

Domoic acid production near California coastal upwelling zones, June 1998

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Abstract

Sea lion mortalities in central California during May and June 1998 were traced to their ingestion of sardines and anchovies that had accumulated the neurotoxin domoic acid. The detection of toxin in urine, feces, and stomach contents of several sea lions represents the first proven occurrence of domoic acid transfer through the food chain to a marine mammal. The pennate diatoms, *Pseudo-nitzschia multiseries* and *P. australis*, were the dominant, toxin-producing phytoplankton constituting algal blooms near Monterey Bay, Half Moon Bay, and Oceano Dunes, areas where sea lions with neurological symptoms stranded. Toxic *Pseudo-nitzschia* were also found near Morro Bay, Point Conception, Point Arguello, and Santa Barbara, demonstrating that these species were widespread along the central California coast in June 1998. Measurements of domoic acid during three cruises in early June showed the highest cellular toxin levels in *P. multiseries* near Point Año Nuevo at 6 pg cell⁻¹ and in *P. australis* from Morro Bay at 78 pg cell⁻¹. Maximum cellular domoic acid levels were observed within 20 km of the coast between 0 and 5 m depth, although toxin was also measured to depths of 40 m. Hydrographic data indicated that the highest toxin levels and greatest numbers of toxic cells were positioned in water masses associated with upwelling zones near coastal headlands. Nutrient levels at these sites were less than those typically measured during periods of active upwelling, due to the 1998 El Niño event. The flow of cells and/or nutrients from coastal headlands into embayments where cells can multiply in a stratified environment is a possible mechanism of bloom development along the central California coast. This coupling of toxic *Pseudo-nitzschia* growth near upwelling zones with physical processes involved in cell transport will be understood only when long-term measurements are made at several key coastal locations, aiding in our capability to predict domoic-acid producing algal blooms.

In May and June 1998, at least 70 California sea lions (*Zalophus californianus*) and one northern fur seal (*Callorhinus ursinus*) were found stranded along the central California coast. This stranding event occurred between 15 May

and 19 June over a 300 km stretch of the California coast from Oceano Dunes to Half Moon Bay (Gulland et al. 1999). All animals were in good body condition but displayed neurological symptoms such as seizures, ataxia, and head weaving. Based on these symptoms, domoic acid (DA) poisoning was suspected.

Several lines of evidence pointed toward DA as the cause of the neurological disorders observed in these animals. Anchovies collected from Monterey Bay in late May had levels of DA over 100 µg g⁻¹ tissue and some were found to contain *Pseudo-nitzschia australis* frustules in their gut (Lefebvre et al. 1999; Scholin et al. 2000; Gulland et al. in press). DA was detected in serum, urine, and feces of several sea lions (Lefebvre et al. 1999; Scholin et al. 2000; Gulland et al. in press). In addition, several affected sea lions showed histological lesions evidenced by neuronal necrosis that was most severe in zone CA3 of the hippocampus and the dentate gyri (Gulland et al. in press), regions of the brain known to be specifically affected by DA (Teitelbaum et al. 1990; True-love et al. 1997). This combination of clinical signs, histo-

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pathology, and toxicology led to a diagnosis of DA toxicity in the California sea lions that stranded during this marine mortality event.

The excitatory amino acid, DA, was first discovered to be produced by the pennate diatom, *Pseudo-nitzschia multiseries*, after a human poisoning event that occurred in Prince Edward Island, Canada, in 1997 (Bates et al. 1989; Wright et al. 1989). Mussels that had accumulated DA by filter feeding large numbers of *P. multiseries* were consumed by humans, which resulted in neurological disorders including short-term memory loss and disorientation (Bird et al. 1988; Wright et al. 1989; Todd 1993). Extreme cases of intoxication resulted in the death of several people during that episode (Perl et al. 1990). Since shellfish were the toxin vector, the intoxication syndrome was named amnesic shellfish poisoning (ASP).

The first measurements of DA in coastal regions of the U.S. were made in 1991 when high levels of toxin were found in sardines and anchovies in the Monterey Bay, California area, resulting in numerous seabird mortalities after their consumption of the fish (Buck et al. 1992; Fritz et al. 1992; Work et al. 1993). A bloom of *P. australis*, identified for the first time as a species producing high levels of DA (Garrison et al. 1992; Villac et al. 1993), was observed in Monterey Bay during this event. Because toxin was transferred through suspension-feeding finfish to seabirds, this intoxication syndrome was termed DA poisoning.

DA levels in sardines and anchovies collected from Monterey Bay in the spring of 1998 corresponded to increasing cell numbers of *P. australis* within the bay (Scholin et al. 2000). As the bloom of *P. australis* reached its maximum, sea lions in the same general area suffered an unusual incidence of morbidity and mortality. However, high densities of toxin-producing *Pseudo-nitzschia* species were probably not restricted to Monterey Bay, since anchovies from Morro Bay (about 160 km south of Monterey Bay) were also found to contain DA (Gulland et al. 1999) and stranded sea lions with symptoms of DA poisoning were found in areas both north and south of Monterey Bay. Specifically, the first sea lion to show signs of neurological disorder was taken for treatment by the Marine Mammal Center (Sausalito, California) on 15 May from the Oceano Dunes area, about 220 km south of Monterey Bay. Since many stretches of beaches along the central California coast are not easily accessible, especially the area south of Monterey Bay to Oceano Dunes, observations of stranded sea lions were localized to areas including Oceano Dunes from 15 to 24 May, Monterey Bay from 20 May to 16 June, and one stranded sea lion in Half Moon Bay on 14 June (Gulland et al. 1999).

We report here the results of three cruises conducted in response to the sea lion seizures and deaths. Our sampling scheme encompassed the coastal areas with the highest reported numbers of sea lions with neurological symptoms. Because DA poisoning was suspected prior to our cruises, our objective was to determine the spatial extent of toxic *Pseudo-nitzschia* blooms. The data presented and samples described in this paper were collected off the central California coast from 3 to 17 June 1998. In addition to *Pseudo-nitzschia* species composition, DA levels, nutrients, chlorophyll levels, and physical data, we also present

oceanographic and meteorological data collected from buoys moored off the California coast and satellite images from the time encompassing both the strandings and our cruises.

Methods

Sampling design—California coastal waters from San Francisco to Santa Barbara were sampled on three cruises (Fig. 1). On the first cruise, which departed from Monterey Bay (PB *Bluefin*) on 3 June, the coastal area from Monterey Bay to Morro Bay was sampled. The second cruise departed on 4 June (RV *Ballena*) and sampled the area from Santa Barbara to Morro Bay. The third cruise (RV *Shana Rae*) departed from San Francisco on 9 June and sampled the area to Point Lobos (just south of Monterey Bay). At most stations only surface water was collected, but at other locations samples were taken at 5 and 15 m using 2.5-liter Niskin bottles. At some of the southern stations, additional samples were collected at 30 and 40 m. Water was processed for the analysis of nutrient concentrations, particulate DA, chlorophyll *a* levels, *Pseudo-nitzschia* cell counts, and *Pseudo-nitzschia* species determination (see below for methods used).

Collection and processing of conductivity, temperature, depth (CTD) data—All CTD data obtained during the coastal DA surveys were collected with a Sea-Bird Electronics SEACAT-SBE-19 profiler, except data collected on the RV *Ballena*, which was obtained using a SBE911 Plus CTD. At each CTD station, the profiler was lowered to 50 m (RV *Shana Rae*), 30 m (PB *Bluefin*), 70 m (RV *Ballena*) or to within 2 m of the bottom. Only data collected on the downcast were preserved for analysis. Data were processed using Seabird's Seasoft data processing program and smoothed by averaging to 1-m depth bins.

Meteorological time series—Meteorological data were obtained from buoys that were operational during May and June in the area of our cruises. These sites include the region's National Data Buoy Center (NDBC) and Monterey Bay Aquarium Research Institute (MBARI) moored buoys—(MBARI M1 buoy: 36.7°N, 122.0°W; Point Arguello NDBC 46023: 34.71°N, 123.97°W; Point Conception NDBC 46063: 34.25°N, 120.66°W). Daily averages of several surface meteorological characteristics, including sea-surface temperature (SST), wind speed, and direction, were collected for the period preceding and during the cruises. Wind speed and direction were converted to eastward and northward components. Some minor gaps in the Point Arguello and Point Conception records were filled by linear interpolation, then a 24-h running average filter was applied for clarity.

Satellite imagery—Advanced very high resolution radiometer (AVHRR) SST imagery originated from the NOAA CoastWatch West Coast regional node in LaJolla, California. Archived NOAA-14 imagery for the region of interest was acquired from NOAA CoastWatch active archive system (NCAAS) and processed using the CoastWatch format (CWF) software library and utilities. Imagery was processed to a Mercator projection with a pixel size of 2.5 km.

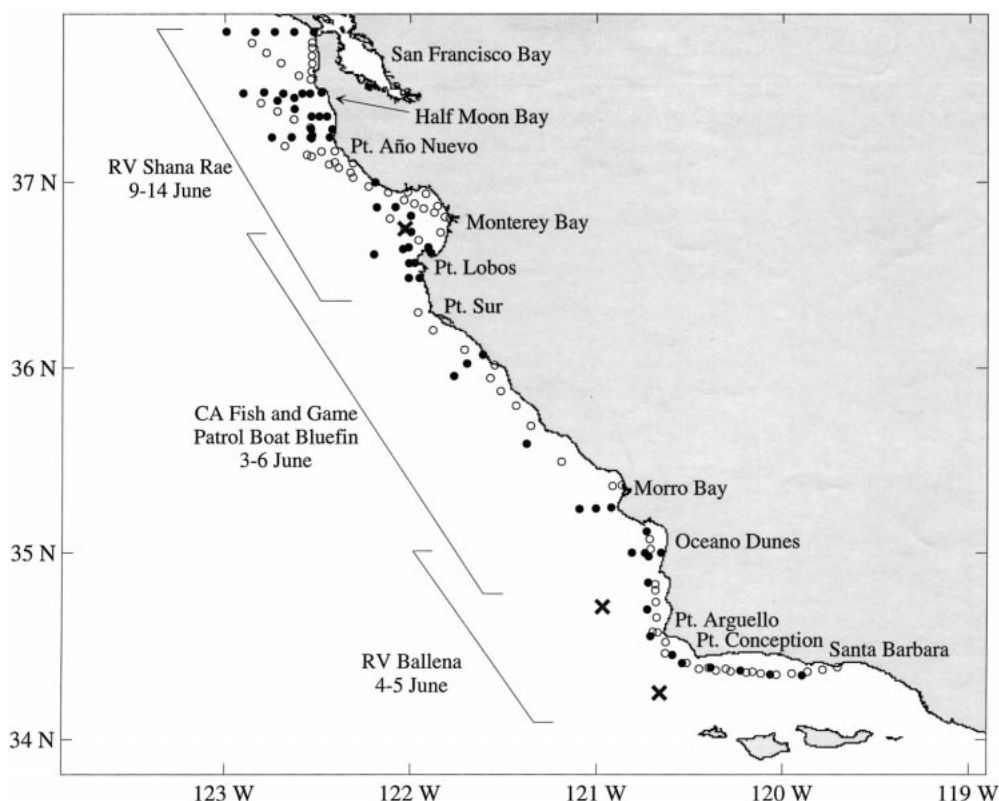


Fig. 1. Locations of central California coastal sampling sites. The vessel names and cruise dates are shown outside the brackets that mark the stations sampled during each cruise. At each station either surface (open circles) or surface and depth samples (closed circles) were taken. Locations of buoys used for wind and SST data are marked by an X.

Domoic acid analysis—Cellular DA was analyzed by receptor binding assay and high performance liquid chromatography (HPLC). Samples were processed by vacuum filtration of 1 liter seawater through a Nucleopore HA filter (0.45 μm pore size, 47 mm diameter) and stored at -20°C until analysis. Receptor binding experiments, in which cellular DA levels in seawater were determined, were performed as described in Trainer et al. (1998). This method is a modification of the high throughput protocol described by Van Dolah et al. (1997) using a glutamate receptor (GluR6) cloned into a viral cell line and expressed in insect cells (Taverna and Hampson 1994). A glutamate decarboxylase digestion step prior to analysis was used to remove endogenous glutamate in all samples. HPLC using the cleanup procedure described in Hatfield et al. (1994) was performed on a subset of the same samples that were prepared for receptor binding analysis. Results obtained using the HPLC method were compared to values obtained using receptor binding analyses. A certified DA standard (DACS-1B, National Research Council, Canada) was used in both receptor binding and HPLC analyses. For quantitation of DA in selected seawater samples, liquid chromatography–tandem mass spectroscopy (LC-MS/MS) was also performed according to standard protocols (Scholin et al. 2000).

Nutrients—Seawater for nutrient determination was filtered through Whatman No. 1 filter (11 μm particle reten-

tion), frozen, and analyzed using autoanalyzer methods (Whitledge et al. 1981).

Chlorophyll a—Aliquots (50 ml) of seawater samples were filtered through Whatman GF/F filters for chlorophyll *a* (Chl *a*) detection. Filters were individually wrapped in aluminum foil and stored at -20°C until analysis. Samples were analyzed using the standard fluorometric method (Welschmeyer 1994) following extraction with 10 ml 90% acetone overnight at 4°C in the dark. An aliquot of the extract was read directly, without acidification, using a Turner Designs fluorometer (TD-700) equipped with narrow band-pass filters. Chl *a* concentration was determined by comparison to a standard curve.

Pseudo-nitzschia cell counts and species identification—Whole water was collected for total *Pseudo-nitzschia* counts. Samples for species identification were collected using 0.25 m diameter 20- μm nets. Whole water and concentrates from net tows were preserved with buffered formalin at a final concentration of 1%. Cells were counted at $100\times$ magnification using the inverted microscope method (Hasle 1978). Species determination was made using scanning electron microscopy (SEM). A KMnO_4/HCL oxidation method (Simonsen 1974; Round et al. 1990; Miller and Scholin 1998) was used to prepare samples for SEM as follows. Disposable filter tubes constructed from 5-ml Eppendorf tubes with the

tips removed were clamped onto a filter system fitted with Millipore RTTP filters (1.2- μm pore size, 13-mm diameter). The filter system was attached to a manifold assembly as described by Miller and Scholin (1998). Samples were added to tubes and rinsed three times with distilled water. Two drops of saturated KMnO_4 were added to each sample and incubated for 1 h. One milliliter of concentrated HCl was then added to each sample and allowed to incubate for an additional 5 min before filtering. The samples were then rinsed twice more with 1 ml of concentrated HCl , followed by three rinses of distilled water. Saturated KMnO_4 was applied again for 15 min, HCl was added for 5 min, and the rinses of concentrated HCl and distilled water were repeated. Filter membranes with processed samples were bonded to aluminum stubs with colloidal graphite, allowed to dry overnight, coated with gold-palladium, and examined with an AMRAY 1000 scanning electron microscope.

Ribosomal RNA analysis—Ribosomal RNA (rRNA) sandwich probe analysis was performed by filtering a total of 500 ml water onto three Durapore (0.65 μm , 25 mm) filters. Filters were frozen at -20°C in the field, then at -80°C in the laboratory until analysis. Sandwich assays were performed by placing filters into 1.5-ml microcentrifuge tubes, resuspending the sample in lysis solution, and processing as described in Scholin et al. (1996) using reagents available from Saigene. Each sample was analyzed using six different treatments: a positive control (a universally conserved sequence of the SSU rRNA), negative control (a sequence targeted toward LSU rRNA specific for *Alexandrium tamarense*), no probe, *P. pungens*, *P. multiseriis*, and *P. australis* (Scholin et al. 1996).

Data processing—Contour maps and vertical sections were constructed using version 6 of the Surfer® three-dimensional plotting software package. All plots were made using the kriging gridding method.

Results

Toxin analysis—Values obtained by both receptor binding analysis and HPLC ($n = 34$) agreed well ($r^2 = 0.92$). Samples with the highest toxin levels from the Point Año Nuevo and Point Conception areas ($n = 12$) were analyzed also by LC-MS/MS. Comparison of values obtained using this method agreed well with those obtained by the receptor binding method ($r^2 = 0.89$).

Meteorological conditions preceding and during the cruises—Meteorological time series of the daily vector mean wind and SST are presented for three buoys in the area of our sample collection (Fig. 2). The buoy at Point Conception was not operational until May. Frequent wind reversals at the M1 buoy in April were accompanied by small variations in SST with a general cooling trend in mid-April. Winds were predominantly southward to southeastward from 6 to 24 April at the Point Arguello site, accompanied by a decrease in SST in mid-April. Just prior to the first reported sea lion stranding on 15 May, winds shifted again to the southeast (8–10 May) and SST decreased by about 1°C at

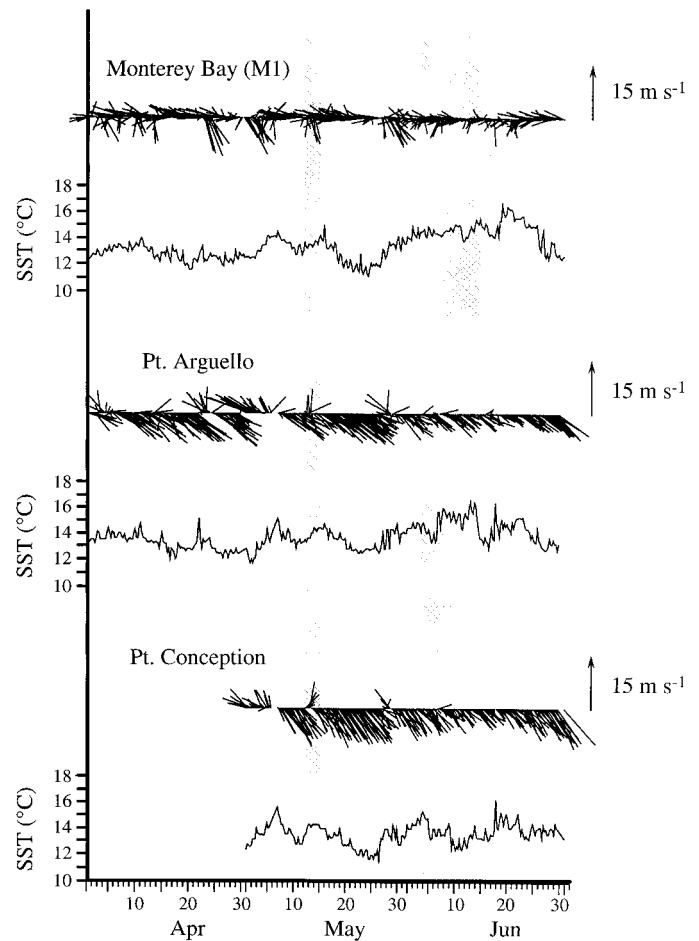


Fig. 2. Vector time series of daily average winds (m s^{-1}) and SST ($^\circ\text{C}$) from NDBC and MBARI buoys, April–June 1998. Lines point in the direction that the wind was blowing. Thin shaded area indicates the date when seizing sea lions were first observed. The wide shaded area denotes the period of sample collection.

all three buoy stations. A wind reversal was observed on 12–15 May, after which time winds were predominantly southward to southeastward marking a period of strong upwelling at Point Arguello and Point Conception. More variable winds were measured during the latter part of May at the M1 buoy where frequent reversals were observed through June. At the southern buoys, the only notable reversal after mid-May occurred on 27 and 28 May (Fig. 2), after which time southeastward winds again dominated. Maximum upwelling on 24 May at the M1 and Point Conception buoys (22 May at the Point Arguello buoy) resulted in a decrease in SST of about 2°C at all three sites. Substantial warming occurred at the end of May and into early June at all three sites. A decrease in SST by more than 1°C was observed in mid-June at the Point Conception buoy.

Horizontal maps of temperature, salinity, chlorophyll a, nutrients, and DA—Monterey Bay and north: The region seaward of San Francisco Bay showed high nitrate and phosphate concentrations with characteristic low salinities and high temperatures indicative of strong freshwater outflow

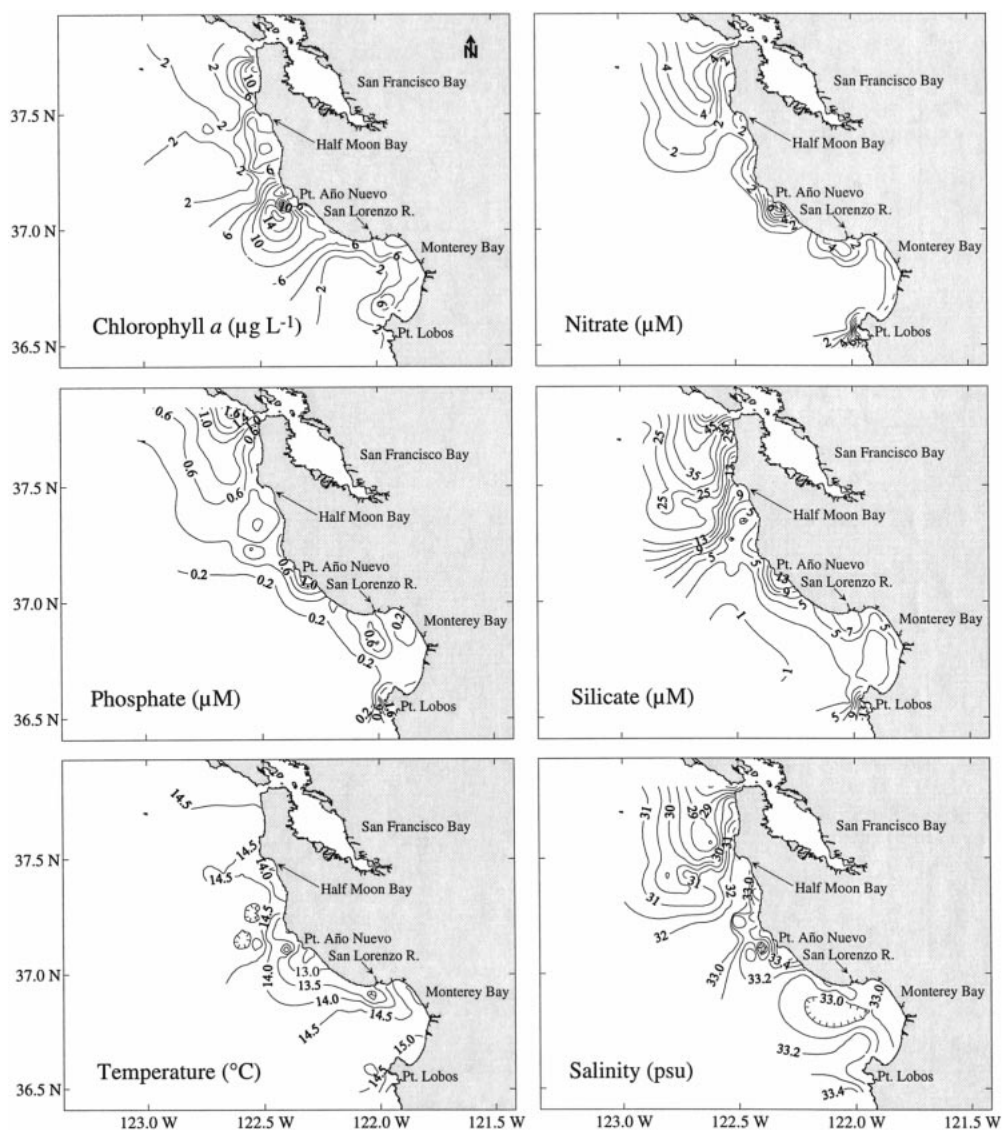


Fig. 3. Surface nutrients, Chl *a*, salinity, and temperature in Monterey Bay and north. Contour intervals vary in each plot.

(Fig. 3). Silicate reached levels greater than $45 \mu\text{M}$, also indicative of strong fluvial input. Although Chl *a* levels were high in the area just south of San Francisco Bay (Fig. 3), *Pseudo-nitzschia* cells were generally absent from this area (not shown). A relatively cool ($12.8\text{--}13.0^\circ\text{C}$) and saline (33.4 psu) area was observed near Point Año Nuevo compared to the water immediately seaward, which was warmer and less saline. Nitrate concentrations were up to $8 \mu\text{M}$, and phosphate was up to $1.0 \mu\text{M}$. Silicate levels reached $13 \mu\text{M}$. Surface Chl *a* concentrations were high near Point Año Nuevo ($10\text{--}14 \mu\text{g L}^{-1}$), which corresponds with the highest surface DA concentrations observed north of Monterey Bay. These Chl *a* levels were the highest measured in the northern areas sampled. The coastal area off Point Lobos, located on the southern Monterey peninsula, also showed evidence of recent upwelling indicated by high salinity (33.4 psu) and nutrient concentrations ($\text{Si}[\text{OH}]_4$, $13 \mu\text{M}$; NO_3 , $8 \mu\text{M}$, PO_4 ,

$1.6 \mu\text{M}$), which were higher than comparable measurements in surrounding waters. Surface DA levels ranged from 180 to 270 ng L^{-1} .

Water within Monterey Bay was comparatively warmer (up to 15.0°C) and more deplete of nutrients. Nearshore Chl *a* values were moderate (about $6 \mu\text{g L}^{-1}$), but DA levels were low (averaging $\sim 100 \text{ ng L}^{-1}$). Results from SEM indicated that the primary species of *Pseudo-nitzschia* within the bay at the time of our sampling was *P. pseudodelicatissima*. Silicate levels were highest (about $7 \mu\text{M}$) in the northern part of Monterey Bay; slightly elevated nitrate and phosphate levels likely indicated influence from the San Lorenzo River.

South of Monterey Bay: The coastal area between Point Lobos and Morro Bay showed wide-ranging levels of nitrate ($2\text{--}9 \mu\text{M}$) and phosphate ($0.4\text{--}0.9 \mu\text{M}$), salinity ($32.7\text{--}35.0$

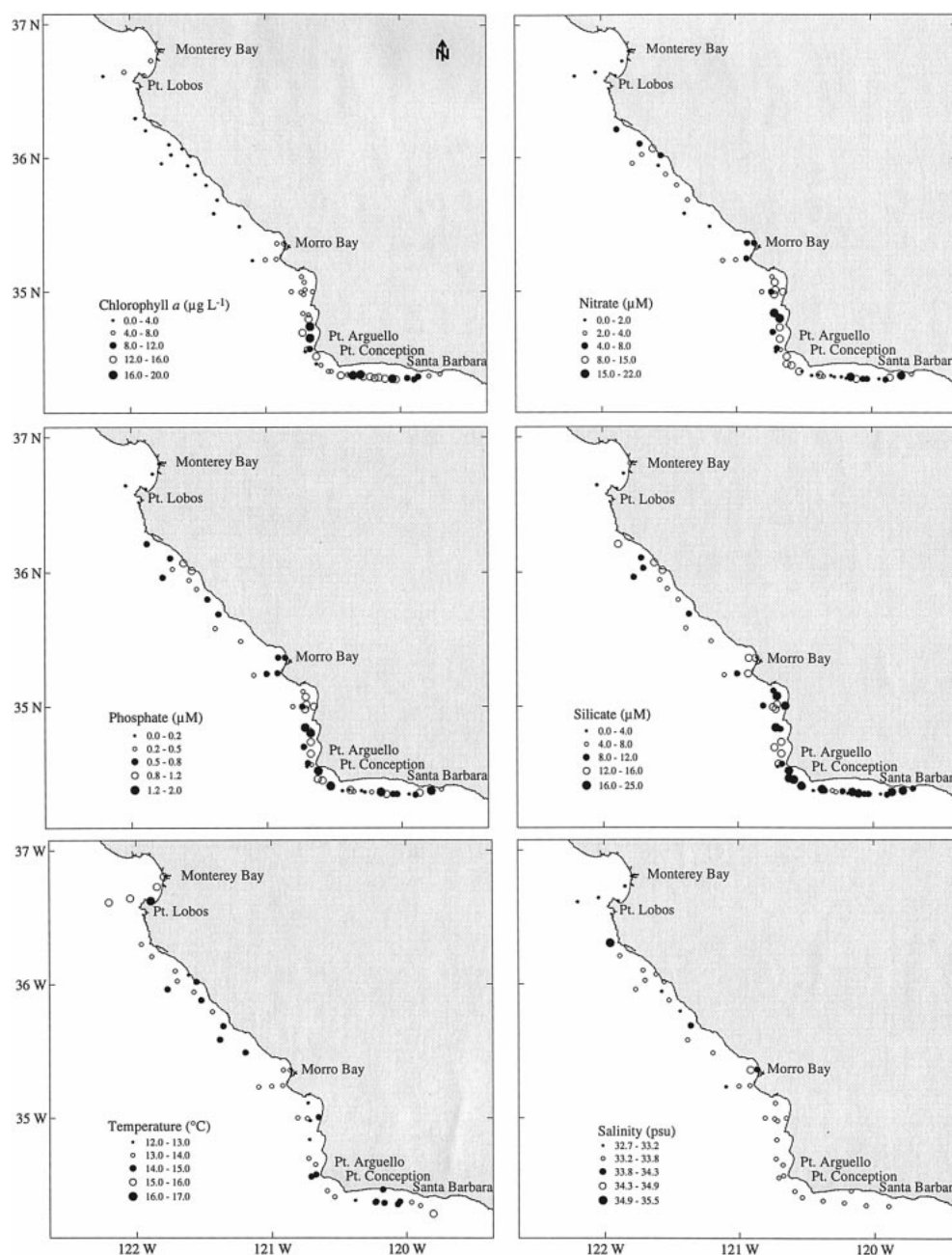


Fig. 4. Surface nutrients, Chl *a*, salinity, and temperature in Monterey Bay and south. Increasing size of circles represent increasing concentrations. Alternating solid and open circles are used for clarity. Legends differ for each plot.

psu), and temperature (12.6–14.2°C, Fig. 4). Throughout this region, Chl *a* values as well as DA levels (averaging ~120 ng L⁻¹, not shown) were consistently low. The sampling sites within Morro Bay showed moderate levels of Chl *a* (4–8 µg L⁻¹), nitrate (6 µM), phosphate (0.7 µM), and silicate (13–14 µM). Temperatures ranged from 13 to 14°C, and salinity averaged 34.3 psu. DA concentrations were ~3,800 µg L⁻¹.

South of Morro Bay, in the classic springtime upwelling regions of Point Arguello and Point Conception, nitrate (<15 µM) and phosphate (<2 µM) levels were among the highest measured over the sampling locations covered by our

cruises. Chl *a*, DA, and silicate values were correspondingly high and among the highest levels measured at all stations (maximum concentrations were 20 µg L⁻¹, 7,300 µg L⁻¹, and 20 µM, respectively). Salinities ranged from 33.3 to 33.5 psu, and temperatures were moderate at 13.5–14.2°C. At sampling sites to the east toward Santa Barbara, surface DA levels (0–2,700 µg L⁻¹) generally decreased, whereas nitrate (2–17 µM), phosphate (0.3–1.5 µM), and temperatures (12–15°C) were more variable. Chl *a* and silicate levels generally remained high (<18 µg L⁻¹ and <20 µM, respectively).

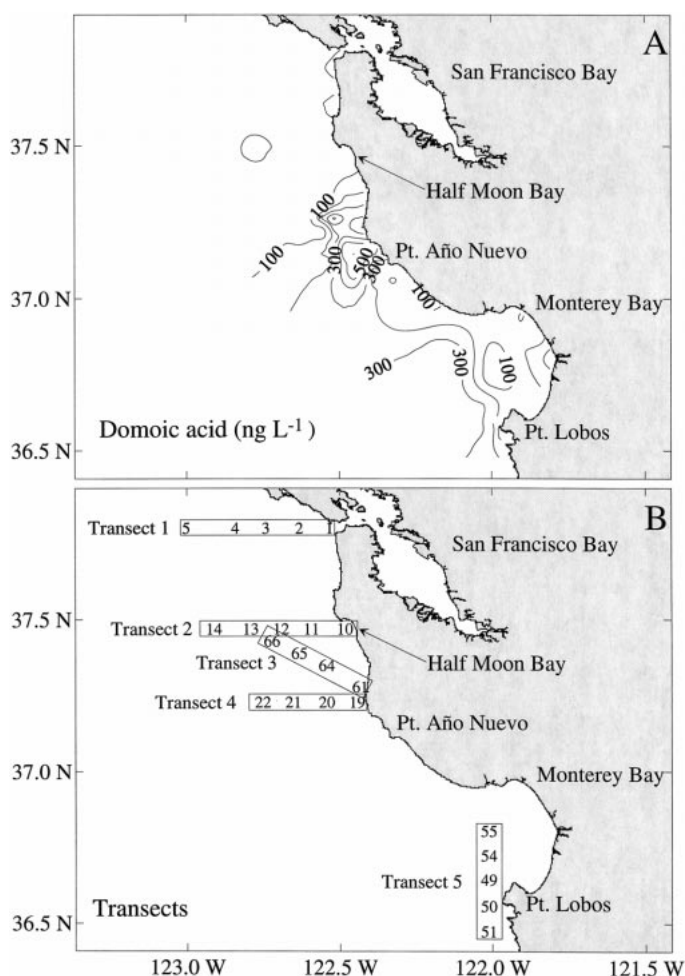


Fig. 5. Surface concentrations of DA in Monterey Bay and north. (A) Surface concentrations of DA are shown in ng L⁻¹. (B) The locations of a series of vertical transects are represented by boxes with stations numbered within the boxes. Vertical transects are shown in Fig. 6.

Vertical cross-shelf sections (density and toxicity)—The horizontal distribution of DA in the northern sampling area is shown in Fig. 5A. This is the only sampling area where weather and time permitted a more comprehensive sampling effort at depth along offshore transects (Fig. 5B). The vertical distribution of density along four representative cross-shelf sections (Fig. 6), extending offshore from San Francisco Bay, Half Moon Bay, and Point Año Nuevo, shows that maximum toxin levels were spatially variable, although associated with higher density (24.5 isopycnal or greater), upwelled water in transects 3 and 4. Strong freshwater influence was seen in transect 1 as isopycnals sloping downward toward the coast. Highest DA levels on this transect (130 ng L⁻¹) were generally associated with denser water a distance of 10 km or more offshore. Significant isopycnal tilting up to the coast was observed in transects 2, 3, and 4; upwelled waters originated from about 20 m depth (not shown). In transects 2 and 3, a well-defined front 8–15 km from the coast separated newly upwelled water from that offshore. Some of the highest toxin levels north of Monterey

Bay were seen at transect 4 (440 ng L⁻¹), associated with the higher density water mass, although the highest surface concentrations of DA (Fig. 5A) were a few kilometers south of station 61. Toxin maxima, with concentrations exceeding 230 ng L⁻¹ at 15 m depth, were seen at 13 km (transect 4) and 40 km (transect 2) offshore, which indicates some offshore movement of toxic cells. The alongshore transect 5 at the mouth of Monterey Bay and south to Point Lobos (station 50 corresponds to the Point Lobos site) showed maximum DA of 380 ng L⁻¹ at 5 m, coincident with elevated nutrient levels (not shown).

Figure 7 shows horizontal distributions of DA in the southern sampling area. The sampling sites within Morro Bay, an area where sea lion strandings had been reported 2 weeks prior to our cruise, showed high levels of DA (up to 3,800 ng L⁻¹). The highest toxin levels per liter seawater were observed within the north/south transect (Fig. 8), at the stations off Point Arguello (7,300 ng L⁻¹) and 30 km to the northwest (6,300 ng L⁻¹). At these stations on the north/south transect, which are 10–25 km offshore, the highest concentrations of toxin were focused at the surface, although DA was measurable also at 40 m depth. In the east/west transect (Fig. 8), the highest DA levels (~2,600 ng L⁻¹) were measured from 0 to 5 m depth at the station about 30 km west of Santa Barbara (southeast of Point Conception) and at about 45–60 km west of Santa Barbara (southwest of Point Conception).

Pseudo-nitzschia species composition and cellular DA levels—Five distinct regions within our cruise sampling area where *Pseudo-nitzschia* cell numbers exceeded 10⁵ cells L⁻¹ and/or DA levels were greater than 0.4 µg L⁻¹ are characterized in Table 1. Although toxin levels were high in the areas just south of Monterey Bay and Morro Bay, samples were not collected for cell counts, so the regions near Point Lobos and Oceano Dunes were not included in this table. The range of cellular DA levels was estimated for each area with the assumption that *P. multiseriata* and *P. australis* were the only toxin-producing species. The majority of phytoplankton in all samples represented in Table 1 were *Pseudo-nitzschia*. In the Morro Bay area, toxin levels were among the highest recorded for field populations of *P. australis* at a maximum of 78 pg DA cell⁻¹. *Pseudo-nitzschia* species were also determined in the Point Año Nuevo, Point Conception, and Santa Barbara areas by rRNA specific probes and compared to SEM results. These molecular probes gave strong positive results for *P. multiseriata* in the Año Nuevo region, *P. australis* in the Point Conception region, and *P. australis* in the Santa Barbara region. Control seawater samples that did not contain toxic species tested negative for those species.

A linear regression of Si:NO₃ molar ratios in *P. australis*-dominated versus *P. multiseriata*-dominated areas showed no significant difference (*t*-test, *df* = 56). Average molar ratios ranged from about 2.1 to 9.1.

Satellite data—Four advanced very high resolution radiometer (AVHRR) satellite images (Fig. 9) are shown, representing dates before, during, and after sample collection. The first image on 15 May shows the SST pattern on the

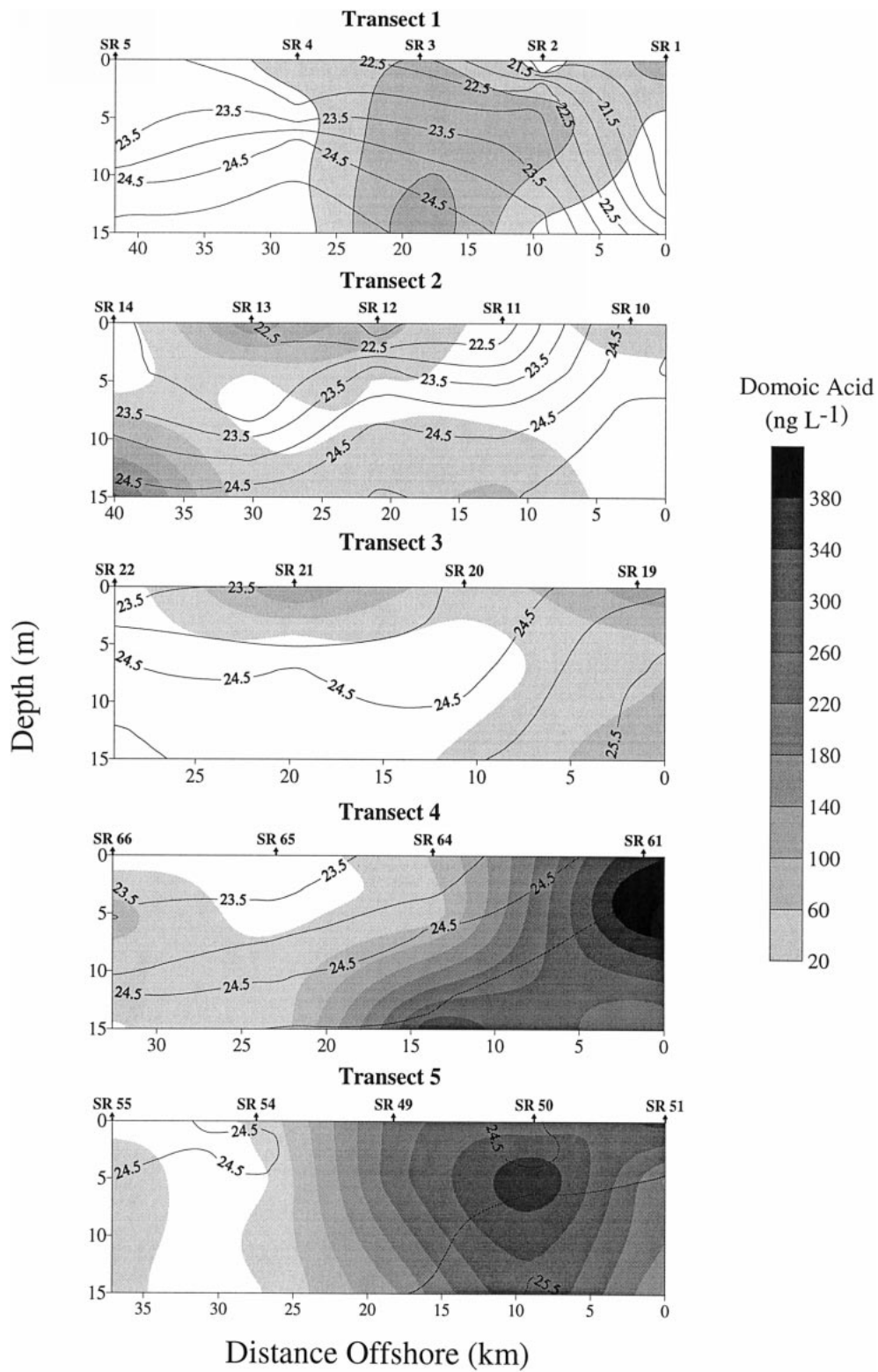


Fig. 6. Vertical profiles of DA in Monterey Bay and north. Vertical transects are represented as plots with increasing toxin concentrations (ng L⁻¹) shown as increasingly shaded areas (see legend for concentrations). Station locations are indicated on the top abscissa. Samples were taken at 0, 5, 10, and 15 m. Transects were occupied on 9 June (transect 1), 10 June (transects 2 and 3), 12 June (transect 5), and 13 June (transect 4). Density (Sigma-T) values are contoured as solid lines.

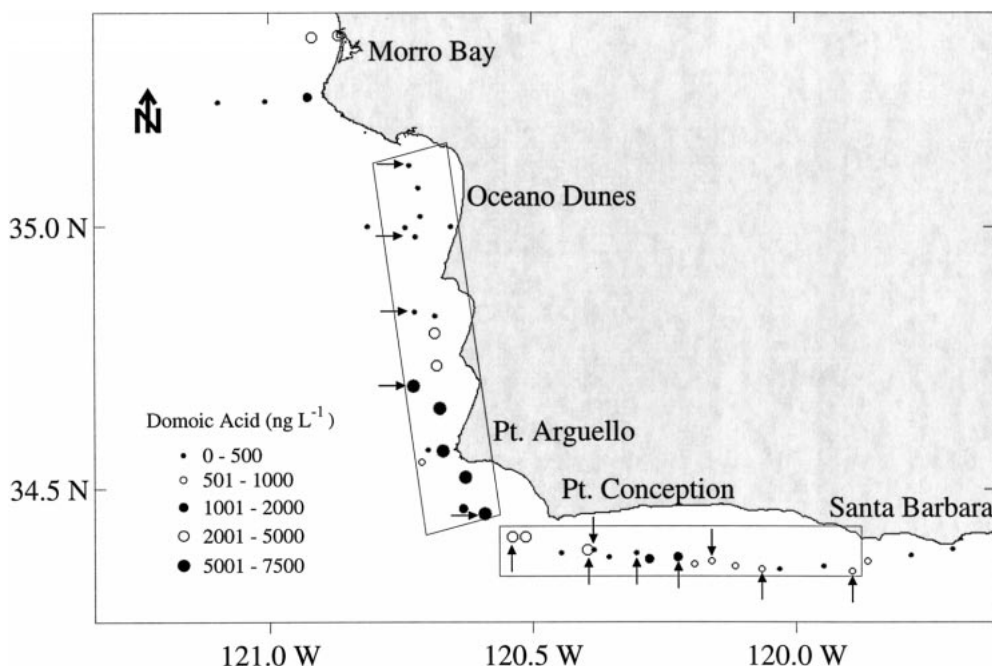


Fig. 7. Surface concentrations of DA in Morro Bay and south. Increasing surface concentrations of DA in ng L^{-1} are shown as dots of increasing size. Solid and open dots are used for clarity. North-south and east-west transects are outlined. Stations used for the vertical profiles shown in Fig. 8 are marked by arrows.

day when the first sea lion stranding in Morro Bay was reported. Cool water (blue color) is evident just north of Morro Bay at Point Estero and further north at Point Piedras Blancas with a plume extending offshore and southward along the coast. This image was collected 3 d after the shift to upwelling-favorable winds in May. The next image in the sequence, collected on 21 May, shows the coolest nearshore waters, which indicates the strongest coastal upwelling, which is supported by SST data in Fig. 2. This image corresponds to the approximate date when the *P. australis* bloom was at its peak in Monterey Bay and when sea lions began stranding in this same area. On 21 May, the colder upwelled water is advected into Monterey Bay as a plume extending along the mouth to an area as far south as $35^{\circ}50'N$. An area of upwelling is also apparent near Point Lobos on 15 May. The intensity of upwelling along the entire coast from Monterey Bay to Point Conception increases from 15 to 21 May. The Point Conception region also shows low SST on 15, 21 May, and 4 June with a filament extending southward past the Channel Islands clearly visible on these dates. The 4 June image corresponds to the start of our two cruises in the area south of Monterey Bay. Although cloud cover prevented a complete image in the offshore areas, it is apparent that upwelling was still intense in the southern regions, especially the onshore areas south of Monterey Bay to Point Conception and toward Santa Barbara to the east. Substantial warming of offshore water is evident on 17 June, and upwelling has largely subsided from areas along the central California coast.

Discussion

Source of bloom nutrients—Every year during spring and summer months, upwelling-favorable winds from the north cause surface divergence on the California coast, injecting nutrient-rich water from depth into the euphotic zone. This seasonal pulse of nutrients supports coastal phytoplankton blooms. During 1998, the relative intensity of the upwelling signal along the California coast was substantially reduced in comparison to previous years due to El Niño conditions (F. Chavez pers. comm.). In addition, rainfall reached record levels in the late winter and early spring of that year, compared to average historical levels (<http://waterdata.usgs.gov/nwis-w/CA/>).

Runoff from rivers during the spring of 1998 has been suggested as a contributing factor to *P. australis* bloom formation in Monterey Bay in May (Gulland et al. 1999; Scholin et al. 2000). Record levels of river runoff were recorded during the winter and spring of 1998 in central California (<http://water.usgs.gov/>). In early May, rainfall and subsequent discharge from the Estrella River (near Morro Bay) and the San Lorenzo River (discharges into northern Monterey Bay) were high due to unusually rainy conditions (<http://waterdata.usgs.gov/nwis-w/CA/>). The appearance of toxic *Pseudo-nitzschia* blooms after a period of heavy rainfall, resulting in freshwater nutrient runoff, has been observed in other geographical areas. Nutrient loading from rivers has been hypothesized to be a possible cause of *Pseudo-nitzschia* blooms in eastern Canada in 1987 (Smith et al.

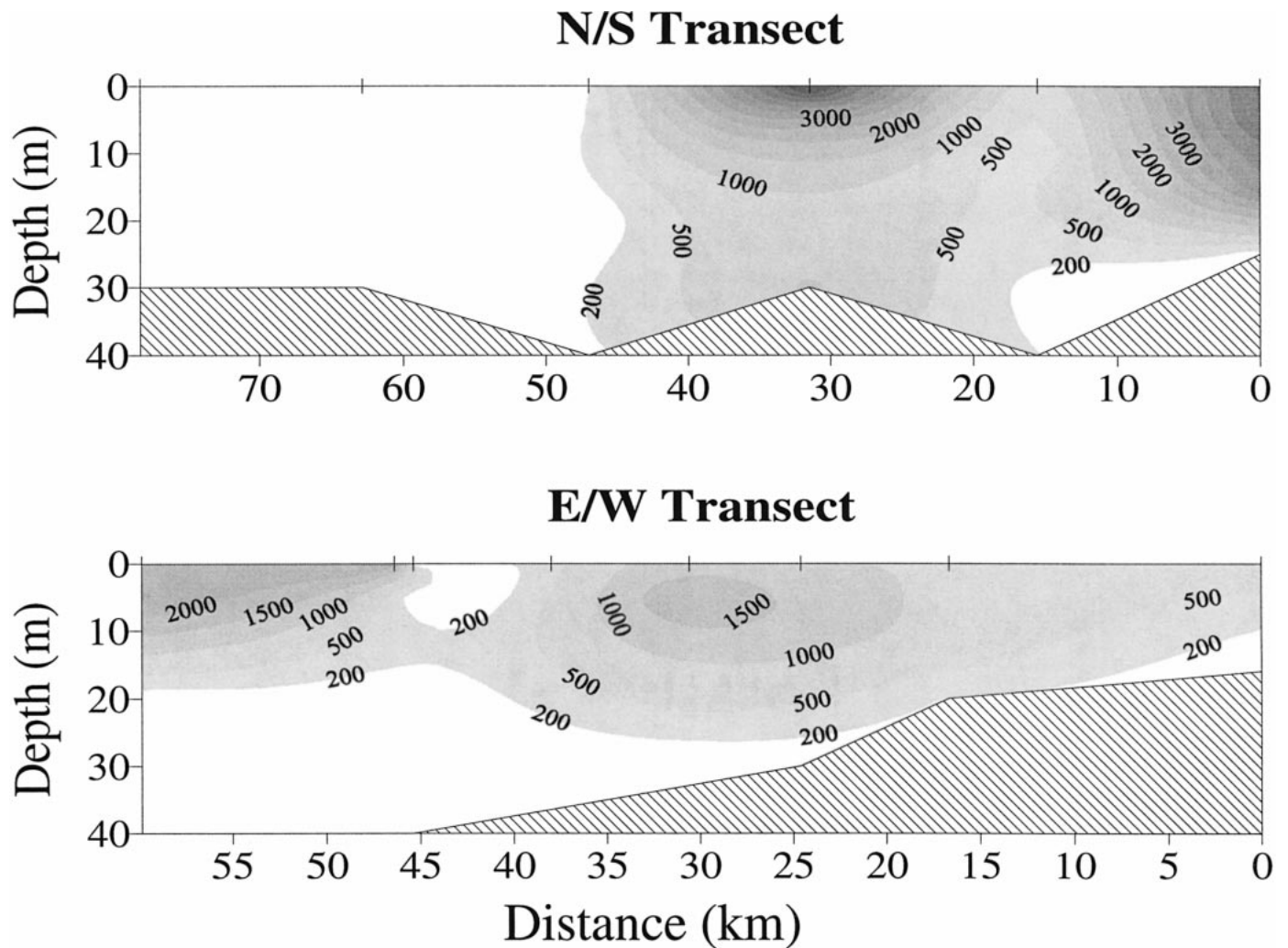


Fig. 8. Vertical profiles of DA in Morro Bay and south. Station locations are indicated as a bar on the upper abscissa of both north-south and east-west transects, and DA concentrations (ng L^{-1}) are shown as contours in linear gray scale. The maximum depth of sampling at each station is bounded by the hatched region. Samples were collected at 0, 5, 15 (all stations), 30, 40 m (some stations). In the north-south transect distance is measured from the southernmost station, and in the east-west transect distance is measured from the station closest to Santa Barbara (upper abscissa, right corner).

1990, 1993; Bates et al. 1998), in the northern Gulf of Mexico in the early 1990s (Dortch et al. 1997), on the Washington coast in 1991 (Horner and Postel 1993), in Monterey Bay in 1991 (Bird and Wright 1989), and in Puget Sound, Washington, in 1997 (Trainer et al. 1998). However, river runoff may not be a factor contributing to every toxic *Pseudo-nitzschia* bloom event. Little river influence was evident in coastal areas at the time of our cruises in early June 1998 (<http://waterdata.usgs.gov/nwis-w/CA/>). In general, very little fluvial input was present in the Point Conception and Morro Bay areas, likewise the temperature and salinity signatures supported oceanic, not fluvial input, so enhanced river flow was likely not a contributing factor to the toxic *Pseudo-nitzschia* blooms observed in those areas during our cruises.

Nutrient input from upwelling, however, did appear to fuel the toxic blooms observed during these cruises. Although upwelling is known to occur all along the central California

coast, preferred sites of intense upwelling associated with capes and headlands have been documented (Reid et al. 1958; Kelly 1985). Specifically, important upwelling centers have been described near Point Año Nuevo, Point Sur/Point Lobos, and Point Conception (Dugdale and Wilkerson 1989; Rosenfeld et al. 1994; Hutchins and Bruland 1998). The chemical and physical characteristics of several central California coastal areas during our June 1998 cruises suggested that recent upwelling had occurred, although the nutrient signal associated with upwelling was reduced, a typical observation in El Niño years (Barber and Chavez 1983).

More specifically, vertical transects north of Monterey Bay near Point Año Nuevo showed the association of the highest DA levels in our northern sampling area with higher density, upwelled waters (Fig. 6). High DA was also measured in areas of elevated nutrients near Point Lobos. Some of the highest cell numbers of *P. australis* and also the highest cellular DA levels were observed between Point Arguello

Table 1. Domoic acid content, *Pseudo-nitzschia* species, and silicate : nitrate ratios in surface samples collected off the central California coast in June 1998.

Area*	Date (1998)	Cellular domoic acid (pg cell ⁻¹)†	Whole water domoic acid (μg L ⁻¹)	Dominant <i>Pseudo-nitzschia</i> species	Maximum toxic <i>Pseudo-nitzschia</i> (cells L ⁻¹)	rRNA probe toxic species determination	Si:NO ₃ ratios‡
Año Nuevo	10–13 June	0.3–6.3 (n = 8)	0.1–0.7	<i>P. pungens</i> <i>P. multiseriis</i>	5.2 × 10 ⁵ <i>P. multiseriis</i>	<i>P. multiseriis</i> (n = 11)	6.7 ± 6.2
Monterey Bay	12 June	0.8–1.2 (n = 2)	0.1–0.4	<i>P. pseudodeli</i>	3.1 × 10 ⁵ <i>P. multiseriis</i>	ND	9.1 ± 8.1
Morro Bay	3 June	37.0–78.0 (n = 3)	1.3–3.8	<i>P. australis</i>	4.9 × 10 ⁴ <i>P. australis</i>	ND	2.1 ± 0.1
Point Conception	4–5 June	15.0–22.0 (n = 3)	2.2–6.3	<i>P. australis</i>	2.3 × 10 ⁵ <i>P. australis</i>	<i>P. australis</i> (n = 6)	7.2 ± 8.5
Santa Barbara	4 June	0.1–0.9 (n = 3)	0.5–1.2	<i>P. australis</i> <i>P. pungens</i> <i>P. pseudodeli</i>	1.1 × 10 ⁷ <i>P. australis</i>	<i>P. australis</i> (n = 3)	5.7 ± 7.8

* Each area encompasses stations that are within Monterey or Morro Bay or located within a 15 km radius of the designated land station.

† Cellular toxin levels were determined by estimating the total percentage of toxic species in a volume seawater using SEM and dividing that value by the concentration of DA in that same volume. The *n* values for given ranges of cellular and whole water DA are the same.

‡ Silicate : nitrate ratios are represented as the average ± standard deviation. *P. pseudodeli* = *Pseudo-nitzschia pseudodelicatissima*. ND = not done.

and Point Conception, an upwelling zone that has been studied in detail (Sverdrup 1938; Sverdrup and Allen 1939; Brink and Muench 1986; Barth and Brink 1987; Dugdale and Wilkerson 1989). Nutrient levels in this region were higher than those in surrounding areas but less than maximum reported values (e.g., surface nitrate concentrations can reach 32 μM at Point Conception; Dugdale 1985). These data indicate that nutrients from cooler, upwelled water sustained the toxic *Pseudo-nitzschia* populations observed during our cruises.

Transport processes—Our field sampling efforts began 2–3 weeks after the peak bloom of *P. australis* cells was observed in Monterey Bay. Since the first sea lion strandings were reported in the Oceano Dunes area on 15 May and high levels of DA in seawater were observed to the north in Morro Bay and to the south near Point Arguello on 4–5 June, it appears that the *P. australis* bloom in this region may have been sustained for 3 weeks or more. Satellite photos also indicated the presence of cooler waters near this area over a sustained period from mid-May to early June (Fig. 9). These cooler waters to the north of Morro Bay may have been a source of upwelled nutrients into the bay.

Transport of upwelled waters near capes or headlands into bays may have fueled blooms of toxic *Pseudo-nitzschia* along the central California coast. Since there is no significant upwelling source within Monterey Bay (Graham et al. 1992), upwelled water originating near Point Año Nuevo, north of Monterey Bay, was likely advected southward into the bay (Rosenfeld et al. 1994; Tracy 1990). The presence of high numbers of *P. multiseriis* near the Point Año Nuevo upwelling area during the mid-June cruise suggests that this may be a bloom initiation site with strong influences on Monterey Bay. Previous research has indicated that upwelled water from Point Año Nuevo is advected to the southeast into Monterey Bay past Santa Cruz (Graham et al. 1992). Satellite imagery of SST, especially on 21 May (Fig. 7), provides evidence that cool water was entering Monterey Bay in late May. A series of wind relaxation events in Mon-

terey Bay during May (Fig. 2) provided the mechanism for onshore transport of cells. However, in early June during our cruise, offshore transport of cells was evident in the toxin data from the Año Nuevo area (Fig. 6, transect 3), indicative of an upwelling event. The balance between a supply of nutrients to surface waters associated with upwelling and transport of cells to inshore areas during wind relaxation events is crucial to the development of toxic *Pseudo-nitzschia* blooms in coastal regions.

Strong winds and currents in coastal areas south of Monterey Bay resulted in coastal upwelling, providing areas of high nutrients in which phytoplankton cells could thrive. Strong southward surface winds and currents were sustained in late May and early June 1998 along the southern California coast from Morro Bay to Point Conception (D. Siegel pers. comm.; <http://www-ccs.ucsd.edu/research/sbcsmb/buoys/>), which supports the possibility that toxic cells were transported southward and fueled by nutrients from upwelling centers. The absence of relaxation events in this region from late May to mid-June, i.e., the observation of consistent southward winds from buoys in this region, indicates that flow was to the south at this time. Separate transport mechanisms and physical conditions may account for the mixed population of *Pseudo-nitzschia* observed from Point Conception to Santa Barbara. A decrease in surface DA levels in this region and knowledge of circulation patterns in the Santa Barbara Channel during upwelling periods (<http://www-ccs.ucsd.edu/research/sbcsmb/>) provide evidence that cells are dispersed from Point Conception toward Santa Barbara via complex physical mechanisms. The use of buoys and drifters as part of large-scale harmful algal bloom (HAB) studies will allow a more complete description of the transport processes instrumental to the spreading of toxic blooms to key coastal locations.

Sinking of *Pseudo-nitzschia* cells—Evidence from sediment traps (Alldredge and Gottschalk 1989; Buck et al. 1992) suggests that vertical export of primary production from the upper ocean to depths may occur as seasonal events

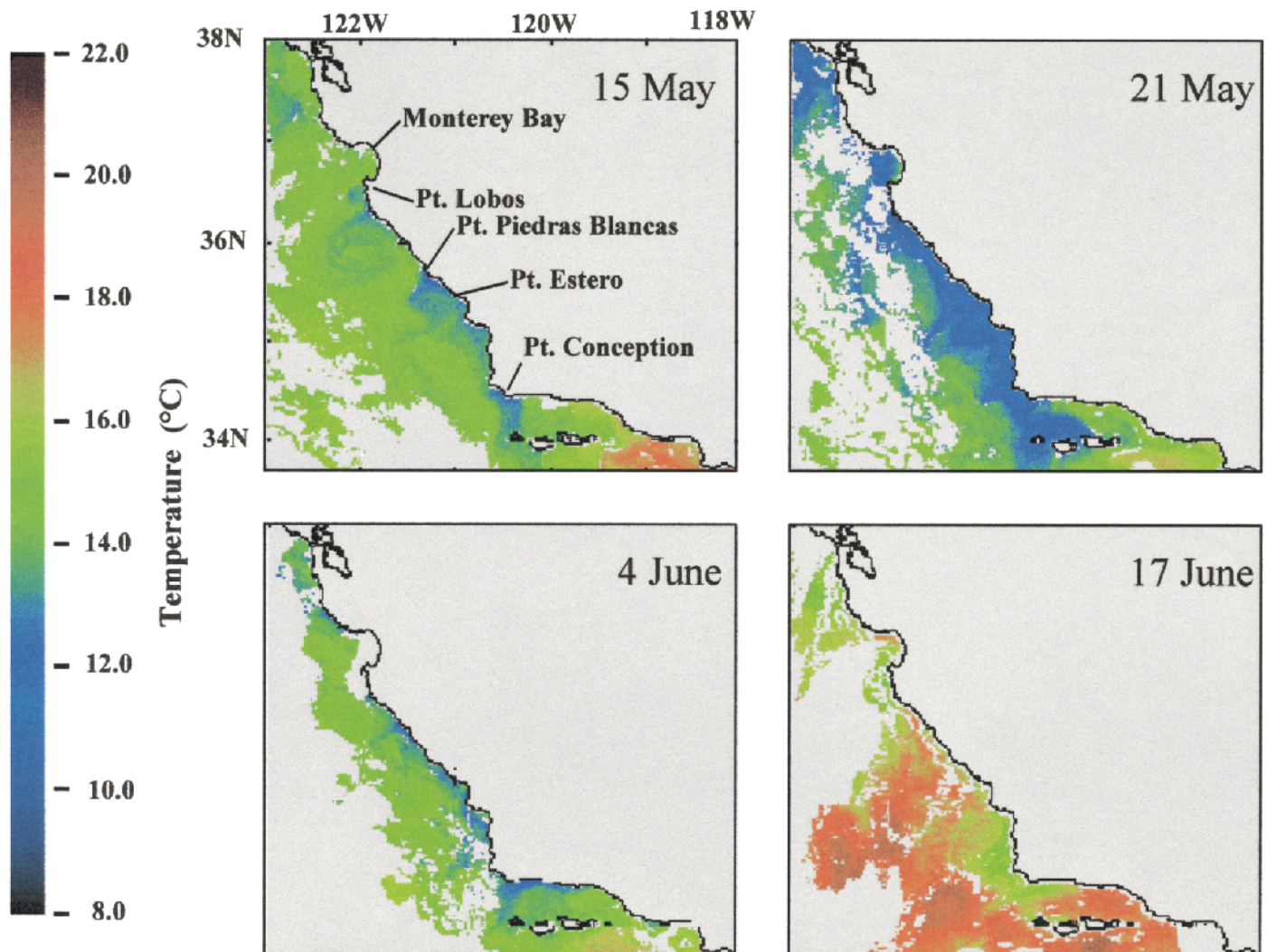


Fig. 9. AVHRR sea-surface temperature (SST) images of the survey region along the central California coast from 15 May through 17 June. Color scale ($^{\circ}\text{C}$) for SST is shown on the left. Ocean cloud cover, west of the land mass containing labeled points of interest on the 15 May image, is shown in off white.

following phytoplankton blooms. This vertical movement of cells through the water column may have contributed to the persistence of *Pseudo-nitzschia* blooms on the coast for several weeks. *Pseudo-nitzschia* cells sink as they become less buoyant when nutrients are depleted; however, these cells can still retain toxin even at depth (Dortch et al. 1997; Trainer et al. 1998). The measurement of cellular DA below the photic zone to 40 m near Point Conception demonstrates that intact, toxic cells sank to depth in this area. Previous studies have shown that *Pseudo-nitzschia* species (previously characterized as *Nitzschia* spp.) are major components of marine snow in the Santa Barbara region, especially during seasonal upwelling (Alldredge and Gotschalk 1989). These authors suggest that flocculation may confer nutrient advantage to diatoms as they sink through the mixed layer and into the thermocline. We theorize that *Pseudo-nitzschia* cells may reach depths and enter a resting state of slow metabolism in a nutrient-rich environment until they are brought to the surface by the next upwelling event. This replenishment of cells

to the surface may also explain why some blooms appear to persist for up to 3 months in the field (Bates et al. 1989; Smith et al. 1990).

Nutrient requirements of Pseudo-nitzschia—A 10-yr time series from Monterey Bay (F. Chavez pers. comm.; <http://www.mbari.org/bog/Projects/CentralCal/>) indicated that pennate diatoms are generally at their maximum levels in the late summer and fall when upwelling has subsided and nutrients are declining. In another study, Walz et al. (1994) documented the occurrence of the most persistent DA-producing blooms in Monterey Bay during the late summer and autumn at a time when seasonal, coastal upwelling was sporadic or had ceased. During this time, hydrographic conditions were characterized by warmer water ($14.5\text{--}17.5^{\circ}\text{C}$) and lower nutrients (NO_3 $0.2\text{--}3.0\ \mu\text{M}$; $\text{Si}[\text{OH}]_4$ $1.0\text{--}7.0\ \mu\text{M}$). However, in this same study, DA was also measured during springtime *Pseudo-nitzschia* blooms at a time when nutrients were plentiful, which indicates that these cells were produc-

ing toxin under a range of environmental conditions. It has been suggested that spring blooms may be retained in near-shore regions where they are subjected to nutrient stress similar to that observed during postupwelling conditions in later summer and autumn (Walz et al. 1994). In support of these previous observations, results from our cruises indicated that areas where toxic *Pseudo-nitzschia* were focused contained moderate nutrient levels corresponding to springtime, post-upwelling events. However, nutrient levels were anomalously low during the spring transition in 1998 due to El Niño conditions (F. Chavez pers. comm.; <http://www.mbari.org/bog/Projects/CentralCal/>). We suggest that nutrients that favor toxic *Pseudo-nitzschia* growth appear to be at concentrations less than the maxima observed at upwelling sites during non-El Niño years.

Past studies have indicated that silicate to nitrate ratios are important in the control of DA production and that silicate limitation may be important in the control of toxin production, at least in cultured *P. multiseriis* (Bates et al. 1991). However, in all areas shown in Table 1, average Si:NO₃ molar ratios were 6 (in areas where *P. australis* was the dominant toxin producer) to 8 (in areas where *P. multiseriis* was the dominant toxic species). This indicates that silicate was sufficient for diatom growth and that silicate enrichment was probably occurring in these upwelling areas by mineralization processes (Alvarez-Salgado et al. 1998). These observations indicate that nutrient requirements for toxin production in situ may not always be effectively predicted by laboratory experiments using cultured isolates of *Pseudo-nitzschia*.

Trace elements may be important in the ability of toxic *Pseudo-nitzschia* to outcompete other phytoplankton species. Iron addition experiments have shown that Fe levels in upwelling regions off the California coast are important in the promotion of *Pseudo-nitzschia* blooms (Hutchins and Bruland 1998). Recent studies have also indicated that upwelling areas with sufficient silicate were not iron limited (Hutchins and Bruland 1998), which suggests that both iron and silicate were sufficient in the areas of our cruises where toxic *Pseudo-nitzschia* were abundant. Clearly, long-term field measurements are needed to fully describe the dynamics of nutrient concentrations and trace elements in *Pseudo-nitzschia* bloom formation.

Cellular toxin levels—Cellular levels of DA differed significantly in field populations of *P. multiseriis* (0.3–6.3 pg cell⁻¹) and *P. australis* (0.1–78 pg cell⁻¹). These levels agree well with previously reported values for field populations of *P. multiseriis* in eastern Canada (up to 9 pg cell⁻¹; Smith et al. 1990) and of *P. australis* in Monterey Bay (0.4–32.6 pg cell⁻¹, Walz et al. 1994; up to 34 pg cell⁻¹, Buck et al. 1992; up to 75 pg cell⁻¹, Scholin et al. 2000) and values for cultured *P. multiseriis* from eastern Canada (up to 21 pg cell⁻¹; Bates et al. 1989) and *P. australis* isolated from Monterey Bay (37 pg cell⁻¹ maximum, Garrison et al. 1992). The cellular levels of toxin documented in Morro Bay during our cruises (up to 78 pg cell⁻¹) are the highest yet recorded for a field population of *P. australis*. These data suggest that lower cell densities of *P. australis* than *P. multiseriis* may be required to produce similar amounts of DA.

Food chain transfer of toxin—Originally named amnesic shellfish poisoning in 1987 because of the transfer of DA from mussels to humans causing loss of short-term memory (Todd 1990), it is now clear that toxin can enter the food web by a variety of vectors. Over the past several years, examples of DA transfer through suspension-feeding fish and shellfish to higher levels of the food chain have become evident along the Pacific coast of the U.S. In the 1991 DA outbreak in Monterey Bay, cormorants and pelicans were the only animals directly examined (Work et al. 1993). Analysis of the birds' stomach contents revealed that they had been feeding on anchovies and sardines from the bay that contained high levels of DA. In the fall of 1991, razor clams on the Washington coast were found to contain levels of DA above the regulatory limit (Wekell et al. 1994). These DA events on the Washington coast and in Monterey Bay clearly demonstrate that DA has at least two means of entering the higher food web, through filter-feeding molluscan shellfish and suspension-feeding finfish.

Since shellfish and finfish can contain DA and are staples in the diet of some marine mammals, these filter feeders were suspected vectors of toxin to higher organisms. The possibility that DA could cause the disoriented behavior of marine mammals, including whales, has been suggested in the past (Fritz et al. 1992). Indeed, during the 1998 DA episode, pelagic finfish were shown to play a crucial role in the transfer of toxins to sea lions. In addition, other vectors have been found to be important in the transfer of toxin to other marine mammals, especially those that feed on benthic invertebrates. *Pseudo-nitzschia* cells that retain significant levels of DA are known to sink to the bottom of enclosed bays (Trainer et al. 1998) where filter-feeding benthic organisms could play a role in toxin transfer. Indeed, sea otters, known to feed on small benthic crustaceans, were observed to have neurological symptoms during the first 2 weeks of June in Monterey Bay and during the last 2 weeks of June in Morro Bay (F. Gulland pers. comm.). Samples of urine, feces, and stomach contents from some otters tested positive for DA (M. Chechowitz et al. unpubl. data). Antennae from the spiny mole crab (*Blepharipoda occidentalis*) were found in the stomach of one sea otter that tested positive for DA in its gut and urine (Trainer et al. unpubl. data). It is possible that the mole crab, a common food of sea otters, retains DA and can act as a vector in their poisoning. Clearly, the entry of DA into both the benthic and pelagic food webs can have a negative impact on the nearshore ecosystem.

Since 1978, there have been documented cases of marine mammals along the California coast suffering from unexplained neurological dysfunction (Scholin et al. 2000; Gulland et al. in press). Although a direct link of DA poisoning in sea lions via consumption of sardines and anchovies was not proven in 1991, a number of unexplained seizure events occurred in these mammals in the Santa Barbara, Oceano Dunes, and Monterey Bay area during September (J. Cordaro pers. comm.). At the peak of the September 1991 event, DA levels in coastal waters reached 10 µg L⁻¹ and abundances of *P. australis* were up to 10⁶ cells L⁻¹ (Walz et al. 1994). Since cellular levels of DA were similar to those during the 1998 event, it is reasonable to suspect that at least some of the 1991 sea lion strandings were also caused by DA poi-

soning. In addition, there were reports of sea lions poisoned by DA in central and southern California after the event in May and June 1998. Several sea lions were poisoned by DA in Monterey Bay in October 1998, coincident with a *P. australis* bloom in the fall (Gulland et al. 1999). Sea lions suffering from seizures were also observed in San Diego county in March 1999, and subsequently DA was measured in the urine of two animals (T. Reidarson pers. comm.; Trainer et al. unpubl. data). As this work was going to press, over 90 sea lions with neurological symptoms were reported to be stranding again in the summer of 2000 off the coast of central California (F. Gulland pers. comm.). These combined observations support the possibility that mammal strandings due to DA exposure are relatively common events.

Detection of toxic events—The ecological significance of the 1998 DA event in central California became a subject of study only after reports of marine mammal deaths were made. These reports resulted from sporadic human observations along the accessible stretches of the California coast. Although these observations were an incomplete means of initially documenting this DA episode, they did eventually lead to the measurement of toxin in the affected animals. On the other hand, DA levels in mussels, the sentinel species that was used as an indicator of DA in coastal California, did not effectively predict the toxic potential of this bloom event. Past studies have indicated the difficulty in using mussels to detect toxic events due to their fast depuration of toxin (Novaczek et al. 1991; Langlois et al. 1993). During the spring 1998 episode, levels of DA in mussels reached $2.1 \mu\text{g DA g}^{-1}$ tissue, whereas toxin levels in sardines and anchovies reached $257 \mu\text{g DA g}^{-1}$ tissue (G. Langlois and S. Luscutoff, California Department of Health). However, because these herbivorous finfish can move rapidly from one coastal location to another, they can not be used as an effective indicator of toxin levels in a given area. In contrast, the rapid detection of *P. australis* cells using molecular methods and of toxin levels using high throughput biochemical techniques during this event showed that these methods have great potential for early warning of toxic events in a localized coastal region (Scholin et al. 2000; this paper). The modification of these methods for in situ use with real-time output would provide an early warning system at key coastal locations. Both current and future DA monitoring programs could consider these molecular and biochemical techniques as adjuncts to current laboratory methods.

The complete ecological significance of DA in these coastal regions will only be understood when the diversity of organisms involved in the complex chain of toxin transfer is rigorously studied. This will lead to information about rates of depuration and accumulation in a variety of species, leading to the discovery of an effective sentinel organism. However, the most effective prediction of toxic bloom events will be made possible through an understanding of the complex series of factors that influence toxic bloom development. The presence of *Pseudo-nitzschia* in central California waters, often in high numbers, during all but the most extreme winter months (C. Scholin pers. comm.), allows investigation of bloom dynamics during much of the year. The physical and chemical conditions contributing to harmful al-

gal bloom initiation and maintenance will be fully understood only when long-term data sets detailing bloom dynamics are collected and high throughput, automated techniques are used for the detection of toxins and toxigenic cells.

Conclusions

DA measured in seawater by three different methods confirmed its presence at high levels in California coastal waters during early June 1998. Cellular levels of DA in *P. australis* reached 78 pg cell^{-1} in Morro Bay and in *P. multiseries* reached 6 pg cell^{-1} near Point Año Nuevo. Maximum cellular DA levels in the areas sampled were observed within 20 km of the coast and from 0 to 5 m depth, however toxin was measured to 40 m depth.

Toxic *Pseudo-nitzschia* blooms were widespread along the central California coast during June 1998. *Pseudo-nitzschia* cells were positioned in water masses near upwelling zones during late spring 1998. A supply of increased nutrients from upwelling zones may be an important trigger of toxic *Pseudo-nitzschia* blooms. High numbers of *P. multiseries* and *P. australis* were associated with nitrate, silicate, and phosphate levels that were less than the maximum concentrations typically found in upwelling zones. The flow of cells and/or nutrients from coastal headlands into embayments where cells can multiply in a stratified environment is a possible mechanism of bloom development along the central California coast.

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