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Domoic Acid-Producing Diatom Blooms in Monterey Bay, California: 1991–1993

P.M. Walz, D.L. Garrison, W.M. Graham, M.A. Cattey, R.S. Tjeerdema, and M.W. Silver
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ABSTRACT During the autumn of 1991, numerous seabird fatalities in Monterey Bay, California, led to the discovery of a new domoic acid-producing diatom, *Pseudonitzschia australis.* Since this initial event, sizable populations of *P. australis,* as well as other likely toxin producers, *P. pungens* f. *multiseries* and *P. pseudodelicatissima,* have occurred biannually in Monterey Bay. Using the highly sensitive FMOC-HPLC method, we detected domoic acid whenever *Pseudonitzschia australis* was found in the plankton, even at densities as low as 4.0×10^3 cells/L. Based on correlations of domoic acid and *P. australis* abundances and the overwhelming biovolume dominance of *P. australis,* we conclude that *P. australis* has been the major domoic acid producer during the period of our study.

Our study suggests that *P. australis* cells may always be toxic in natural populations and that toxin concentrations on a per cell basis have no statistically significant relationship to population density or to nutrient concentrations other than silicate. Cellular levels of domoic acid were positively correlated with silicate concentrations, which is at variance with reports from prior culture experiments. These conclusions must be tentative because of the limited extent of our sampling. Nevertheless, these preliminary data indicate that further investigations of environmental conditions affiliated with cell growth and toxin production in *P. australis* are warranted.

As a practical matter, domoic acid in the pelagic environment cannot be reliably or consistently detected by monitoring domoic acid levels in intertidal mussels. Direct measurement of domoic acid using sensitive HPLC methods is probably the most cost-effective and accurate approach for an ongoing phycotoxin monitoring program. © 1994 Wiley-Liss, Inc.

Key Words: Amnesic shellfish poisoning (ASP), Neurotoxin, Phytoplankton ecology, *Pseudonitzschia*

INTRODUCTION

Domoic acid (DA) poisoning first became a concern along the west coast of North America in September of 1991, when over 100 brown pelicans and cormorants were found dead or suffering from unusual neurological symptoms in Monterey Bay, California [Fritz et al., 1992; Work et al., 1993]. This event was followed by another occurrence of DA poisoning along the coast of Oregon and Washington, in which humans were afflicted after consuming domoic acid-contaminated razor clams [Todd, 1993].

The 1991 domoic acid-poisoning event in Monterey Bay was attributed to a bloom of *Pseudonitzschia australis* [Buck et al., 1992; Fritz et al., 1992], and isolates of *P. australis* from Monterey Bay were subsequently shown to produce DA in culture [Garrison et al., 1992]. *P. australis* is now the third species of the genus *Pseudonitzschia* found to produce domoic acid [Bates et al., 1989; Martin et al., 1990]. Although several *Pseudonitzschia* species are common in California coastal waters [Bolin and Abbott, 1963; Garrison, 1979; Schrader, 1981; Smith, 1991; Villac et al., 1993; Scholin et al., 1994], their population dynamics and the hydrographic conditions that support their growth are poorly characterized, particularly those conditions that favor blooms associated with high concentrations of DA. The objectives of this investigation were to identify *Pseudonitzschia* species affiliated with occurrences of DA in the waters of Monterey Bay and to document seasonal patterns and hydrographic conditions associated with *Pseudonitzschia* growth.

MATERIALS AND METHODS

Domoic Acid Analysis

Plankton samples were collected at approximately 1–2 week intervals from October 1991 through March 1993 with a break in the sampling program from mid-March through July 1992 (Table I). During periods when *Pseudonitzschia* species were present in abundance, samples were collected more frequently. Stations were located at the Santa Cruz

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TABLE I. Samples Collected During the 1991–1993 Study and Concentrations of Domoic Acid (DA), Nutrients (NO_3 + NO_2, SiO_2, NH_4), and Chlorophyll *a (*)**

Date	Station	Depth (m)	Type	DA (μg/L)	NO_3	SiO_2 (μM/L)	NH_4	Chl *a* (mg/m^3)	Comments
1991 Autumn bloom									
21-OCT-91	SCP	0	Water		0.7	8.9	8.0*	2.7	No DA sample
23-OCT-91	SCP	0	Water	0.29	2.4	10.9	7.6*	3.1	
25-OCT-91	SCP	0	Water	0.54	0.8	5.9	6.0*	2.5	
28-OCT-91	SCP	0	Water	0.78	1.3	10.3	5.2*	1.6	
30-OCT-91	SCP	0	Water	0.74	2.8	13.8	5.3*	2.5	
11-NOV-91	SCP	0	Water	0.33	5.8	14.6	5.8*	1.8	
22-NOV-91	SCP	0	Water	0.67	4.3	4.1	2.6*	1.5	
29-NOV-91	SCP	0	Water	12.30	3.1	7.1	0.9	8.2	
19-DEC-91	05	0	Water		11.8	28.1	1.3	0.3	No DA sample
1992 Spring bloom									No DA sample
03-FEB-92	05	0	Water						
19-FEB-92	05	0	Water						No DA sample
	05	15→0	Net						
25-FEB-92	05	0	Water	(0.02)					
	05	15→0	Net	[1.5]					
28-FEB-92	05	5	Water	(0.02)	3.3	15.5	2.0	1.6	
	05	15→0	Net	[1.4]					
12-MAR-92	05	5	Water	(0.60)	0.5	24.3	0.9	0.8	
	05	15→0	Net	[4.5]					
1992 Autumn bloom									
04-AUG-92	05	5	Water		0.6	1.5	0.0	2.7	
	05	15→0	Net	[+]					
13-AUG-92	SCP	0	Water						
	SCP	10→0	Net	[+]					
20-AUG-92	SCP	0	Water	(0.00)					
	SCP	10→0	Net	[0.0]					
27-AUG-92	SCP	0	Water						
	SCP	10→0	Net	[+]					
04-SEP-92	05	5	Water		4.7	10.9	0.8	0.6	
	05	15→0	Net	[+]					
10-SEP-92	SCP	0	Water						
	SCP	10→0	Net	[+]					
16-SEP-92	SCP	0	Water						
	SCP	10→0	Net	[+]					
02-OCT-92	SCP	0	Water						
	SCP	10→0	Net	[+]					
07-OCT-92	05	5	Water		5.1	9.0	0.1	3.0	No DA sample
12-OCT-92	05	5	Water		0.1	4.8	0.0	0.4	
	05	15→5	Net	[+]					
	2206	5	Water		1.3	6.3	0.1	1.3	
	2206	15→0	Net	[+]					
16-OCT-92	SCP	0	Water	(0.00)					
	SCP	10→0	Net	[0.0]					

Date	Station	Depth (m)	Type	DA (µg/L)	NO_3	SiO_2 (µM/L)	NH_4	Chl *a* (mg/m^3)	Comments
24-OCT-92	SCP	0	Water	(0.00)					
	SCP	10→0	Net	[0.0]					
30-OCT-92	SCP	0	Water						No DA sample
08-NOV-92	SCP	0	Water						No DA sample
10-NOV-92	05	5	Water	(0.20)	1.4	4.9	0	1.0	
	05	15→0	Net	[10.3]					
	2206	5	Water	(0.07)	1.3	8.9	0.3	1.3	
	2206	10→0	Net	[25.4]					
13-NOV-92	SCP	0	Water	(0.10)					
	SCP	10→0	Net	[23.0]					
15-NOV-92	SCP	0	Water	(0.05)					
	SCP	10→0	Net	[10.7]					
18-NOV-92	05	5	Water	(0.06)	2.7	7.6	0.3	2.6	
	05	15→0	Net	[2.4]					
20-NOV-92	SCP	0	Water	(0.04)					
	SCP	10→0	Net	[7.2]					
27-NOV-92	SCP	0	Water	(0.00)					
	SCP	10→0	Net	[0.0]					
02-DEC-92	05	5	Water		2.4	10.8	0.1	0.9	
	05	15→0	Net	[+]					
1993 Spring bloom									
07-FEB-93	05	5	Water	(0.00)					
	05	15→0	Net	[0.0]					
13-FEB-93	05	5	Water	(0.02)	6.3	10.7	0.1	2.1	
	05	15→0	Net	[6.3]					
27-FEB-93	05	0	Water	(0.03)	2.1	11.1	0.2	2.0	
	05	15→0	Net	[6.1]					
	2206	5	Water	(0.40)	1.9	13.4	0.0	2.0	
	2206	15→0	Net	[86.5]					
04-MAR-93	05	5	Water	(0.25)					
	05	15→0	Net	[37.8]					
10-MAR-93	05	5	Water	(0.11)	3.0	16.6	0.5	1.7	
	05	15→0	Net	[26.4]					
18-MAR-93	05	5	Water	(0.00)	5.4	7.8	0.1	1.7	
	05	15→0	Net	[0.0]					

*NH_4 values appear anomalously high. In 1992 and 1993, domoic acid concentrations were measured in net samples (shown in brackets) and domoic acid per cell values (see Fig. 3) were calculated from cell counts using an aliquot of the net sample. DA per cell times cells per liter resulted in DA per liter (values shown in parentheses). These later extrapolated values are comparable to domoic acid determinations on discrete water samples during 1991. +: Domoic acid was detected but could not be quantified.

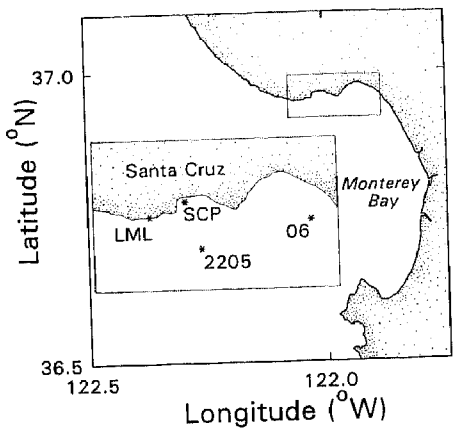

Fig. 1. Monterey Bay, California, showing sampling locations. Santa Cruz Pier (SCP), permanent nearshore hydrographic stations (2205, 06), and the UC Santa Cruz Long Marine Lab (LML) are indicated.

Municipal Pier (SCP) and at nearshore, long-term hydrographic sampling sites (2205, 06) in northern Monterey Bay (Fig. 1).

DA was measured in plankton concentrated from the upper water column with a 37 μm-mesh net, in water samples collected at 5 m with a 1.7 L Niskin bottle, or at the surface with a bucket (Table I). When phytoplankton were sparse, replicate net tows were pooled to obtain a concentrated sample. For both net- or bottle-collected samples, an aliquot (usually 100–400 ml) of fresh sample was gently concentrated onto a moistened 5.0 μm Nuclepore membrane filter [Bates et al., 1991]. Both cells and filters were transferred to a graduated plastic tube and either immediately processed for DA analysis or stored at −15°C. To extract domoic acid, 5 or 10 ml of distilled water were added to filters which were then sonicated for 90 s at 50 W. Microscopic examination confirmed that this was sufficient to break the frustules and lyse all cells. Samples were then filtered with a disposable 0.22 μm filter to remove particulate material prior to HPLC analysis.

Domoic acid concentrations were measured using a modification of the FMOC-derivitization procedure of Pocklington et al. [1990]. Briefly, samples were analyzed using a Hewlett-Packard (HP) model 1090L/series II high pressure liquid chromatography system with a 1040 diode-array-detector (DAD) and a model 1046A fluorescence detector. A Vydac 201TP (Separations Group, Hesperia, CA) reverse-phase column (25 cm × 2.1 mm I.D., 5 μm) was used with a mobile phase of aqueous acetonitrile (ACN) containing 0.1% trifluoroacetic acid (0.2 ml/min; 48°C). Separations were performed using 2 or 10 μl injections and discontinuous gradient elution over 42 min; initial composition of 30% ACN was increased to 50% over 15 min, then to 100% over 2 min, which was held for 7 min before returning to 30% ACN over 3 min.

Samples containing less than 200 ng/ml of DA were quantified by fluorescence, while highly concentrated samples (those with high fluorescent background) were quantified via DAD. Calibration curves (1 ng/ml–1 μg/ml) were constructed from a DA calibration standard (DACS-1; National Research Council of Canada, Marine Analytical Chemistry Standards Program, Halifax, Nova Scotia). Peak purity was assessed using DAD comparison of spectra stored during individual runs and signal wavelength ratio plots. The method limit of detection was 1 ng/ml (10 pg of DA injected). Our detection limit in plankton samples was approximately 0.025 μgDA/L of seawater when 200 ml were concentrated and extracted in 5 ml. (Our ability to detect domoic acid is much greater when examining net-concentrated phytoplankton, i.e., more cells present.)

Species Abundance

Aliquots of water (quantitative samples) and net tows (qualitative samples) were preserved with acid Lugol's iodine solution. Both net and water samples were concentrated using Utermöhl settling chambers (net = 10 ml chamber, water = 50 ml chamber) and counted using an inverted microscope. The number of *Pseudonitzschia* cells counted was usually >300 (range: 7–935). Confidence limits on cell counts were estimated from formulas given by Lund et al. [1958] and Venrick [1978], and species abundances were considered significantly different when there was no overlap of 95% confidence levels on counts [see Lund et al., 1958].

When there was more than one toxic species present in natural populations (the usual case in our study), it was impossible to determine directly the relative contribution of each species to the total domoic acid concentrations without making some assumptions. We assumed that domoic acid would be distributed among the potentially toxic species present based on the biovolume of each species in the water (i.e., cells/L × avg. cell volume). From cell measurements, we determined that typical cell volumes were 4,084, 1,944, and 113 μm^3 for *P. australis*, *P. pungens* f. *multiseries* and *P. pseudodelicatissima*, respectively, or 36:17:1 for the largest to the smallest cells, and we assumed each cellular μm^3 produced the same amount of DA in toxin-producing species of *Pseudonitzschia*. Using these assumptions about species-specific domoic acid concentration, we estimated quantitative domoic acid concentrations (μg/L) for sample dates when domoic acid was measured from net samples and cell abundances were determined from unconcentrated water samples (Table I). Since DA concentrations were derived from net tow samples, and cell counts from water samples, our calculation also presumes that the populations in net and water samples are the same with respect to cellular domoic acid concentrations. Because both net and bottle samples were collected within the surface mixed layer, this assumption seems reasonable.

Species Identification

Preliminary identifications of *Pseudonitzschia* species were made with phase contrast light microscopy and confirmed using scanning electron microscopy (SEM). For SEM examination, preserved net samples were cleaned using $KMnO_4$ and concentrated HCl [Hasle and Fryxell, 1970]. A few drops of this cleaned material were then air dried onto glass cover slips, which were affixed to aluminum stubs and coated with gold-palladium. Hasle [1965, 1993] was referenced for species descriptions; species identifications were also confirmed by Dr. C. Lange (Scripps Institution of Oceanography, pers. comm.).

Hydrographic Data

Sea-surface temperatures and samples for nutrient analysis were collected as often as possible from SCP during the early part of the sampling program. More complete, but less frequent, hydrographic observations (CTD casts, Chl *a*, and nutrients) were collected during monthly routine hydrographic sampling cruises in Monterey Bay on the R/V *David Johnston*. A continuous record of sea surface temperature was obtained from the University of California, Santa Cruz Long Marine Laboratory.

Nutrient and Chl *a* samples were taken at 5 m using Niskin bottles or from surface, bucket-collected samples (only at SCP). A 250 ml portion of this sample was filtered through a GF/F glass fiber filter. The filters (for Chl *a* analysis) and the filtrate (for nutrient analysis) were kept on ice until frozen upon arrival at the laboratory. Colorimetric techniques for the determination of silicate, ammonia, and the combined concentration of nitrate and nitrite were those of Parsons et al. [1984]. During 1993, nitrate and nitrite concentrations were determined using a Technicon autoanalyzer. Chlorophyll *a* was extracted for 24 h in 10 ml of 90% acetone and measured with a Turner Designs fluorometer [Parsons et al., 1984].

RESULTS

Domoic acid has been detected in northern Monterey Bay in both autumn and early spring since the start of our monitoring program. The highest concentrations were measured on November 29, 1991, during a pronounced *Pseudonitzschia* "bloom" (i.e., cell numbers of 10^5–10^6 cells/L) (Table I; Fig. 2).

During all periods when domoic acid was detected, *P. australis* was present and usually numerically dominant among *Pseudonitzschia* species, but at least one of the two other potentially toxic species (i.e., *P. pungens* f. *multiseries*, and *P. pseudodelicatissima*) were also present in moderate to high densities (Fig. 2a–c). For example, during the fall bloom of 1991, both *P. australis* and *P. pseudodelicatissima* were present at densities usually exceeding 10^4 cells/L. October 23, 30, and December 19, 1991 were the only dates when abundances of these two species were statistically indistinguishable. At the peak of the bloom and

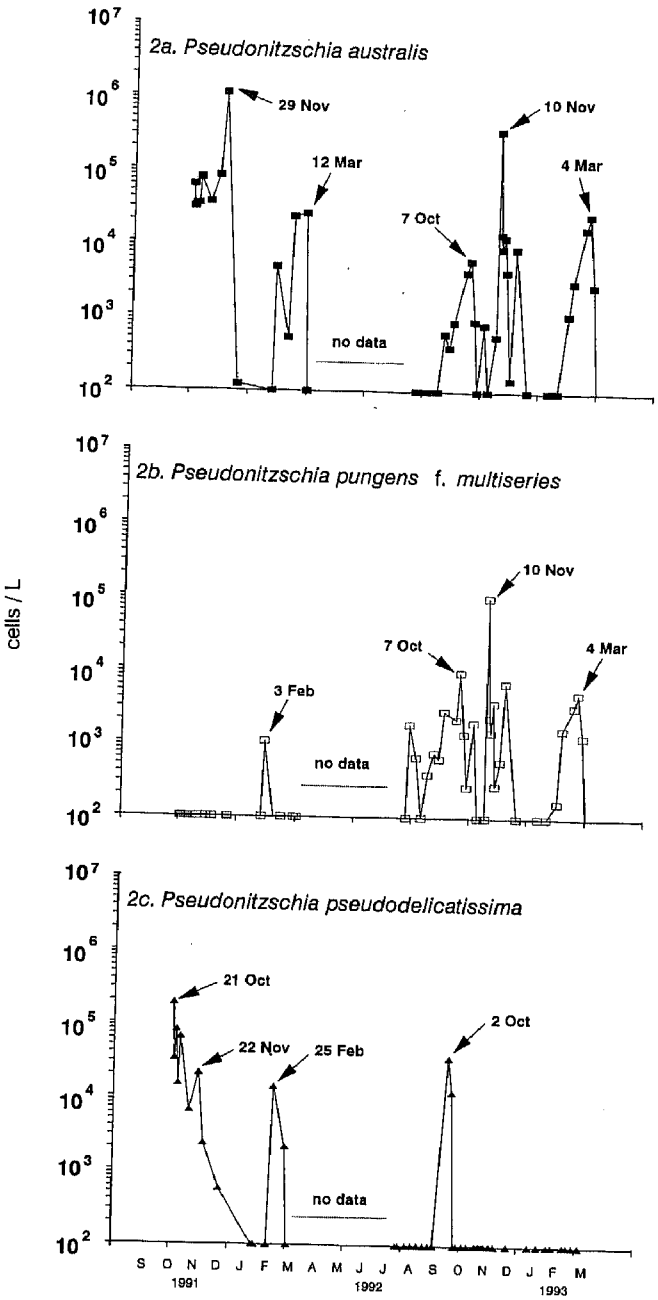

Fig. 2. a–c: Abundances of *Pseudonitzschia australis*, *Pseudonitzschia pungens* f. *multiseries*, and *Pseudonitzschia pseudodelicatissima*, respectively, determined from cell counts in near surface waters (0 or 5 m). Note that the y-axis is $\log_{10}$ scaled.

when domoic acid reached its highest level (November 29; see Table I), *P. australis* abundance exceeded 10^6 cells/L (Fig. 2a). In the spring of 1992, all three toxic species were present (Fig. 2a–c). *P. australis* and *P. pseudodelicatissima* reached densities exceeding 10^4 cells/L during the February to March period, but *P. pungens* f. *multiseries* was only detected on one sampling day (Feb. 3, see Fig. 2b). Cell abundances for *P. pungens* f. *multiseries* were significantly

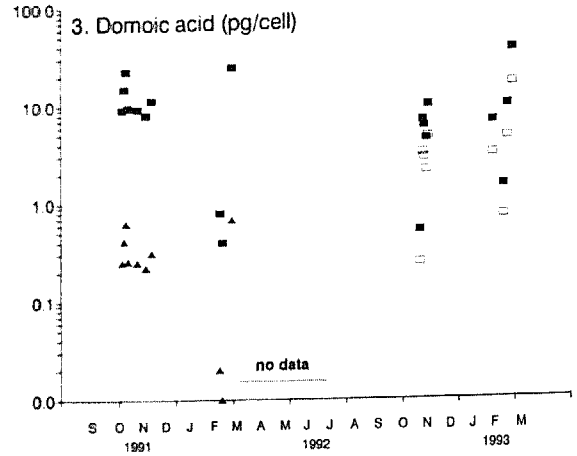

Fig. 3. Domoic acid per cell calculated from species abundances and measurements of DA in either water or net collected samples. The symbols indicating the three potentially toxic species are the same as those used in Figure 2a–c. Values assume that DA is apportioned among species by biovolume and cell abundance (see Discussion in text).

lower than those for *P. australis* and *P. pseudodelicatissima* and these two later species were also numerically distinct from one another.

Pseudonitzschia species reappeared in August 1992 and were found sporadically until early December (Fig. 2a–c). *P. australis* and *P. pungens* f. *multiseries* were most abundant from November 10 to December 2, 1992, when cell densities approached or surpassed 10^5 cells/L for both species (Fig. 2a,b). Aside from December 2, 1992, when the 95% confidence levels for both species overlapped, abundances were significantly different. The third species, *P. pseudodelicatissima*, was briefly observed October 2–7 at moderate densities (approx. 10^4 cells/L, Fig. 2c). SEM analysis of phytoplankton net tows also confirmed the presence of a fourth form, *P. pungens* f. *pungens*, during the fall of 1992. Observations of this non-toxic form [see Villac et al., 1993] indicated its occurrences usually coincided with *P. pungens* f. *multiseries*, but was numerically far less common among the *Pseudonitzschia* species previously described.

In the spring of 1993, *P. australis* and *P. pungens* f. *multiseries* were recognized once again (Fig. 2a,b) but population densities remained between 10^3 and 10^4 cells/L and never achieved bloom densities (i.e., 10^5–10^6 cells/L). Domoic acid was detected, however, at concentrations equivalent to those found during periods of higher *Pseudonitzschia* abundance (Table I). During the entire spring event of 1993, species abundances were significantly different. *P. pungens* f. *pungens* was minimally present in SEM prepared samples at this time (as it was during fall 1992) in conjunction with appearances of *P. pungens* f. *multiseries*.

Estimated cell concentrations of DA derived from either net or water samples are shown in Figure 3. All nutrient and chlorophyll *a* concentrations are summarized in Table I. Temperature data is illustrated in Figure 5. The occurrence of domoic acid (measured as μg DA/L, Table I) was strongly correlated with abundances of *P. australis* (Pearsons $r = 0.93$, $P = 0.05$). Interestingly, our data showed no statistically significant relationship between total domoic acid concentrations and nutrient concentrations (i.e., NO_3, SiO_2, NH_4). However, we did find that per cell concentrations of domoic acid in *P. australis* were positively correlated with silicate ($r = 0.57$, $P < 0.05$, $n = 16$). Over the wide range of *Pseudonitzschia* spp. abundances observed, there was no apparent statistical relationship between species abundance and per cell domoic acid concentrations. Similarly, per cell domoic acid concentrations for the three species were uncorrelated with total plankton biomass, as estimated by chlorophyll *a*.

DISCUSSION

One objective of our study was to assess the frequency of domoic acid-producing blooms in Monterey Bay and to determine which species likely contribute to toxin production. Since we initiated our phytoplankton monitoring program, we have detected domoic acid several times in plankton samples collected from shallow waters of northern Monterey Bay (Table I). Maxima occurred in both late autumn and early spring.

We found the highest concentration of domoic acid was present in late November of 1991. There was no sampling for domoic acid in plankton during the September period when seabird mortalities were first reported. However, domoic acid and *P. australis* cells were found in anchovies recovered from the stomachs of seabirds that had died from domoic acid intoxication in September [Fritz et al., 1992]. The November 1991 DA peak in Monterey Bay occurred at approximately the same time that domoic acid was detected in razor clams from Washington and Oregon; therefore the toxin may have been widely distributed at the time. Levels of domoic acid in 1992 and 1993 never reached those measured in late 1991 (Table I), and, with the exception of a bloom on November 10, 1992, populations of potentially toxic *Pseudonitzschia* species were much smaller (see Fig. 2a–c).

It has been generally assumed that *P. australis* is the dominant domoic acid-producing species on the west coast [e.g., Fritz et al., 1992; Garrison et al., 1992; Buck et al., 1992; Work et al., 1993]. We have found two other known domoic acid-producing *Pseudonitzschia* species in Monterey Bay, but their actual contributions to toxin production in this area have not yet been quantified. Domoic acid production has been confirmed in culture for clones of *P. australis* and *P. pungens* f. *multiseries* from Monterey Bay [Garrison et al., 1992; Villac et al., 1993] but toxin production has not been demonstrated for local clones of *P. pseudodelicatissima*, a species that has been reported to produce toxin elsewhere [Martin et al., 1990]. In July 1993, a predomi-

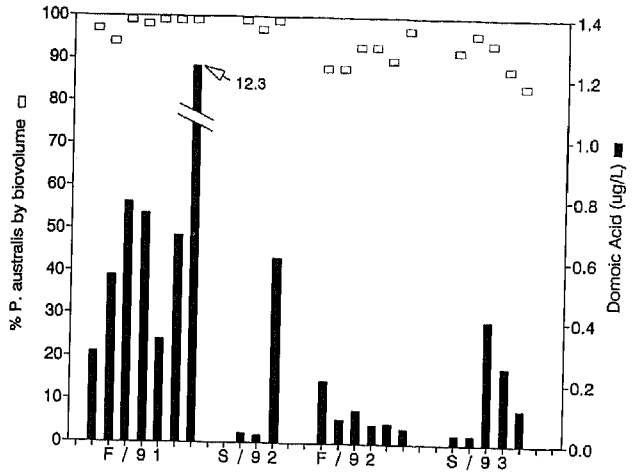

Fig. 4. Total DA present (μg/l, histogram bars) and percent biovolume *P. australis* (open squares) among all *Pseudonitzschia* cells observed.

nantly *P. pseudodelicatissima* bloom occurred in the Berkeley Marina (San Francisco Bay, California), with cell densities reaching 4.6×10^6 cells/L (Pat Smith, California Department of Health Services); however, domoic acid was not detected in samples from this bloom (Gregg Langlois, unpublished data). Therefore, the toxicity of *P. pseudodelicatissima* in local waters remains uncertain.

Although multiple potentially toxic species have been present during our study, our data implicate *P. australis* as the major domoic acid contributor. For example, we found that DA concentrations were strongly positively correlated with *P. australis* abundances ($r = 0.95$; $P < 0.01$) but not with the abundances of other potentially toxic species. *P. australis* dominated the *Pseudonitzschia* population biovolume in Monterey Bay throughout our study (Fig. 4), with total population contributions ranging from 1.13×10^7 to 4.50×10^9 μm^3. On average, *P. australis* accounted for 94.5% of the total *Pseudonitzschia* biomass present (Fig. 4). This biovolume dominance among *Pseudonitzschia* species substantiates our hypothesis that domoic acid is principally associated with *P. australis*.

DA per cell values ranged from 0.4 to 32.6 pg/cell for *P. australis* during our observations (Fig. 3). Given the assumptions we have made regarding apportioning of DA among cells, these values closely approximate DA concentrations estimated from field populations in other Monterey Bay samples [Buck et al., 1992; 3–31 pg/cell] and from culture observations using Monterey Bay isolates [Garrison et al., 1992; max. of 37 pg/cell].

Using the highly sensitive FMOC-HPLC technique, we have detected domoic acid whenever *P. australis* has been evident among locally monitored phytoplankton. Furthermore, and most importantly, per cell concentrations of DA have not been dependent upon abundances of field populations. Over a wide range of *P. australis* concentrations in the water column (Fig. 2a) we have seen relatively similar per cell concentrations of domoic acid (Fig. 3, avg. = 10 pg/cell). These data apparently indicate that cells produce DA regardless of population density.

Based on our relatively limited survey, it is premature to conclude that domoic acid production in this region depends mostly on a single species. A number of *Pseudonitzschia* species are widely distributed throughout the California Current system [Cupp, 1943; Scholin et al., 1994], and only a few of these have been assayed for their ability to produce domoic acid. Long-term plankton surveys, covering at least four decades, show that members of this group (previously called "Nitzschia" spp. in most local accounts) are present periodically throughout the year and form blooms that show little seasonal regularity [Bolin and Abbott, 1963; Garrison, 1979; Schrader, 1981; Smith, 1991]. Systematic analyses performed during these previous studies were not sufficiently detailed to resolve individual species cycles. Within the short period of our study, however, the timing and duration of *P. australis* domoic acid-producing blooms have followed a similar pattern: abundance peaks have been observed in the October–November and February–March periods, with autumn domoic acid occurrences persisting over 3–4 months. In contrast, spring blooms have been of shorter duration (see Table I and Fig. 2a–c).

A second objective of our program was to examine the environmental conditions associated with the occurrence of domoic acid and *Pseudonitzschia* growth. Based on laboratory batch culture studies on *P. pungens* f. *multiseries*, domoic acid production is expected to be limited to the stationary growth phase when cells are limited by some factor other than nitrogen [e.g., silicate; Subba Rao et al., 1988; Bates et al., 1991]. Although experiments performed by Garrison et al. [1992] on *P. australis* were equivocal with respect to whether domoic acid production was restricted to the stationary growth phase, the results appeared to be in general agreement to what has been previously reported from the Canadian studies with *P. pungens* f. *multiseries* (i.e., that the highest levels of domoic acid production occur at high cell densities when nutrient levels are becoming depleted).

If conditions that promote domoic acid production in culture are similar to those that also produce domoic acid in natural populations, then it is not particularly surprising that we have observed the highest concentrations of domoic acid and the most persistent domoic acid-producing blooms in the late summer and autumn (see Table I). It is during this period in the Monterey Bay region when seasonal, coastal upwelling is sporadic or ceases altogether [e.g., Bolin and Abbott, 1963], and hydrographic conditions are characterized by warmer water (14.5 to 17.5°C) and lower nutrients (NO_3 0.2–3.0 μM; SiO_2 1.0–7.0 μM, Garrison [1979]. During the present study, autumn domoic acid-producing blooms were characterized by relatively warm sea surface temperatures (Fig. 5) as well as NO_3 concentrations that were generally <5.0 μM/L (Table I), all suggestive of post-upwelling conditions.

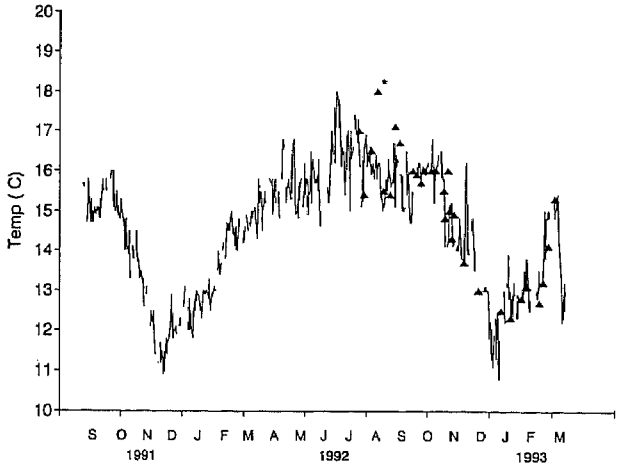

Fig. 5. Daily sea surface temperatures recorded at the UCSC Long Marine Lab (—) and on dates when discrete samples were obtained at stations SCP, 2205, or 06 (▲). See Figure 1 for relative locations of these sites. *High temperature may be an indication of long residence time and decreased circulation in the area near SCP. As discussed in the text, temperature is a good indicator of hydrographic events (i.e., upwelling) and, in general, temperature is inversely related to nutrient concentrations.

It was somewhat surprising to have found domoic acid present during the early spring because this is usually the season of active upwelling, characterized by lower temperatures and high levels of inorganic nutrients [Bolin and Abbott, 1963]. Local circulation patterns and coastal topography, however, create regions near shore where nutrients are depleted [Broenkow and Smethie, 1978]. For example, upwelled water originates north of Monterey Bay, and this cold (approx. 12°C), nutrient-rich ($NO_3 > 10.5$ μM/L, $SiO_2 > 20$ μM/L) water is advected into the bay by local circulation patterns [see Garrison, 1979, regarding water characteristics; and Tracy, 1990; Rosenfeld et al., 1994, for information about local circulation]. Water eventually reaching the shallow regions of northern Monterey Bay where our monitoring stations are located, often shows marked nutrient depletion as high-density plankton blooms develop [Garrison, 1976, 1979, 1981; Waidelich, 1976; Broenkow and Smethie, 1978]. This phenomena may be enhanced when water is trapped near the coast because of peculiarities in local upwelling circulation [Graham, 1993]. Thus, if spring blooms either develop in shallow waters or are advected into nearshore regions, where they are subsequently retained, they are likely to be subjected to environmental stress similar to that observed during the post-upwelling conditions in late summer and autumn. The relatively low temperatures in the February–March periods (see Fig. 5) are apparently an indication of recent upwelling, but nutrient concentrations are markedly reduced from those usually associated with upwelling source waters (Table I). The absence of significant statistical relationships between domoic acid and nutrient concentrations, and the unexpected positive correlation of silicate and cellular levels of domoic acid in *P. australis*, may be an artifact of our limited nutrient data and/or the fact that nutrient samples and cells harvested for DA were not necessarily taken from the same exact depth. It is equally likely that domoic acid production in natural populations may not follow predictions from culture studies. Further examination of conditions that stimulate *Pseudonitzschia* growth and levels of domoic acid production appear to be warranted.

From a public health and safety perspective, it is significant that we have been able to measure domoic acid over prolonged periods in plankton samples, but, with the exception of a brief period in the autumn of 1991, domoic acid has not been detectable in intertidal mussels (*Mytilus californianus*), the locally monitored bioindicator organism collected at Natural Bridges, California [Langlois et al., 1993]. In particular, mussel samples taken on November 14, 1992, and March 10, 1993, at Natural Bridges (adjacent to the UCSC Long Marine Laboratory, see Fig. 1) contained no detectable DA, despite these being times when *Pseudonitzschia* were abundant and DA was present in our phytoplankton samples (Fig. 2a–c; Table I). Clearly, the mussel beds at Natural Bridges would be exposed to DA-producing populations similar to those found at our nearby sampling sites. It is possible that high depuration rates may account for the absence of DA in these filter-feeding bivalves [Novaczek et al., 1992; Wekell, 1992]. Nonetheless, it is perplexing to find DA in the plankton but not in these filter feeders, especially when *Pseudonitzschia* cells were present for prolonged periods (days–weeks).

Although our studies have been restricted to a two-year period and are limited in spatial coverage, our data suggest some tentative conclusions. Domoic acid will likely be present in coastal areas when toxic species are present, regardless of cell densities. Monitoring of intertidal mussels is not a particularly sensitive method for detecting low but significant levels of domoic acid in plankton, missing all but the most extreme events. Direct analysis of phytoplankton samples for domoic acid will probably remain the fastest and most reliable method to confirm the presence of domoic acid. Because historical data suggest that potentially toxic *Pseudonitzschia* may bloom at any season (although they may be more prevalent in late autumn and spring as indicated by the present study), year-round monitoring must be maintained. It is not clear whether domoic acid is always present in cells in natural populations or whether some environmental "stress," normally present in our sample region, induces domoic acid production. Our results further indicate that the domoic acid phenomenon is one that will require a combination of laboratory and field investigations if we hope to predict future outbreaks and to understand the underlying causes of such events.

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