>
<td></td>
</tr>
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</table>

*Pearson’s coefficients for the correlation between mean *Pseudo-nitzschia* spp. densities in the period April 15–May 15 at sites along the Fort Morgan peninsula and mean discharge from the Styx River, by year (2004–2008; *n* = 5). Sites are grouped according to the cluster analysis of relative frequency distributions (Fig. 3). All data were log-transformed; log(n + 1) for cell densities vs log(discharge), prior to comparison. Discharge was compared for the same period and for 30-day periods advanced by 1, 2 and 3 weeks ahead of the cell data. Significance levels: NS, *p* > 0.10; † *p* < 0.05; ** *p* < 0.01.

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**Fig. 6.** (A) Daily discharge at the USGS Styx River gauging station for the period April 1–30, by year. (B) Abundance of *Pseudo-nitzschia* spp. at sites AP, GSP, GSBP and LLP for the period April 15–May 15, by year. Only samples in which *Pseudo-nitzschia* spp. were detected are shown. The numbers of samples in which *Pseudo-nitzschia* spp. were not detected (n, ND) and total sample number (n, total) are indicated between the panels. Note log scales. (C), Relationship between mean log-transformed *Pseudo-nitzschia* spp. density (B) and discharge from the Styx River (A), by site. Grouping of AP, GSP and GSBP is based on cluster analysis of relative frequency distributions (Fig. 4). Correlation coefficients are given in Table 1.
Nitzschia pungens f. multiseries. The relationship resembled the Arrhenius function over the experimental temperature ranges (15–25 °C). Estimates of the Q10 quotient were 1.8 and 2.8 over the 5–15 °C range and 2.1 for 10–20 °C (Bates et al., 1998). A similar temperature-dependence might underlie the maxima in abundance at 22–28 °C in our data. We note that a reduction in light-saturated photosynthesis above a temperature of c. 25 °C has been observed in natural populations of benthic diatoms (Blanchard et al., 1996) and above 29–30 °C in cultures of the pennate diatom Cylindrotheca closterium (Morris and Kromkamp, 2003). While the reduction was a response to short-term temperature stress, the same response was found when natural populations were growing under temperatures that varied from 1.0 to 10.5 °C or from 16.4 to 24.6 °C (Blanchard et al., 1996). The response might therefore be an intrinsic feature of photosynthetic response.

The effect of temperature on growth and photosynthesis is likely to be one of the factors that drive the temporal distribution of Pseudo-nitzschia spp. in coastal Alabama (Fig. 4C and D). The average temperatures in the spring and fall periods, when Pseudo-nitzschia spp. are most likely to be present and abundant, are 22.7 ± 3.6 °C and 25.3 ± 4.1 °C, respectively. It is equally unlikely that temperature would be the only factor driving Pseudo-nitzschia spp. abundance. The inter-annual variation in abundance indicates the limited effect: Pseudo-nitzschia spp. were far more abundant in 2005 and 2008 than it was in other years (Fig. 6B), while annual temperature trends were similar through the study period (Fig. 2A).

4.2. Discharge

Styx River discharge (Fig. 6A) can be used not only as a proxy for the relative discharge of all the rivers within its hydrologic unit, but also as a proxy for runoff from Baldwin County entering the Gulf of Mexico via Perdido Bay and along the Fort Morgan peninsula (Fig. 1). Although Baldwin County lacks major rivers, the primary river in this hydrologic unit is the Perdido, the discharge of which is carried to the sites along the Fort Morgan Peninsula (OB, AP, GSPB and LLP) via long-shore currents. A similar mechanism is likely for the Dauphin Island sites (DIEE and DIPB), which lie west of the mouth of Mobile Bay and would receive its discharge (represented by the discharge of Tensaw River in Fig. 2D) via the same long-shore currents.

Correlation between Styx River discharge and the groundwater level in Baldwin County (see Section 2) indicates that the discharge data can also be used as a proxy of groundwater flow and subsequently, groundwater discharge. Baldwin County has high rainfall (167 cm/year, Robinson et al., 1996) and lacks major river systems, so the recharge rate of the aquifers is high (Dowling et al., 2004). Most of the water in the aquifers in Baldwin County enters the Gulf of Mexico as SGD, making this pathway the most likely source of terrigenous nutrients to the five sites in Baldwin County (Dowling et al., 2004). The high rate of agricultural fertilizer application and its well-drained soils place Baldwin County in the upper quartile of risk for groundwater nitrate contamination in the USA (Nolan et al., 1997). Many sites in Baldwin County had a high probability (>67%) of nitrate contamination, defined as concentrations above 63 μmol L⁻¹ in the surficial groundwater (Nolan et al., 2002). We have measured concentrations of 170 μmol L⁻¹ nitrate and 249 μmol L⁻¹ total nitrogen in the surficial ground-water adjacent to Little Lagoon (unpublished data).

The correlation between Pseudo-nitzschia spp. and discharge, on an inter-annual basis (Fig. 6A and B) is likely the result of increased nutrient availability due to SGD following the observed river discharge events. This is comparable to blooms of P. multiseries in Prince Edward Island, Canada, which have been associated with periods of high precipitation and river discharge (Bates et al., 1998; Smith et al., 1990). Several studies on the west coast of the United States have shown Pseudo-nitzschia spp. to be associated with coastal upwelling systems (Walz et al., 1994, Trainer et al., 2000, 2002). These are analogous to discharge events in that both usually supply relatively large amounts of new nutrients over a short period of time. Large discharge and upwelling events are also similar in that they provide a high degree of physical forcing that could favor diatoms in general (Margalef, 1978; Huisman et al., 2004) and Pseudo-nitzschia spp. in particular (Trainer et al., 2000, 2002).

4.3. Salinity

River discharge events can also cause local decreases in salinity, which would seem to discourage Pseudo-nitzschia spp. growth based on the relationship of abundance and salinity observed here (Fig. 5C). The salinity at which the mean population density was maximum was 30.1 ± 3.2 and, with one exception, all bloom densities in this study occurred at salinities higher than 28. In general, Pseudo-nitzschia spp. have been shown to prefer higher, oceanic salinities (Bates et al., 1998). Other studies in the northern Gulf of Mexico have also shown this relationship, with Pseudo-nitzschia spp. growing best in field and laboratory studies at salinities of 30–45 (Dorch et al., 1997; Thessen et al., 2005). The apparent preference of Pseudo-nitzschia spp. for higher salinities shown in this study reflects the spatial distribution of observations. The site with the lowest average salinity and most variable salinity (OB) had the greatest proportion of absences of Pseudo-nitzschia spp., while the site furthest from any surface discharge point (LLP) had the lowest proportion number of absences and highest occurrence of bloom densities (Figs. 3 and 4A and B).

4.4. Little Lagoon Pass as a Pseudo-nitzschia spp. “hot spot”

Notable features in the spatial distribution of Pseudo-nitzschia spp. were the low percentage of absences (23%) and high proportion of sub-bloom and bloom densities observed at LLP. A factor likely to contribute to these is the consistent, relatively high salinities observed at this site. LLP connects the Gulf of Mexico to the poorly flushed Little Lagoon, which has no surface connection to local rivers. LLP is also furthest from the mouth of Perdido River among sites in Baldwin County. These geographic features insulate LLP from the variations in salinity observed at other sites as a result of surface-water discharge events.

The spatial distribution of Pseudo-nitzschia spp. indicates that LLP is a “hot-spot” for this potentially toxic diatom genus. Pseudo-nitzschia spp. abundance at LLP was correlated with river discharge (Fig. 6, Table 1) and bloom densities were preceded by two of the three highest discharge events from the Styx River (Figs. 2D and 3). The hydrology of Baldwin County would cause the discharge observed from the Styx River to occur in parallel with SGD, which dominates the discharge to the Gulf of Mexico from Baldwin County aquifers (Dowling et al., 2004). Baldwin County releases an amount of nitrate to the northern Gulf of Mexico via SGD along the Fort Morgan Peninsula that is equivalent to 50% of the nitrate supplied by surface flow through Mobile Bay (Dowling et al., 2004). This nitrate flux is attributed to the high groundwater nitrate levels observed in Baldwin County coupled with the porous, siliciclastic nature of its aquifers (Dowling et al., 2004). This is notable in that SGD from Baldwin County represents only ~3% of the total discharge from Mobile Bay (Dowling et al., 2004), which is the drainage point for the nations 4th largest watershed by area (Bricker et al., 2007). Consequently, we would expect SGD to deliver nitrate with less dilution of salinity than occurs with river discharge. The observation that the highest groundwater elevation in coastal Baldwin County is adjacent to Little Lagoon (Margulet and Tick, 2007) is consistent with nutrient supply by SGD playing a
role in the elevated densities of *Pseudo-nitzschia* spp. at LLP. Murgueit and Tick (2007) also showed the occurrence of salt-water intrusion to the near-surface aquifer underlying Little Lagoon, which implies direct hydrologic connectivity between this aquifer and the Gulf of Mexico waters near LLP. Thus LLP may receive greater nutrient inputs than the other sites following recharge of aquifers in Baldwin County. The proposed local maxima in the discharge of nutrient-rich groundwater at LLP and its relative isolation from surface water discharge may be optimal conditions for *Pseudo-nitzschia* spp. growth and abundance.

A link between *Pseudo-nitzschia* spp. abundance and SGD in coastal Alabama is important given the current trends in land use in Baldwin County. In addition to being a productive agricultural area, Baldwin County is experiencing some of the highest population growth rates in the nation, with some of the highest rates in the coastal communities (US Census data). Nitrogen and phosphorus loading are expected to increase in tandem with population growth (Vitousek et al., 1997, Lee et al., 2001). The relative abundance of *Pseudo-nitzschia* spp. has been shown to increase with both nitrate loading over decadal time-scales and with a decrease in the Si:N ratio (Sommer, 1994, Parsons et al., 2002). The high submarine groundwater discharge and associated nitrate flux from coastal Baldwin County, coupled with the prospect of growing development and residential population indicates that this area may be at risk for greater frequencies and densities of *Pseudo-nitzschia* spp.

5. Conclusions

We have analyzed a 5-year data set from coastal Alabama containing 829 records of *Pseudo-nitzschia* spp. density with supporting data for temperature, salinity, and local river discharge from USGS monitoring stations. *Pseudo-nitzschia* spp. were present in 59% of these samples and were observed at all sites and through the year. While being present along a wide range of temperature and salinity, high densities of *Pseudo-nitzschia* spp. were most likely to be found at temperatures between 22 and 28 °C and salinities greater than 26. Inter-annual river discharge, which is used as a proxy for groundwater discharge and nutrient loading, was correlated with *Pseudo-nitzschia* spp. abundance at sites along the Fort Morgan peninsula, where submarine discharge has previously been shown to dominate surface water inputs. *Pseudo-nitzschia* spp. were most abundant in the spring and fall months and at the LLP site. We hypothesize that LLP is a local “hot spot” for *Pseudo-nitzschia* spp. due to a relatively high average salinity and an inferred greater nutrient supply via submarine groundwater discharge.

Acknowledgments

We thank personnel at the Alabama Department of Environmental Management and Baldwin County Health Department, particularly Camilla English, Suzie Rice and Byron Webb, for sample collection. JDL was supported by NSF’s Research Experience for Undergraduates program and by a graduate fellowship from the Baldwin County Health Department, particularly Camilla English, Suzie Rice and Byron Webb, for sample collection. JDL was supported by NSF’s Research Experience for Undergraduates program and by a graduate fellowship from the Baldwin County Health Department, particularly Camilla English, Suzie Rice and Byron Webb, for sample collection. JDL was supported by NSF’s Research Experience for Undergraduates program and by a graduate fellowship from the Baldwin County Health Department, particularly Camilla English, Suzie Rice and Byron Webb, for sample collection.


