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Abundance and vertical flux of *Pseudo-nitzschia* in the northern Gulf of Mexico

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ABSTRACT: Many species of the ubiquitous pennate diatom genus *Pseudo-nitzschia* have recently been discovered to produce domoic acid, a potent neurotoxin which causes Amnesic Shellfish Poisoning (ASP). *Pseudo-nitzschia* spp. were extremely abundant (up to 10^8 cells l^{-1} ; present in 67% of 2195 samples) from 1990 to 1994 on the Louisiana and Texas, USA, continental shelves and moderately abundant (up to 10^5 cells l^{-1} ; present in 18% of 192 samples) over oyster beds in the Terrebonne Bay estuary in Louisiana in 1993 and 1994. On the shelf there was a strong seasonal cycle with maxima every spring for 5 yr and sometimes in the fall, which were probably related to river flow, water column stability, and nutrient availability. In contrast, in the estuary there was no apparent seasonal cycle in abundance, but the time series of data is relatively short and the environment highly variable. At one site on the shelf, where sediment traps were deployed from spring to fall and sampled at frequent intervals in both 1990 and 1991, approximately 50% of the *Pseudo-nitzschia* spp. cells present in the water sank into sediment traps. *Pseudo-nitzschia* spp. were also abundant in surficial sediments. The species of *Pseudo-nitzschia* present during this study were not routinely identified with the methods employed. However, toxin-producing *P. multiseries* has been identified previously from Galveston Bay, Texas, and cells from a bloom on the shelf in June 1993 were identified by scanning electron microscopy as *P. pseudodelicatissima*, which is sometimes toxic. Although there have been no known outbreaks of ASP in this area, historical data suggests that *Pseudo-nitzschia* spp. abundance may have increased on the shelf since the 1950s. It is hypothesized that the increase is due to doubling of the nutrient loading from the Mississippi and Atchafalaya rivers and increased eutrophication on the shelf.

KEY WORDS: *Pseudo-nitzschia* Gulf of Mexico Harmful algal bloom

INTRODUCTION

A group of common coastal diatoms from the genus *Pseudo-nitzschia* have recently been found to contain a potent neurotoxin, domoic acid (summarized in Villac et al. 1993b). A wide range of organisms, such as mussels, oysters, razor clams, anchovies, smelt, Dungeness crabs or rock crabs, concentrate domoic acid in their stomach contents and/or body tissue by direct or indirect consumption of *Pseudo-nitzschia* spp. (Buck et al.

1992, Fritz et al. 1992, Drum et al. 1993, Garrison & Walz 1993, Horner et al. 1993, Langlois et al. 1993, Roelke 1993, Work et al. 1993, Wood et al. 1994). From these organisms it can be passed on to higher trophic levels such as humans or marine birds (Villac et al. 1993b).

The toxicity of *Pseudo-nitzschia multiseries* (formerly *Nitzschia pungens* var. *multiseries*, Hasle 1995) was first demonstrated when 107 people became ill and 3 died from eating mussels cultured on Prince Edward Island, Canada (Bates et al. 1989, Todd 1993). Since one of the characteristic symptoms is permanent short-term memory loss, the syndrome has been called

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Amnesic Shellfish Poisoning (ASP). Subsequently, sea-bird deaths off the coast of California (USA) in 1991 and human illness after consuming razor clams in Washington State (USA) were related to the occurrence of high numbers of *Pseudo-nitzschia australis* (Buck et al. 1992, Fritz et al. 1992, Horner & Postel 1993, Work et al. 1993). Several other species of *Pseudo-nitzschia* produce domoic acid, but not all species nor all clones of sometimes toxic species produce it (Fryxell et al. 1990, 1991, Martin et al. 1990, Villac et al. 1993a, b, Lundholm et al. 1994) and production may be dependent on environmental conditions (Bates et al. 1991, Bates & Douglas 1993, Lewis et al. 1993, Smith et al. 1993, Pan et al. 1996a, b). Thus, the widespread occurrence of potentially toxic *Pseudo-nitzschia* spp. poses a significant potential human health problem and it is essential to determine the distribution of *Pseudo-nitzschia* spp. and the environmental factors which control them in order to predict toxic outbreaks.

During routine sampling for other purposes it was observed that *Pseudo-nitzschia* spp. were abundant in the water column, sediment traps, and surficial sediments on the Louisiana (USA) continental shelf in an area influenced by the Mississippi River (Dortch et al. 1992). Sampling on the Louisiana and Texas shelf was continued, so that there is now a 5 yr time series of data. Because toxic forms of *Pseudo-nitzschia* were also identified in Galveston Bay (Fryxell et al. 1990, 1991), additional sampling was begun at 3 estuarine locations with extensive oyster beds, for which there are now approximately 1.5 yr of data.

Harmful algal blooms (HABs) have become a major problem in the coastal waters of many countries, with serious economic and human health consequences. A major need is to 'determine and parameterize the environmental factors that govern the initiation, growth, maintenance, dissipation and impact of HABs' (Anonymous 1995, p. 4), but this has been difficult due to the sporadic and ephemeral nature of their occurrence.

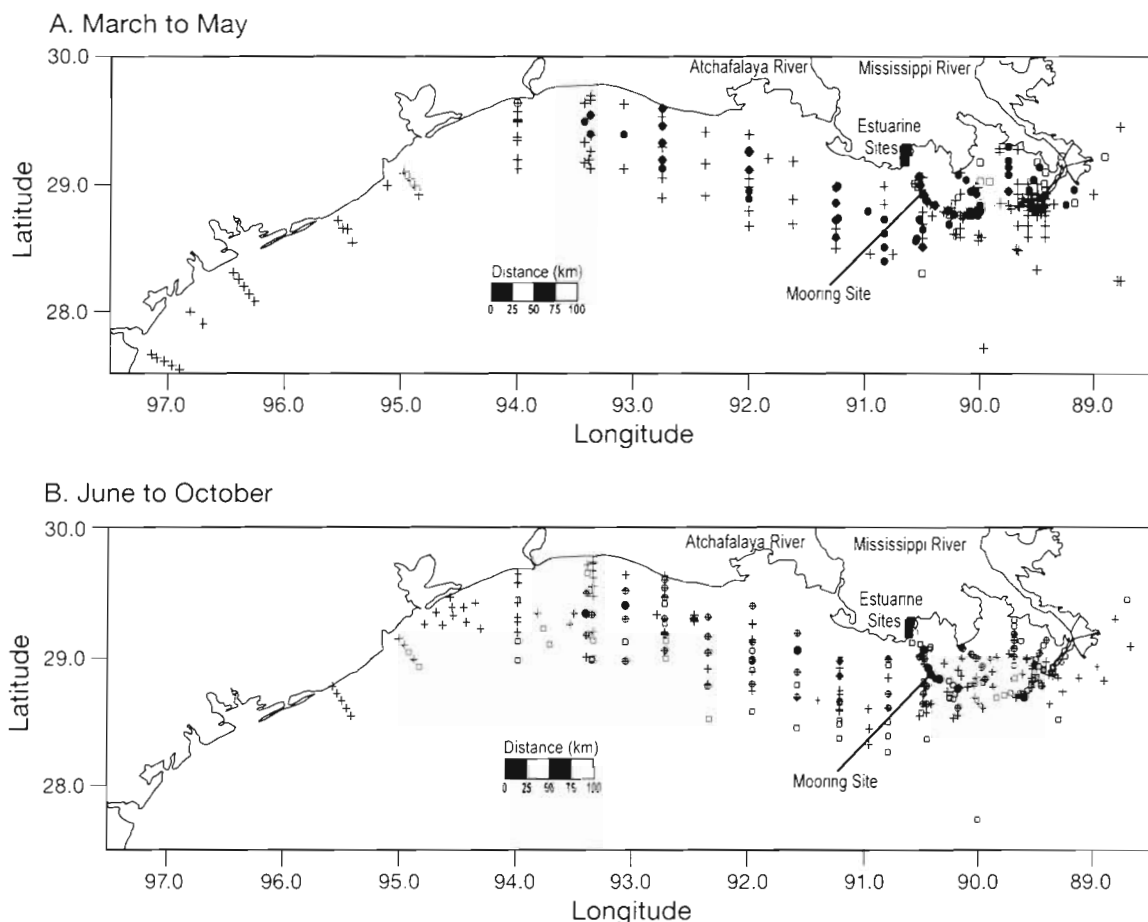


Fig. 1. *Pseudo-nitzschia* spp. Composite seasonal distribution maps along the Louisiana/Texas shelf. (A) March to May, $n = 335$; % frequency [number of samples with *Pseudo-nitzschia* spp. (total number of samples) $^{-1} \times 100$] = 92.2. (B) June to October, $n = 502$; % frequency = 53.6. (□) Stations at which *Pseudo-nitzschia* spp. were never observed, (+) stations at which *Pseudo-nitzschia* spp. were present but numbers were $<10^6$ cells l^{-1} , (●) stations at which *Pseudo-nitzschia* spp. abundance $>10^6$ cells l^{-1}

Clearly, *Pseudo-nitzschia* spp. are abundant in the northern Gulf of Mexico and, as a result of the influence of the Mississippi River and the proximity of the oligotrophic Gulf of Mexico, Louisiana and Texas coastal waters experience extreme ranges of all environmental variables. Surface salinities can vary from 0 to >36‰ within relatively short distances, horizontally. Vertically, salinity differences are also often quite large, so that except in very shallow areas, in the winter, or in areas distant from the Mississippi River the region is also characterized by an extremely stable water column. Dissolved inorganic nitrogen (DIN) and silicate concentrations often exceed 100 µM in or near the mouth of the Mississippi River, but drop to low, and sometimes undetectable, concentrations within short distances (<100 km). Due to the wide range of environmental conditions and frequent occurrence of *Pseudo-nitzschia* spp., this is an ideal area for examining the relationship between abundance and environmental factors.

This report describes the spatial and temporal variability of *Pseudo-nitzschia* spp. abundance and the temporal variability of *Pseudo-nitzschia* spp. vertical flux into sediment traps and abundance in surficial sediments, and begins to examine the environmental factors which influence *Pseudo-nitzschia* spp. distributions.

METHODS

Field sampling. Cruises were conducted along the Louisiana/Texas, USA, shelf from 1990 to 1994 (Fig. 1), as outlined in Table 1. Eight cruises, spanning 3 seasons, focused solely on the Mississippi River plume (MR plume, Table 1) and only a few samples were taken west of the Atchafalaya River. Six cruises traversed the Louisiana shelf between Southwest Pass of the Mississippi River and the Louisiana/Texas border (LA shelf); all but 1 cruise was in the middle of summer. Four cruises covered the western half of the Louisiana shelf (from slightly east of the Atchafalaya river) and approximately half of the Texas shelf (LA/TEX shelf), focusing on the extended plumes of the Mississippi and Atchafalaya Rivers and the coastal current. Finally, from 1990 to 1994, an offshore transect through an area of persistent bottom water hypoxia (Hypoxia transect; Rabalais et al. 1991, 1992, 1994) was made approximately monthly. In 1990 and 1991 samples were taken at 5 stations, including a station with an instrument and sediment trap mooring (see below), and from 1992 to 1994 samples were taken only at the station with the mooring. Sampling was conducted at the mooring location whenever possible on other cruises or when servicing the mooring, so data from this location represent the most detailed time series. Water samples were

Table 1. Cruises on the Louisiana/Texas continental shelf. See 'Methods: field sampling' for explanation of sampling location. Hydrographic, nutrient, and chlorophyll data are contained in the data sources. N. N. Rabalais, R. E. Turner & W. J. Wiseman Jr unpubl. (Rabalais et al. unpubl.) data are deposited with the NOAA Nutrient Enhanced Coastal Ocean Productivity (NECOP) Data Management Program (NDMP; Hendee 1994) and the National Oceanographic Data Center (NODC). N. N. Rabalais & R. E. Turner unpubl. data are deposited with NODC. *Phytoplankton data deposited in NDMP and NODC; **data deposited in NODC

Date	Sampling location	Number of samples	Data source
Jul–Aug 1990	MR plume	80*	Hitchcock & Whitledge (1992)
Jul 1990	LA shelf	88*	Rabalais et al. (unpubl.)
Aug 1990	MR plume	66	Rabalais et al. (unpubl.)
Oct 1990	MR plume	46	Toon & Dagg (1991)
18 Mar–16 Nov 1990	Hypoxia transect	125*	Rabalais et al. (unpubl.)
Mar 1991	MR plume	77*	Hitchcock & Whitledge (1992)
Jul 1991	LA shelf	94*	Rabalais et al. (unpubl.)
Sep 1991	MR plume	50	Toon & Dagg (1992a)
2 Feb–7 Dec 1991	Hypoxia transect	119*	Rabalais et al. (unpubl.)
Apr 1992	LA/TEX shelf	79**	Rabalais & Turner (unpubl.)
May 1992	MR plume	116*	Toon & Dagg (1992b)
Jul 1992	LA shelf	123*	Rabalais et al. (unpubl.)
Oct 1992	LA/TEX shelf	79**	Rabalais & Turner (unpubl.)
23 Feb–27 Oct 1992	Hypoxia transect	63*	Rabalais et al. (unpubl.)
Apr 1993	MR plume	74*	Toon & Dagg (1993)
Apr 1993	LA/TEX shelf	93**	Rabalais & Turner (unpubl.)
Apr 1993	LA shelf	138*	Rabalais et al. (unpubl.)
Jul 1993	LA/TEX shelf	130**	Rabalais & Turner (unpubl.)
Jul 1993	LA shelf	124*	Rabalais et al. (unpubl.)
19 Mar–18 Nov 1993	Hypoxia transect	74*	Rabalais et al. (unpubl.)
Apr 1994	MR plume	120**	Rabalais & Turner (unpubl.)
25 Jan–20 Oct 1994	Hypoxia transect	43*	Rabalais et al. (unpubl.)

usually obtained with Niskin bottles, after selecting appropriate depths based on hydrographic data obtained with a CTD (either Seabird SBE or Hydrolab Surveyor II or 3). However, due to the extreme vertical gradients in this region, surface samples were often taken with a plastic bucket. Nutrient, chlorophyll *a*, and suspended particulate matter (SPM) concentrations were measured on the same samples as the phytoplankton. Chlorophyll *a* was measured fluorometrically after filtration onto a Whatman GF/F filter and extraction with 40:60 DMSO:90% acetone, as modified from Parsons et al. (1984). Nitrate, nitrite, ammonium, silicate, and phosphate were measured using either a Technicon AutoAnalyzer II or an Alpkem RFA/2 Rapid Flow Analyzer. When possible, samples were analyzed on board ship, but if necessary they were frozen and analyzed later in the lab. SPM was measured gravimetrically after filtration onto weighed Whatman GF/F filters.

Sediment traps, designed according to Prior et al. (1987), were deployed on a mooring with other instruments (Rabalais et al. 1992, 1994, Qureshi 1995) at a station in 20 m water depth in the center of a region where bottom water hypoxia is often observed in the summer (Fig. 1). Sediment trap data were analyzed only for 1990 and 1991. One trap was mounted at 5 or 7 m (depending on the year), above the usual location of the pycnocline (approx. 10 m), and the other was mounted in the lower water column where hypoxia develops in the summer (at 15 or 16 m, depending on the year). Weather permitting, the traps were serviced by divers at time intervals ranging from 1 to 3 wk. In 1990 the traps contained brine (45‰) and in 1991 they contained brine and 2.0% glutaraldehyde. Surficial sediments and overlying water were obtained from syringe cores taken either by divers or from box cores with overlying water still present.

Sampling in the Terrebonne Bay estuary over 3 oyster beds (Fig. 1) was conducted weekly from January 28, 1993 until December 29, 1994, with a hiatus from April 1, 1993 to August 26, 1993. The waters were quite shallow (0.3 to 1 m) and generally well mixed, so that only 1 sample was taken on each date at each site using a hand-operated peristaltic pump with the intake located just above the oyster bed. Salinity was measured with a Reichert refractometer.

Phytoplankton identification and enumeration. Phytoplankton in the water were preserved in 0.5% glutaraldehyde and refrigerated for 1 to 24 h. The samples were size-fractionated by filtration onto 0.2, 3, and 8 μm polycarbonate filters, with 0.03% proflavine hemisulfate used to stain the latter 2 fractions, and then the filters were mounted in immersion oil (Murphy & Haugen 1985, Shapiro et al. 1989). The 0.2 to 3 μm fraction was counted immediately on board ship, the

3 to 8 μm fraction was counted immediately if possible or otherwise refrigerated and counted within several days of returning. The slide with the >8 μm fraction was frozen and counted later. All samples were counted using an Olympus BH2-RFCA epifluorescence microscope (EFM) with blue and green excitation light, and, when necessary, transmitted light. The phytoplankton were identified to the nearest possible taxon. As described in detail by Buck et al. (1992), the EFM is not usually used for enumerating the entire size range of phytoplankton, but diatoms can certainly be identified to the genus and often species level. In this case the genus *Pseudo-nitzschia* was readily identified, but not the individual species, which usually require scanning electron microscopy (SEM).

Sediment trap material was split up to 5 or 6 times in a small Folsom Plankton Splitter immediately after collection. One of the splits was preserved with 2.5% glutaraldehyde and stored in a refrigerator. Samples were later split between 1 and 11 more times, depending on the amount of material present. Slides were made on an aliquot of the last split and counted in exactly the same manner as for the water column phytoplankton, except that in 1991 live and dead cells were distinguished on the basis of cell contents which were stained with proflavine. Chains of *Pseudo-nitzschia* were readily distinguished, but single cells were more problematic. The counts reported include only those single cells which were likely to be *Pseudo-nitzschia* and may be an underestimate.

The top 1 cm of water and the top 2 mm of sediment were removed from syringe cores using a core extruder. The sample was diluted to 20 ml in filtered seawater, made up to 2.5% glutaraldehyde, and stored in the refrigerator. The samples were split in a Folsom Plankton Splitter and slides were made and counted in the same manner as the sediment trap samples. Because short-term sedimentation rates are seasonally extremely variable (B. M. McKee unpubl. data), the time over which the top 2 mm was deposited is unknown and it is not possible to calculate flux to the sediment. The surficial sediment data are reported as cells area^{-1} and cannot be compared directly with fluxes into the sediment traps except when the data are expressed as % abundance.

Samples were collected for species identification during a *Pseudo-nitzschia* bloom in June 1993. Preserved samples were centrifuged, the frustules cleaned using nitric acid in a boiling water bath, and identified using SEM.

Statistics. Normality of the data was determined based on a consensus of results from normal probability plots, frequency histograms, measures of skewness and kurtosis, chi-squared tests, and the Kolmogorov-Smirnov 1-sample tests. *Pseudo-nitzschia* spp.

abundance data were often not normally distributed and sometimes could not be transformed to fit a normal distribution, due to the number of samples in which no *Pseudo-nitzschia* spp. were present (approx. 30% overall). Several approaches were taken in order to report results and comparisons in a statistically appropriate manner when the data were not normally distributed. Medians are reported as well as means and standard deviations (e.g. see Tables 3 & 4). Non-parametric tests were used when necessary, as indicated in the text. Results were considered significant when $p < 0.05$. Significant figures are reported according to Sokal & Rohlf (1969).

RESULTS

Abundance and taxonomy

Pseudo-nitzschia spp. were observed, at some time, everywhere on the Louisiana/Texas shelf (Fig. 1). The maximum concentration exceeded 10^8 cells l^{-1} (Table 2), and concentrations $>10^6$ cells l^{-1} were not unusual (7.2% of samples). *Pseudo-nitzschia* spp. were present, at some level, in almost 70% of samples collected on the shelf. They made up a large fraction (16%) of the diatoms (Table 2), which were the dominant group in terms of biomass (Dortch 1994).

Pseudo-nitzschia spp. were present, but much less abundant, in the Terrebonne Bay estuary. The maximum cell concentration was 3 orders of magnitude lower in the estuary in comparison with the shelf (Table 2). While *Pseudo-nitzschia* spp. cells were present in 18% of samples, their abundance relative to other diatoms was low ($<1\%$). However, the period of study was much shorter in the estuary than on the shelf, so that the full range of variation in the estuary is not known.

Much of the sampling on the shelf was conducted before the importance of *Pseudo-nitzschia* was widely known. Thus, no systematic effort was made to identify *Pseudo-nitzschia* to the species level. However, surface samples from a bloom at the mooring site on May

23, 1993 (7.85×10^6 cells l^{-1}) were digested to remove organic matter, leaving cleaned frustules, and examined by SEM. The species was identified as *Pseudo-nitzschia pseudodelicatissima* (Fig. 2).

Temporal and spatial variation on the shelf

The best temporal coverage was at the mooring site, which was sampled approximately monthly at 4 depths for 5 yr (Fig. 3, Table 3). Despite considerable variability (Fig. 3), there were significant differences in *Pseudo-nitzschia* spp. abundance between the seasons both in the upper and lower layers (Kruskal-Wallis test). The only seasons for which the *Pseudo-nitzschia* spp. abundances are not significantly different from each other (Mann-Whitney *U*-test) are winter-fall in the upper layer and winter-spring and winter-fall in the lower layer, probably because there are so few data in winter (Table 3). Thus, the annual maximum in *Pseudo-nitzschia* spp. abundance occurred in spring, a minimum was observed in summer and a second peak occurred in the fall (Table 3). Composite surface maps indicate that the seasonal difference in abundance was apparent across the entire Louisiana/Texas shelf (Fig. 1). The greatest likelihood of encountering high numbers of *Pseudo-nitzschia* spp. ($>10^6$ cells l^{-1}) was in the spring when it was present most often in surface samples (Fig. 1A). High concentrations were observed less often in the summer/fall months and they were not present in as many samples (Fig. 1B). The % abundance of *Pseudo-nitzschia* spp. relative to total diatoms was greatest in spring and fall (Table 3). The fall maximum in % abundance occurred because total diatoms decreased but *Pseudo-nitzschia* spp. did not.

Composite surface maps also show that *Pseudo-nitzschia* spp. were widely distributed throughout the Louisiana/Texas coastal zone (Fig. 1). High cell concentrations appeared to occur in the areas directly influenced by the Mississippi and Atchafalaya rivers and were not found farther to the south along the Texas coast, but this may have been a result of de-

Table 2. *Pseudo-nitzschia* spp. Summary of abundance in the Louisiana/Texas shelf and Terrebonne Bay estuary. % Frequency: number of samples with *Pseudo-nitzschia* > 0 /total number of samples; n: total number of samples. % Abundance: number of *Pseudo-nitzschia* spp./total number of diatoms for samples with number of *Pseudo-nitzschia* spp. > 0 ; n: number of samples with *Pseudo-nitzschia* spp. > 0

Area	Maximum concentration (cells l^{-1})	% Frequency	% Abundance	
			Range	Mean
LA/TEX shelf	1.02×10^8	66.7 n = 2195	0.01–100 n = 1389	16.5
Terrebonne Bay estuary	1.09×10^5	18.2 n = 192	0.01–4.67 n = 35	0.4

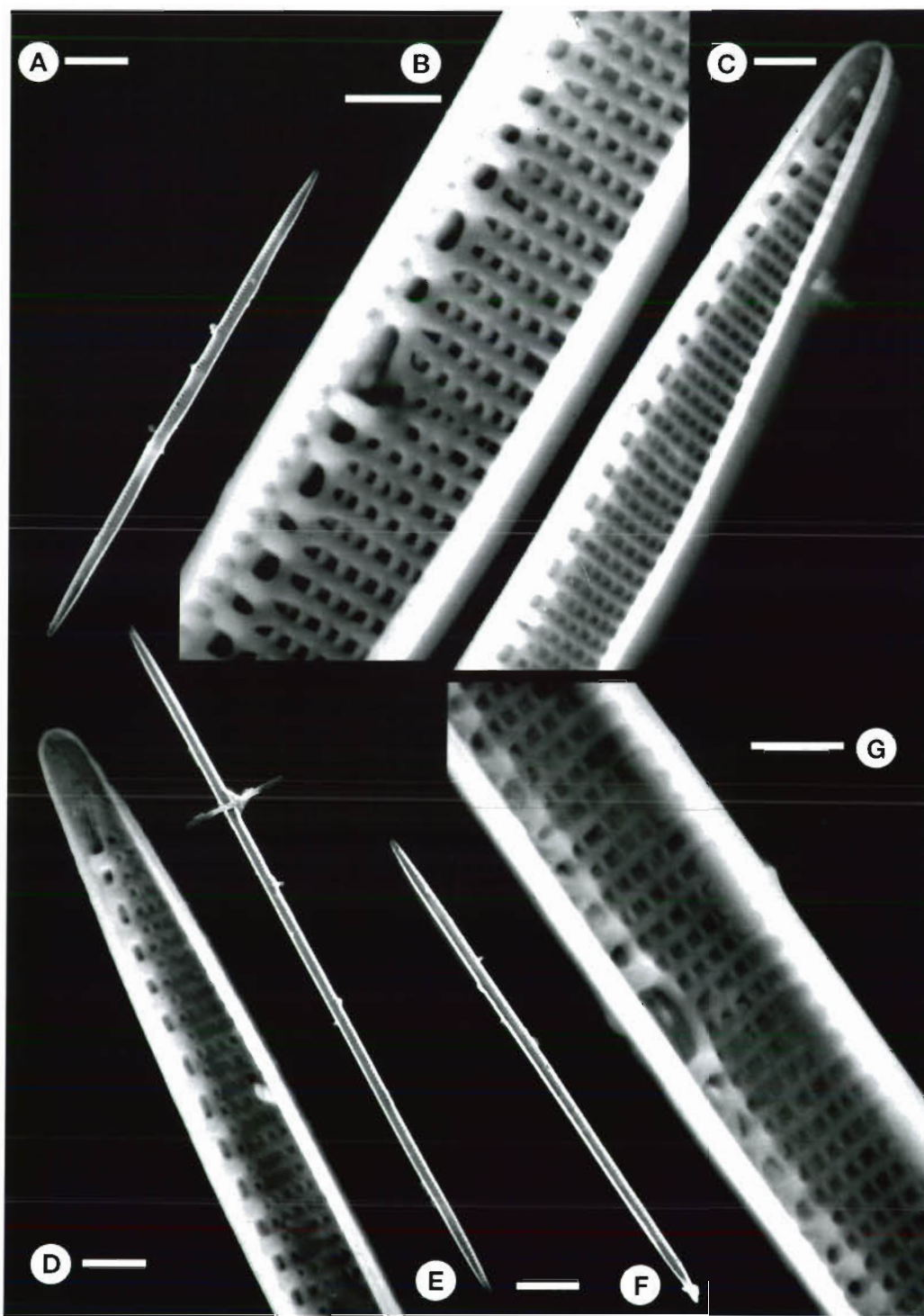


Fig. 2. *Pseudo-nitzschia*. Scanning electron micrographs from surface water sample taken during a bloom at the mooring site, June 23, 1993. All are inside views with siliceous arches (fibulae) overlying slit or raphe at juncture of mantle and valve face on left, except where noted. Note uniformity in width and shape within 1 species, but not in length. (A) *P. pseudodelicatissima*, orientation of whole valve, 86 μm in length, scale bar = 10 μm . (B) Detail of central area of same valve showing wider distance between 2 central fibulae and square appearance of pores in striae, scale bar = 1 μm . (C) Tapered pole of same valve, showing terminus of raphe, scale bar = 1 μm . (D) *P. pseudodelicatissima*, oblique view of pole of longer valve showing slit in raphe running under fibulae, scale bar = 1 μm . (E) Orientation of same valve, 122 μm in length, scale bar = 10 μm . (F) *P. pseudodelicatissima*, orientation of valve, raphe on right, 88.5 μm in length, same scale as E. (G) Detail of central area of same valve, showing vertical nature of mantles, central nodule between the origins of the 2 raphe systems, typical valve structure, scale bar = 1 μm

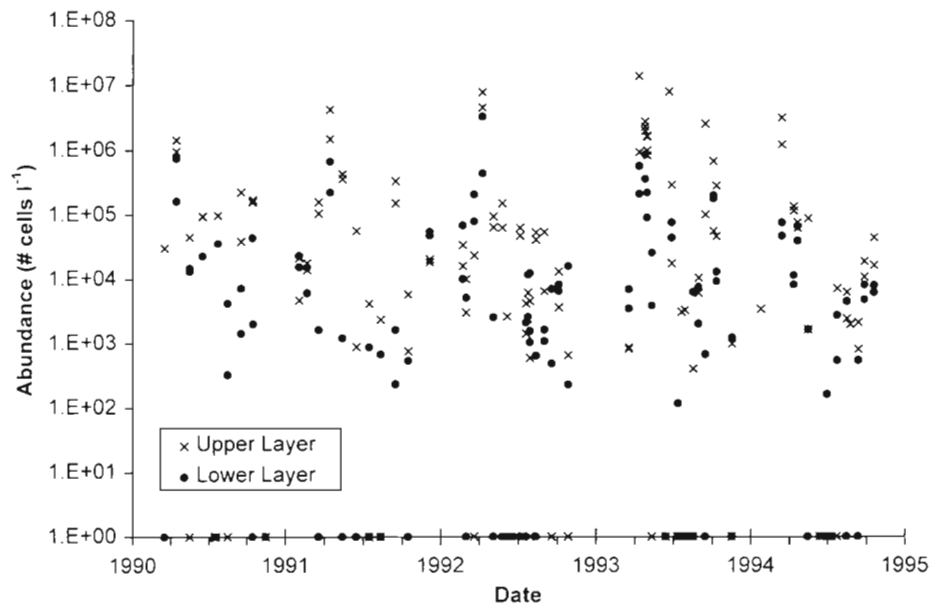


Fig. 3. *Pseudo-nitzschia* spp. Time series of abundance at the mooring site from 1990 to 1994. Upper and lower layer indicate samples above and below 10 m, the approximate depth of the pycnocline when the water column is stratified. $n = 287$, % frequency (see Fig. 1) = 68.3. Y-axis = abundance + 1

creased sampling density. For any given cruise, the distribution of *Pseudo-nitzschia* spp. was quite patchy, with little similarity between cruises even in the same seasons (data not shown).

Pseudo-nitzschia spp. were often abundant in bottom waters. In fact, the highest concentration observed (Table 2) was in a near-shore bottom sample (10.5 m) on April 12, 1994. In these shallow, turbid shelf waters, the euphotic zone is often <5 m, although it is possible for it to be as deep as 20 m (R. E. Turner pers. comm.). At those stations with *Pseudo-nitzschia* spp. in both the upper and lower layers, the concentrations in the lower layer were positively and significantly related to the concentrations in the surface layer. Thus, it seems likely that such high numbers are usually due to sinking rather than *in situ* growth.

Vertical flux of *Pseudo-nitzschia* spp. on the shelf

Pseudo-nitzschia spp. often comprised a large portion of the diatoms which were counted in sediment trap material at both depths and in surficial sediments in 1990 and 1991 (Table 4). Because diatoms made up the largest fraction of the directly sedimenting phytoplankton biomass (Dortch et al. 1992), *Pseudo-nitzschia* spp. contributed substantially to the vertical flux of phytoplankton. There was selective sinking of *Pseudo-nitzschia* into traps in both 1990 and 1991, as indi-

cated by the significantly different median % abundance in the water column and traps (Mann-Whitney *U*-test). Although the % abundance was lower in surficial sediments than traps, the difference was only significant in 1991 (Student's *t*-test).

It is possible to distinguish between apparently dead cells, lacking contents, and living cells, with stainable contents, using epifluorescence microscopy. By these criteria approximately 90% of the *Pseudo-nitzschia* spp. cells in the traps in 1991 were dead (Table 4). Moreover, most of the *Pseudo-nitzschia* spp. cells in

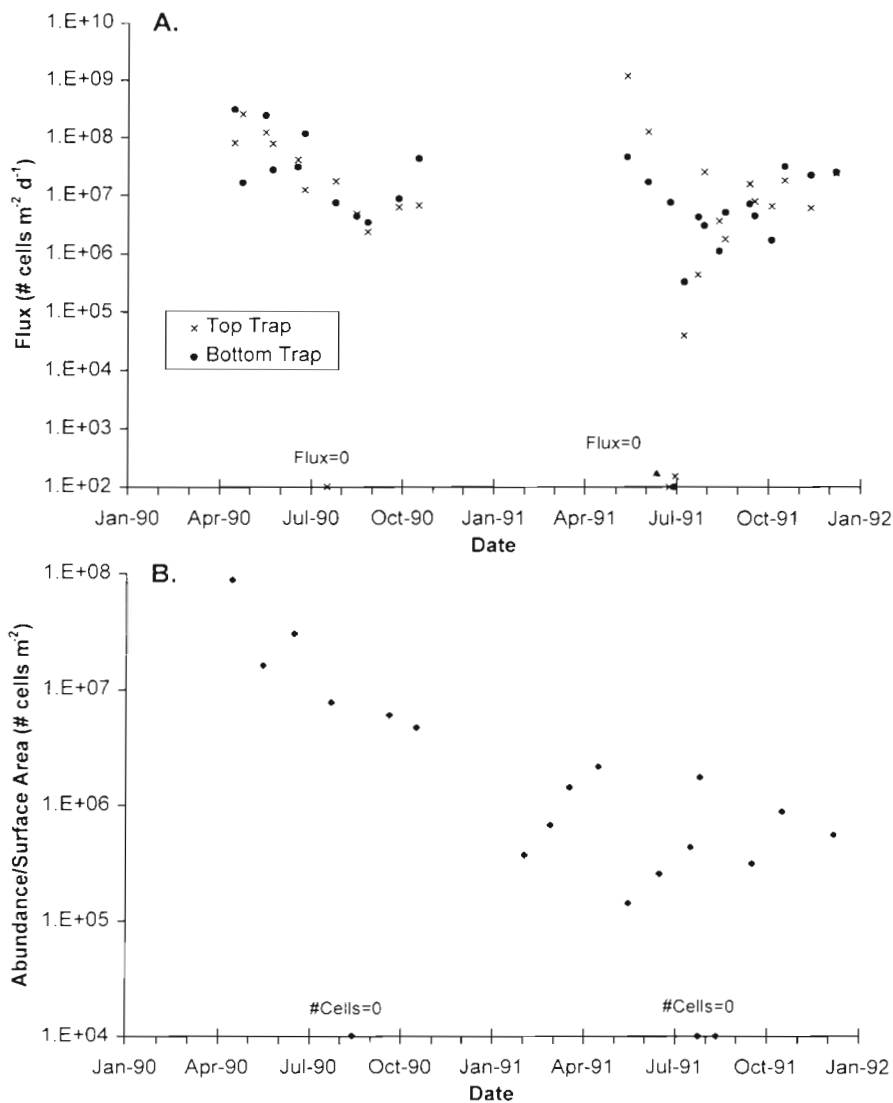
Table 3. *Pseudo-nitzschia* spp. Seasonal variation in abundance (cells l^{-1}) and % abundance [number of *Pseudo-nitzschia* (number of diatoms) $^{-1} \times 100$] in the water at the mooring site. n : number of samples. Seasons based on changes in water column structure and frequency of mixing events. See Fig. 3 for description of upper and lower water layers

Season		Abundance		% Abundance	
		Upper	Lower	Upper	Lower
Spring (Mar–May)	Mean	1.24×10^6	2.11×10^5	17.1	19.7
	Median	1.31×10^5	2.02×10^4	5.2	11.0
	SD	2.49×10^6	5.42×10^5	25.4	25.4
	n	43	40	39	36
Summer (Jun–Aug)	Mean	1.41×10^5	4.30×10^3	5.7	2.7
	Median	2.00×10^2	0	0.3	0.0
	SD	9.96×10^5	1.24×10^4	12.2	6.2
	n	62	61	56	53
Fall (Sep–Oct)	Mean	1.74×10^5	1.8×10^4	17.2	5.7
	Median	3.84×10^4	2.01×10^3	10.2	2.0
	SD	4.69×10^5	4.78×10^4	22.1	8.5
	n	29	29	29	29
Winter (Nov–Feb)	Mean	1.25×10^4	2.19×10^4	2.1	2.4
	Median	1.49×10^4	1.53×10^4	0.8	0.8
	SD	1.06×10^4	2.39×10^4	2.3	3.2
	n	12	11	11	11

Table 4. *Pseudo-nitzschia* spp. in the water column, sediment traps, and surficial sediments in 1990 and 1991. n: number of samples

		1990		1991		
		% Cells in chains ^a	% Abundance ^b	% Cells in chains ^a	% Live cells ^c	% Abundance ^b
Water column	Mean		10.8			14
	Median		1.9			2
	SD		20.34			25.8
	n		85			45
Traps	Mean	23	24	26	9.7	25
	Median	3	10	15	8.2	14
	SD	35.4	25.4	28.4	8.76	27.5
	n	32	34	27	30	25
Surficial sediment	Mean	13	17	14	15	4.8
	Median	1	6	0	0	3.2
	SD	21.6	20.8	21.9	33.5	5.64
	n	7	8	11	11	13

^aNumber of *Pseudo-nitzschia* spp. cells in chains (total number of *Pseudo-nitzschia* spp. cells)⁻¹ × 100
^bNumber of *Pseudo-nitzschia* spp. cells (number of diatom cells)⁻¹ × 100
^cNumber of *Pseudo-nitzschia* spp. cells with stainable contents (total number of *Pseudo-nitzschia* spp. cells)⁻¹ × 100

Fig. 4. *Pseudo-nitzschia* spp. Time series from 1990 to 1991 of (A) flux into sediment traps and (B) abundance in surficial sediments at the mooring site. Flux or abundance equal to zero indicated at appropriate date

trap material were not in their characteristic chains in either 1990 or 1991 (Table 4), but occurred as single cells, which were difficult to identify, so that these counts may underestimate the vertical flux of *Pseudo-nitzschia* spp. There was a significant and linear relationship between the % living cells and the % cells in chains in 1991, suggesting that the chain structure is lost when the cells die.

The maximum flux was observed in the spring and fall in both 1990 and 1991 (Fig. 4A), indicating a seasonal pattern quite similar to that of *Pseudo-nitzschia* spp. abundance in the water (Fig. 3). Consequently, there is a significant, linear relationship between *Pseudo-nitzschia* spp. flux and *Pseudo-nitzschia* spp. concentration in the appropriate water layer ($r^2 = 0.75$, $n = 56$). In contrast, there was no apparent seasonal cycle in the concentration of *Pseudo-nitzschia* spp. cells in surficial sediments (Fig. 4B). The apparent lack of a seasonal cycle in abundance in the top 2 mm of

sediment may result because the time period of accumulation is unknown and probably variable, due to variable sediment accumulation rates.

Temporal and spatial variation in the Terrebonne Bay estuary

Unlike the shelf, a seasonal cycle in *Pseudo-nitzschia* abundance was not apparent in the Terrebonne Bay estuary data (Fig. 5A). This environment is extraordinarily variable, as indicated by the large changes in salinity over short time periods (Fig. 5B), due primarily to changes in wind forcing and runoff rather than tides (Reed 1995). Although the 3 stations were located close together, the % frequency of occurrence of *Pseudo-nitzschia* spp. in all samples decreased from 23.4% at Stn 3, nearest to Terrebonne Bay, to 12.5% at Stn 1, further inland.

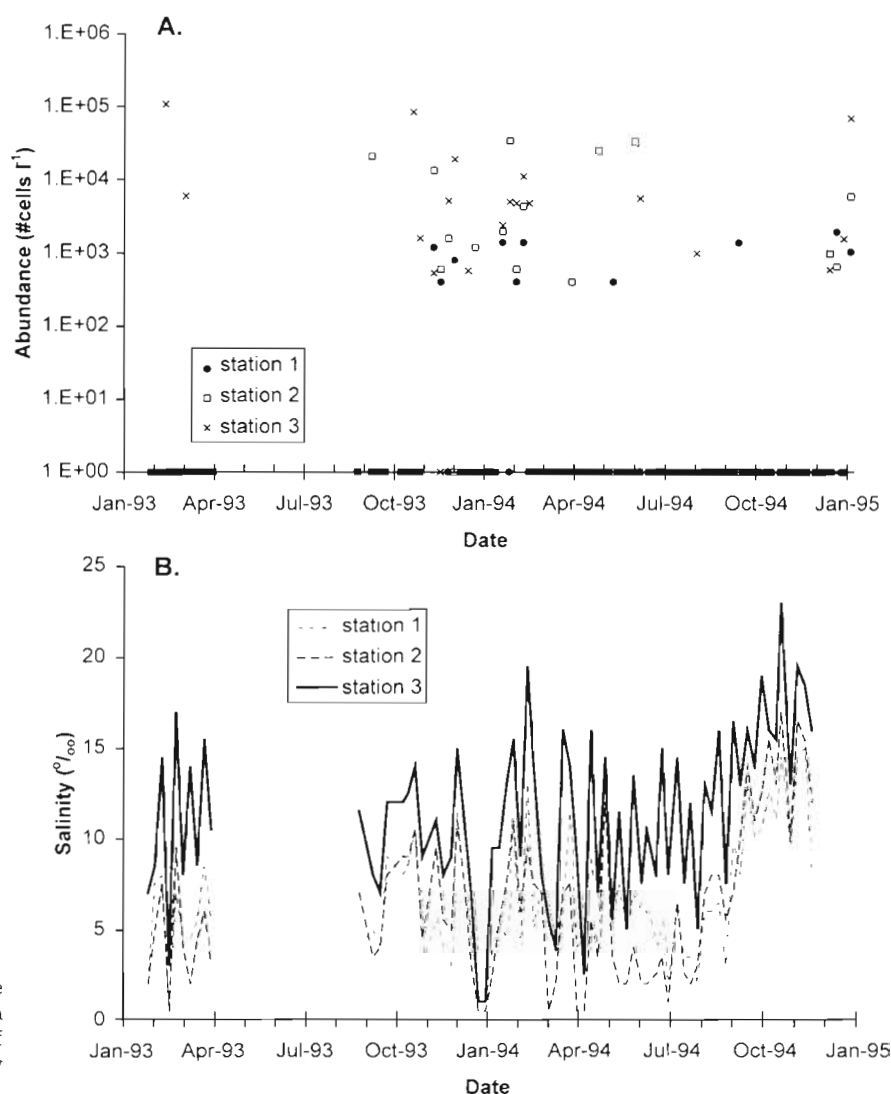


Fig. 5. *Pseudo-nitzschia* spp. Time series in Terrebonne Bay estuary from January 1993 to December 1994 of (A) abundance, $n = 192$, % frequency (see Fig. 1) = 18.2; and (B) salinity

Relationship of *Pseudo-nitzschia* abundance with environmental factors

Pseudo-nitzschia spp. were found in high numbers over the entire salinity range (Fig. 6A). Although the highest numbers tended to occur at intermediate salinities and the lowest numbers at low salinities, this genus appeared to be extremely halotolerant. Similarly, *Pseudo-nitzschia* spp. are found over the full range of temperatures observed in this region (Fig. 6B).

Because *Pseudo-nitzschia* spp. abundance was not normally distributed and often could not be transformed to fit a normal distribution (see 'Methods: Statistics'), 2 approaches were taken to examine the relationship between *Pseudo-nitzschia* spp. abundance and environmental conditions (Table 5); only data from the well-mixed estuary and the upper water layer (0 to 1 m) on the shelf were considered: (1) The environ-

mental data for the upper water layer were separated into 2 groups, defined by the presence or absence of *Pseudo-nitzschia* spp. cells. Unlike *Pseudo-nitzschia* spp. abundance, the environmental data either were normally distributed or could be transformed to fit a normal distribution. Student's *t*-test was used to compare the means of environmental data between the 2 groups (Table 5). (2) Using the same data, 2 non-parametric methods of calculating correlation coefficients were used to examine the relationship between *Pseudo-nitzschia* spp. abundance and various environmental variables: Spearman's rank correlation coefficient and Kendall's tau, based on concordance. Almost identical results were obtained for the 2 correlation coefficients, so that only the Spearman's rank correlation coefficients are reported (Table 5).

According to both approaches *Pseudo-nitzschia* spp. abundance is negatively related to $\Delta\sigma_t$ (difference in

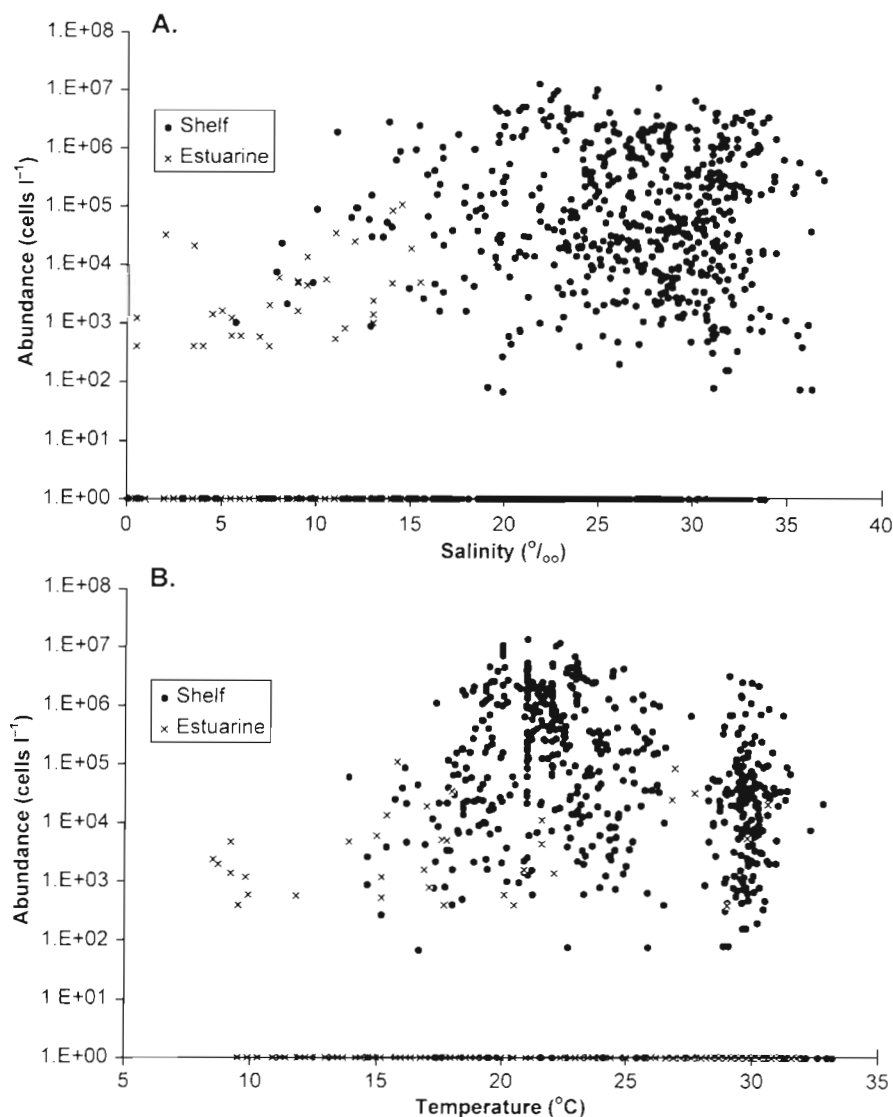


Fig. 6. *Pseudo-nitzschia* spp. abundance in the surface layer of shelf waters and in Terrebonne Bay estuary as a function of (A) salinity, $n = 1025$, % frequency (see Fig. 1) = 59.7; and (B) temperature, $n = 856$, % frequency = 59.5

Table 5. Mean environmental conditions \pm standard deviation (number of samples) when *Pseudo-nitzschia* spp. are present or absent and Spearman's rank correlation coefficient for *Pseudo-nitzschia* spp. abundance and environmental variables (n = sum of the 2 n numbers for presence/absence analysis). Includes all estuarine data and shelf data from 0 to 1 m depth. n varies because data are not available for all variables for all samples. *Means are significantly different (Student's *t*-test, non-normal data transformed^{a,b,c,d} to test significance; means and standard deviations are presented using untransformed data); **Spearman's rank correlation coefficients are significant

	Variable	<i>Pseudo-nitzschia</i> spp.		Spearman correlation Coefficients
		Absent	Present	
Hydrography	Salinity (‰)	15.7 \pm 9.27(397)	* 24.07 \pm 7.457(552)	0.4110**
	Temperature (°C)	26.0 \pm 6.01(340)	* 24.41 \pm 4.886(462)	-0.2273**
	$\Delta\sigma_t$	9.7 \pm 5.40(156)	* 6.10 \pm 4.942(285)	-0.2714**
Turbidity and light	Transmittance (%) ^a	82.6 \pm 10.17(122)	79.3 \pm 15.94(226)	-0.1322**
	Secchi disk depth (m) ^b	3.8 \pm 3.49(62)	3.44 \pm 3.004(148)	-0.0903
	Suspended particulate matter (mg l ⁻¹) ^c	13 \pm 18.9(39)	10.1 \pm 8.90(81)	-0.1542
Location	Longitude	90.72 \pm 0.952(411)	* 91.27 \pm 1.689(578)	-0.0229
	Latitude	29.059 \pm 0.2294(411)	* 28.973 \pm 0.3078(578)	-0.2145**
Nutrients	Phosphate (μ M) ^d	0.59 \pm 0.794(218)	* 0.361 \pm 0.4661(437)	-0.1385**
	Silicate (μ M) ^d	16.4 \pm 26.37(221)	* 7.0 \pm 10.35(442)	-0.2792**
	DIN (μ M) ^{d,e}	16.1 \pm 24.47(210)	* 8.5 \pm 11.74(413)	-0.1593**
	Si/DIN (by moles) ^d	2.11 \pm 3.234(209)	2.13 \pm 3.645(412)	-0.1792**
Algal biomass	Chlorophyll <i>a</i> (μ g l ⁻¹) ^d	8.3 \pm 9.98(409)	* 7.3 \pm 9.47(577)	0.05612
	Total autotrophs (cells l ⁻¹) ^d	8.3 $\times 10^8$	* 2.19 $\times 10^8$	-0.4160**
		$\pm 10.78 \times 10^8(402)$	$\pm 3.658 \times 10^8(568)$	
	Diatoms (cells l ⁻¹) ^a	3.5 $\times 10^6$	3.52 $\times 10^6$	0.2354**
		$\pm 7.33 \times 10^6(402)$	$\pm 6.057 \times 10^6(568)$	
^a <i>t</i> -test performed on transformed data (T = x^3)		^d <i>t</i> -test performed on transformed data (T = log(x + 0.1))		
^b <i>t</i> -test performed on transformed data (T = $x^{0.5}$)		^e DIN = nitrate + nitrite + ammonium		
^c <i>t</i> -test performed on transformed data (T = $x^{0.33}$)				

σ_t from surface to bottom), which is an approximation of the water column stability. The observed positive relationship with salinity and negative relationship with temperature (Table 5) would be expected if decreased stability was a major factor determining abundance. Based on comparisons of latitude and longitude where *Pseudo-nitzschia* spp. were observed, they are more likely to occur in the western portion of the sampling area and somewhat offshore. *Pseudo-nitzschia* spp. abundance was significantly and negatively related to all nutrient concentrations, including nitrate and ammonium, separately, which are not shown. In general there were no differences in the measures of turbidity (transmittance and SPM) or light (Secchi disk depth) when *Pseudo-nitzschia* spp. were present or absent, but there was less data available for these analyses. Finally, abundance of total autotrophs was negatively related to abundance of *Pseudo-nitzschia* spp., because small cyanobacteria are especially abundant in summer (Dortch 1994), when *Pseudo-nitzschia* spp. are not as likely to occur. For other variables, the 2 approaches gave different results.

DISCUSSION

Presence of *Pseudo-nitzschia* spp. in the northern Gulf of Mexico

Pseudo-nitzschia spp. are an abundant and often dominant diatom genus in Louisiana/Texas shelf waters. Maximum concentrations far exceed those reported for any other area (Villac et al. 1993b). Their concentrations in the Terrebonne Bay estuary are considerably lower than in shelf waters; although often present, *Pseudo-nitzschia* spp. also do not dominate the diatom flora as they do on the shelf. Similar results were obtained in Galveston Bay, Texas (Fryxell et al. 1990).

At present 3 species have been identified in this region: *Pseudo-nitzschia multiseries* (Fryxell et al. 1990), which is usually considered toxic, *P. pungens* (Fryxell et al. 1990), which is considered non-toxic, and *P. pseudodelicatissima* (this study), which is sometimes toxic (Martin et al. 1990). Fryxell et al. (1990, 1991) have hypothesized that there may be a suite of *Pseudo-nitzschia* spp. present in this area with differing growth

requirements and toxicities. In particular temperature appears to be a major factor determining species composition (Fryxell et al. 1990, Villac et al. 1993b, Hasle & Syvertsen 1996).

Role of environmental factors

On the shelf there is a clear seasonal cycle in both *Pseudo-nitzschia* spp. abundance and vertical flux, with a large peak in the spring and a smaller peak in the fall. The spring peak corresponds with the average maximum in river flow (Boyles & Humphries 1994). However, during the study period river flow peaked as early as January or as late as June and was often characterized by a series of maxima. Moreover, the fall bloom of *Pseudo-nitzschia* spp. always occurred during a period of low flow. Thus, riverine inputs alone cannot explain the seasonal cycle of *Pseudo-nitzschia* spp.

Another characteristic of the spring and fall periods is the passage of frontal systems and storms which, at least for a short time, break down the stratification, as indicated by data obtained from the instrument array on the mooring (Rabalais et al. 1992, 1994, Wiseman et al. 1992). Since *Pseudo-nitzschia* spp. are present when stability is lower (Table 5), mixing events may be an important mechanism in resupplying nutrients to the surface layer. Despite high nutrient inputs from the river, high nutrient concentrations are usually only observed near the river mouth, nutrient concentrations over the rest of the shelf can be quite low, and regenerated nutrients are surprisingly important (Turner & Rabalais 1991, Dortch & Whittedge 1992, Cotner & Gardner 1993, Bode & Dortch 1996). In the spring, the passage of fronts results in upwelling and corresponding increases in surface nutrients and chlorophyll (Dagg 1988). In the fall, mixing events would advect nutrient rich water from the hypoxic zone below the pycnocline (N. N. Rabalais, R. E. Turner & W. J. Wiseman Jr unpubl. data) up into the nutrient-depleted surface layer. Elsewhere, indirect evidence indicates that nutrient inputs stimulated the development of blooms of *Pseudo-nitzschia* spp. (Buck et al. 1992, Smith et al. 1993, Lange et al. 1994).

While the timing of *Pseudo-nitzschia* spp. blooms in the Louisiana/Texas coastal zone suggests nutrient stimulation, the significant negative relationship between *Pseudo-nitzschia* spp. presence and all nutrient concentrations would appear to argue against it (Table 5). In the field, highest cell numbers are often associated with the lowest nutrient concentrations. Thus, it may be difficult to establish a relationship between abundance and nutrients, based only on nutrient concentrations, when the critical processes of species-specific nutrient uptake and growth cannot

be measured. In mixed species cultures, *P. multiseriis* (formerly *P. pungens* f. *multiseriis*, Hasle 1995) tended to dominate under low light and at relatively low Si/N ratios (Sommer 1994), and in single species cultures, it grew well with all N sources except ammonium (Hillebrand & Sommer 1996).

The other difficulty is that a suite of species is being treated as a single entity. The very broad salinity and temperature ranges over which *Pseudo-nitzschia* spp. were observed suggest there is more than 1 species present. Autoecological studies with isolates from a variety of areas indicate that individual species/clones have much narrower temperature and salinity preferences (Fryxell et al. 1990, Reap 1991, Jackson et al. 1992, Hasle & Syvertsen 1996). It is likely that they will also have different nutrient requirements. Consequently, it will be necessary to identify the species present in all field samples in order to fully understand how environmental conditions affect the abundance of the *Pseudo-nitzschia* group and, thus, the potential for toxic events.

Historical data and eutrophication

Nitrogen and phosphorus inputs to the coastal zone from the Mississippi and Atchafalaya rivers have increased since the 1950s (Turner & Rabalais 1991). This has led to increasing eutrophication in the coastal zone, as indicated by increased biogenic silica deposition in sediment cores (Turner & Rabalais 1994, Rabalais et al. 1996). Eutrophication has also increased in several Louisiana estuaries (Rabalais et al. 1995, 1996, Parsons 1996). If there is a link between nutrient availability and *Pseudo-nitzschia* spp. abundance, then abundance should have increased both on the shelf and in estuaries, at least in proportion to diatom abundance. Further, Si inputs have decreased substantially as N inputs have increased, decreasing the Si/N ratio of riverine nutrient input by a factor of 4 (Turner & Rabalais 1991). Since mixed species culture experiments indicate that *P. multiseriis* (formerly *Nitzschia pungens* f. *multiseriis*, Hasle 1995) dominates when Si/N ratios are low (Sommer 1994), the relative abundance of *Pseudo-nitzschia* spp. in comparison with total diatoms may have also increased.

Pseudo-nitzschia spp. have been observed in this area since the earliest studies in the 1950s and have been identified as *Nitzschia pungens*, *N. pungens* var *Atlantica*, and *N. seriata*. They have been observed in estuaries, such as Barataria Bay, Louisiana (Day et al. 1973, Hart 1979), Bay St. Louis, Mississippi (Housely 1976), Lake Calcasieu, Louisiana (Maples et al. 1982, 1983, Fay & Schnitzer 1984), and Galveston Bay, Texas (Fryxell et al. 1990) and on the northern Gulf of Mexico shelf (Simmons & Thomas 1962, Housely 1974, Fucik &

El-Sayed 1979). Most of these earlier studies give insufficient data to determine whether the abundance of *Pseudo-nitzschia* spp. has increased over time, although individual *Pseudo-nitzschia* species are sometimes listed as abundant.

Two studies on the shelf provide some quantitative data for comparison with data collected in 1990–1994. In the period 1955 to 1957, phytoplankton were identified in numerous samples from the Louisiana coastal zone off the Mississippi River delta (Simmons & Thomas 1962). Abundances of *Pseudo-nitzschia* spp. were generally much lower than observed at present in similar places and times. For example, the maximum observed concentration of *Pseudo-nitzschia* spp. was 2×10^5 cells l^{-1} at a salinity of 23.5‰ (Simmons & Thomas 1962). Presently, at this salinity *Pseudo-nitzschia* spp. maximum concentrations exceed 10^7 cells l^{-1} (Fig. 6A). While this suggests that *Pseudo-nitzschia* spp. abundance has increased since 1955–1957, differences are difficult to quantify because Simmons & Thomas (1962) conducted their sampling to the east of the Mississippi River delta, whereas sampling in this study has focused primarily on the region to the west of the delta. Further, they did not sample during April when the peak in *Pseudo-nitzschia* spp. abundance was observed in 1990–1994. Fucik & El-Sayed (1979) sampled phytoplankton on 12 cruises from January 1972 to January 1974 at 2 stations near the present mooring site. During that period, the relative abundance of *Pseudo-nitzschia* spp. to the total phytoplankton ranged from 0 to 17%, with most of the values <1%. Most of the species reported by Fucik & El-Sayed (1979) were diatoms, so their % abundance based on total phytoplankton is equivalent to that based on diatoms presented here. In the present study, the % abundance of *Pseudo-nitzschia* spp. at the mooring site relative to diatoms ranged from 0 to 100% and averaged 10%. Consequently, the published historical data, especially those of Fucik & El-Sayed (1979), support the hypothesis that *Pseudo-nitzschia* spp. have become more abundant. At the same time nutrient input and eutrophication have increased substantially (Turner & Rabalais 1991, 1994, Rabalais et al. 1996). The hypothesis that the increase in *Pseudo-nitzschia* abundance was related to increasing eutrophication requires rigorous testing, but is important because of the growing suspicion that harmful algal blooms are increasing worldwide as a result of coastal eutrophication (Anonymous 1995).

Flux of *Pseudo-nitzschia* spp.

The vertical flux of *Pseudo-nitzschia* spp. was extremely high. Maximum rates exceeded those in traps

in Monterey Bay, California, by more than an order of magnitude, although the % abundance was not as great (Buck et al. 1992). To put these fluxes in perspective, the abundance of *Pseudo-nitzschia* spp. can be integrated over depth at the mooring site for the upper and lower water layers and the daily flux over a specific period can be compared with the average integrated abundance for the same time period. On average 46% of the *Pseudo-nitzschia* present in a particular water layer sank into that trap each day. However, the variability was high and the % trapped sometimes exceeded what was available in the overlying water column. This could be an artifact of the sampling because the data on concentrations in the water were usually only available for the days the traps were put in and taken out and undetected blooms could have occurred during the interim period. It could also be due to the horizontal advection of cells or by over-collection in the traps. Surficial sediments also contained substantial numbers of *Pseudo-nitzschia*. Because of the difficulty calculating short-term sedimentation rates, it is not possible to calculate a daily loss rate to the sediment.

Most of the sinking *Pseudo-nitzschia* did not occur in chains in either the traps or surficial sediments. Further, in 1991 there was a correlation between the % of cells in chains and the % of cells which were alive. This is consistent with the observations from clonal cultures isolated from the northern Gulf of Mexico that when cells ceased growing, the chains disassociated into single cells or doublets, sank to the bottom, and lost their color (Fryxell et al. 1990).

Threat posed by *Pseudo-nitzschia* spp. in the northern Gulf of Mexico

The exact distribution of toxic and non-toxic species has not been determined, but at least 2 potentially toxic species have been observed in the northern Gulf of Mexico. Toxic and non-toxic clones of *Pseudo-nitzschia multiseries* (formerly *Nitzschia pungens* f. *multiseries*) were isolated from Galveston Bay (Fryxell et al. 1990). Toxic clones were more likely to be isolated during cool months (Fryxell et al. 1990) and their growth requirements suggest they prefer lower temperatures (Reap 1991). A bloom of *P. pseudodelicatissima* was identified by SEM at the mooring site in May 1993. This species has been identified as the source of domoic acid in the Bay of Fundy, Canada (Martin et al. 1990).

It is critical to determine the species present on the shelf and their toxin production because of the extremely high numbers which are frequently encountered, especially in the spring. Moreover, environmental conditions may enhance toxin production if

toxin-producing species are present. Despite high nutrient inputs, the surface waters become severely Si-depleted in the spring in comparison with summer (Dortch & Whitledge 1992, Dortch et al. 1995, Smith & Hitchcock 1994, Nelson & Dortch 1996). Further, dissolved silicate/inorganic nitrogen ratios tend to be quite low in the upper water layer, indicating an excess of nitrogen. Such conditions in cultures enhance domoic acid production (Bates et al. 1991, Bates & Douglas 1993, Pan et al. 1996a, b). As a result, domoic acid production could be at its highest level when cell abundance is maximal.

Domoic acid could enter both pelagic and benthic food chains on the shelf. The direct sinking into traps and surficial sediments makes the material available to an array of benthic organisms. Taylor (1993) hypothesized that Dungeness and rock crabs on the west coast of the USA and Canada became toxic by consuming, either directly or indirectly, a *Pseudo-nitzschia* bloom that sank to the bottom. Along the Gulf coast, shrimp and demersal fish, such as drum, are the commercially harvested species that might acquire toxin from sinking *Pseudo-nitzschia*. The most likely pelagic grazers are large copepods, such as *Eucalanus*, which are major consumers of phytoplankton (Dagg 1995), and menhaden, which are extremely abundant (Deegan & Thompson 1985) and fill the niche equivalent to anchovies on the west coast (E. M. Chesney pers. comm.). Because both are consumed by a variety of other organisms, the toxin could become widely distributed and ultimately affect humans and other consumers.

The maximum *Pseudo-nitzschia* spp. abundance in the estuary was 10^5 cells l^{-1} . Although these abundances are much less than on the shelf, they are as high or higher than *Pseudo-nitzschia* spp. abundances that led to toxicity in blue mussels (Hanic 1989, cited in Dickey et al. 1992) and anchovies/seabirds (Garrison & Walz 1993). Further, these samples were taken 0.3 m above oyster beds in a region with a substantial oyster industry (Dugas et al. 1982). Oysters accumulate toxin, although perhaps not to the high levels observed in blue mussels (Roelke 1993). Identification of the species of *Pseudo-nitzschia* and measurement of domoic acid in phytoplankton, oysters, and other higher trophic levels are essential in assessing the potential risk.

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