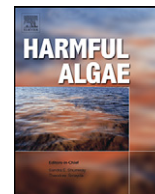




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A shift in the dominant toxin-producing algal species in central California alters phycotoxins in food webs

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ABSTRACT

In California, the toxic algal species of primary concern are the dinoflagellate *Alexandrium catenella* and members of the pennate diatom genus *Pseudo-nitzschia*, both producers of potent neurotoxins that are capable of sickening and killing marine life and humans. During the summer of 2004 in Monterey Bay, we observed a change in the taxonomic structure of the phytoplankton community—the typically diatom-dominated community shifted to a red tide, dinoflagellate-dominated community. Here we use a 6-year time series (2000–2006) to show how the abundance of the dominant harmful algal bloom (HAB) species in the Bay up to that point, *Pseudo-nitzschia*, significantly declined during the dinoflagellate-dominated interval, while two genera of toxic dinoflagellates, *Alexandrium* and *Dinophysis*, became the predominant toxin producers. This change represents a shift from a genus of toxin producers that typically dominates the community during a toxic bloom, to HAB taxa that are generally only minor components of the community in a toxic event. This change in the local HAB species was also reflected in the toxins present in higher trophic levels. Despite the small contribution of *A. catenella* to the overall phytoplankton community, the increase in the presence of this species in Monterey Bay was associated with an increase in the presence of paralytic shellfish poisoning (PSP) toxins in sentinel shellfish and clupeoid fish. This report provides the first evidence that PSP toxins are present in California's pelagic food web, as PSP toxins were detected in both northern anchovies (*Engraulis mordax*) and Pacific sardines (*Sardinops sagax*). Another interesting observation from our data is the co-occurrence of DA and PSP toxins in both planktivorous fish and sentinel shellfish. We also provide evidence, based on the statewide biotoxin monitoring program, that this increase in the frequency and abundance of PSP events related to *A. catenella* occurred not just in Monterey Bay, but also in other coastal regions of California. Our results demonstrate that changes in the taxonomic structure of the phytoplankton community influences the nature of the algal toxins that move through local food webs and also emphasizes the importance of monitoring for the full suite of toxic algae, rather than just one genus or species.

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1. Introduction

California has a long-standing history of toxic phytoplankton blooms that date back to the early 1900s (Meyer et al., 1928). In the past, the toxic species of primary concern has been the dinoflagellate *Alexandrium catenella*, producer of a suite of highly toxic compounds referred to as paralytic shellfish poisoning (PSP) toxins, and human deaths related to PSP can be traced back over a century. After a major PSP outbreak in 1927, the California Department of Public Health

(CDPH) recognized the continuing threat to shellfish consumers and initiated a statewide marine biotoxin monitoring program (Price et al., 1991). Since the inception of the monitoring program, over 500 human illnesses and 39 deaths have been reported, that last of which occurred in the 1980s (Price et al., 1991). Interestingly, and despite its routine presence in California, PSP toxins have not been associated with any marine animal mortality events, as occurs in other regions afflicted by PSP (Geraci et al., 1989; Reyero et al., 1999; White, 1981). Aside from shellfish, sand crabs (*Emerita analoga*) are the only other organisms in which PSP toxins have been reported in California (Bretz et al., 2002).

More recently, members of the diatom genus *Pseudo-nitzschia* were discovered to be problematic on the US west coast, when two

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species, *Pseudo-nitzschia australis* and *P. multiseriata*, were linked to the production of domoic acid (DA) in 1991. The event that led to this discovery involved the death of hundreds of brown pelicans (*Pelecanus occidentalis*) in Monterey Bay, CA (Fritz et al., 1992; Work et al., 1993). Since then, repeated DA poisoning events throughout central and southern California have resulted in the closure of commercial fisheries and the deaths of hundreds of pinnipeds and cetaceans (Langlois, 2003, 2004; Scholin et al., 2000). Many potential vectors of DA to secondary predators have been identified (Bargu et al., 2002; Goldberg, 2003; Powell et al., 2002; Wekell et al., 1994), but planktivorous fish, such as northern anchovies (*Engraulis mordax*) and Pacific sardines (*Sardinops sagax*), are the most frequent vector implicated in animal mortalities in this region (Lefebvre et al., 1999; Scholin et al., 2000; Work et al., 1993). To date, there have not been any incidences of human illness related to DA in California, likely the result of prompt inclusion of DA monitoring by CDPH into the statewide biotoxin monitoring program.

In addition to these well-known taxa, two genera known to be toxic in other regions are present in California coastal waters. *Dinophysis* species such as *D. fortii*, *D. acuminata* and *D. rotundata*, globally recognized as producers of diarrhetic shellfish poisoning (DSP) toxins (Hallegraeff, 1993), are routinely present along our coastline. In addition, DSP toxins have been detected in surface water samples (Weber, 2000) and a recent study has confirmed for the first time the presence of low levels of DSP toxins in mussels from Monterey Bay (Sutherland, 2008). Another potentially problematic dinoflagellate, *Cochlodinium*, has become more frequent in central California (Curtiss et al., 2008), causing visible red tides and attracting the attention of the media. The presence of this alga has raised concerns, as members of this genus have caused fish mortalities in the coastal Pacific waters of Mexico, Central America, Canada and Asia (Garate-Lizarraga et al., 2004; Kim et al., 1999; Lara et al., 2004; Vargas-Montero et al., 2006; Whyte et al., 2001). There have not yet been any published reports of human illnesses or animal mortality events caused by *Dinophysis* or *Cochlodinium* in California, though there is unpublished circumstantial evidence that a recent bloom of *Cochlodinium* caused mass shellfish mortalities (G. Langlois pers. commun.).

The coastal upwelling region of Monterey Bay, the focal point of this study, supports a rich and diverse marine ecosystem and is valued not only economically for its fisheries, but also for its importance to marine wildlife. The primary production level in Monterey Bay differs significantly in the three well-described hydrographic “seasons” of the Bay (Bolin and Abbott, 1963; Breaker and Broenkow, 1994) and is usually highest during the diatom-dominated spring to summer “upwelling season” (Bolin and Abbott, 1963). In contrast, periods of dinoflagellate dominance are thought to be temporary and associated with intrusions of more nutrient-depleted, offshore waters as can occur during the oceanic and Davidson Current “seasons” (Bolin and Abbott, 1963; Garrison, 1979). The seasonal high productivity that characterizes this region is particularly important to the variety of resident and migratory animals, such as sea birds, marine mammals and turtles, that depend on this area as a feeding ground (Benson et al., 2007; Croll et al., 2005; Hyrenbach et al., 2006; Yen et al., 2004). Given that a major prey item for many of these predatory animals is planktivorous fish, exposure to algal toxins may be common, given the efficiency with which these fish vector toxins (Lefebvre et al., 1999; Scholin et al., 2000).

As part of our ongoing work in Monterey Bay monitoring the phytoplankton community (especially harmful algal bloom [HAB] taxa) and examining the presence of algal toxins in marine food webs, we observed an unexpected shift in the phytoplankton community. This shift was characterized by the unusual dom-

inance of dinoflagellates in this typically diatom-dominated system. As such, in this study we compare the taxonomic structure of the phytoplankton community before and after this change in the dominant flora of Monterey Bay using samples collected over a 6-year period, with particular attention paid to toxin-producing taxa and the presence of DA and PSP toxins in the food web.

2. Materials and methods

2.1. Field sampling

2.1.1. Water sample collection

From January 2000 to December 2006 weekly surface water samples were collected using a bucket at 2 sites within Monterey Bay, CA: nearshore at the Santa Cruz wharf (SCW) (36.95 N, 122.02 W) and offshore at the Monterey Bay Aquarium Research Institute's M1 mooring (36.75 N, 122.02 W). SCW water samples were immediately transported to the lab for processing, while M1 samples were collected midday aboard the R/V Pt. Lobos, stored in a dark cooler, and transported to the lab for processing approximately 6 h after collection. The three best known toxin-producing species in Monterey Bay, *P. australis*, *P. multiseriata* and *A. catenella*, were identified and enumerated on an epifluorescent compound microscope (Zeiss Axio Imager) using whole-cell oligonucleotide probes as described in Miller and Scholin (1998, 2000) and Scholin (1994). *P. australis* and *P. multiseriata* densities are summed and presented as “toxic *Pseudo-nitzschia*.” At SCW, net plankton samples, used for phytoplankton community assessments, were collected with a 20- μ m mesh net hauled through the upper 3 m of the water column. Using live net tow material viewed under a dissecting microscope (Olympus SZH StereoZoom) and magnified 64 $\times$, the relative abundance (present, common or dominant) of the most commonly observed genera of dinoflagellates and diatoms were recorded. Aliquots of both water and net tow samples were preserved in 4% formalin for archival purposes.

2.1.2. Fish and shellfish collection

Northern anchovies (*E. mordax*) and Pacific sardines (*S. sagax*) caught commercially within Monterey Bay were collected opportunistically from the landings of local fishing boats during periods of elevated cell densities between January 2003 and December 2005. Freshly caught fish were collected dockside, packaged whole and promptly placed in a -20°C freezer until processing. In preparation for toxin analysis, slightly thawed fish were dissected and the viscera of multiple specimens from each species were pooled and homogenized to provide one representative sample per sampling day. The species and quantity of fish varied based on the day's catch and ranged from 8 to 90 individuals for anchovies and 4 to 34 individuals for sardines. Aliquots of homogenized viscera were placed in 50 ml conical tubes and frozen for later DA and PSP toxin analysis.

Shellfish samples were collected as part of the statewide CDPH marine biotoxin monitoring program. Since our nearshore site, SCW, is not in close proximity to accessible shellfish beds, mussels (*Mytilus californianus*) were harvested from a rocky intertidal beach north of Santa Cruz (Davenport Landing; 37.02 N, 122.21 W), placed in mesh bags (ca. 30 mussels) and suspended from the wharf a minimum of 7 days before collection. Each week one mesh bag of SCW mussels were shucked, homogenized using a standard kitchen blender, frozen at -20°C and shipped to CDPH for toxin assay. Data were also used from shellfish samples gathered statewide, including intermittently elsewhere in Santa Cruz County, and submitted by the CDPH volunteer network. CDPH analyzes all submitted shellfish samples for PSP toxins, whereas samples are only analyzed for DA when *Pseudo-nitzschia* (all

species combined) densities begin to increase steadily, a signal of potential bloom formation. These data are available online in the form of monthly reports at <http://www.cdph.ca.gov/healthinfo/environhealth/water/Pages/Shellfish.aspx>.

2.2. Toxin extraction and analysis

2.2.1. DA extraction and analysis

DA was extracted from aliquots of pooled, homogenized fish viscera (usually 4.0 g) and cleaned of interfering compounds using solid-phase extraction columns according to Hatfield et al. (1994) and Quilliam et al. (1995). Extracts were analyzed for DA using an isocratic gradient profile on a Hewlett-Packard 1050 HPLC. The specific HPLC conditions are detailed in Vigilant and Silver (2007).

Analysis of shellfish samples for DA was carried out in the CDPH Food and Drug Laboratory in Richmond, CA. DA concentrations were determined by HPLC following the extraction and analytical method outlined in Quilliam et al. (1995). All DA concentrations are reported as micrograms of DA per gram of tissue ($\mu\text{g g}^{-1}$). The current regulatory limit for DA in shellfish and in whole fish is 20 $\mu\text{g g}^{-1}$.

2.2.2. PSP toxins extraction and analysis

The same fish viscera homogenates from which aliquots were taken for DA analysis were subsampled and analyzed for PSP toxins, except for samples collected in 2005, which were not analyzed for DA because of the absence of toxin-producing *Pseudo-nitzschia* in the water. PSP toxins were extracted from fish viscera within 4 weeks prior to analysis following the AOAC (2000) protocol used for analyzing shellfish. Briefly, equal volumes of extraction solvent (0.1N HCL) to sample weight (usually 10.0 g) were well mixed, pH adjusted to 3 ± 0.5 and boiled for 5 min. If necessary, samples were pH adjusted again then centrifuged for 15 min at 3800 rpms. The resulting supernatant was passed through a 0.45- μm mixed cellulose ester syringe filter (Millipore Corp., Bedford, MA) and frozen at -20°C until analysis. The concentration of PSP toxins were determined using a receptor-binding assay described in Trainer and Poli (2000). In this assay scintillation spectroscopy was used to quantify the competitive displacement of toxin in the sample by [^3H] saxitoxin (STX; Amersham, Buckinghamshire, England) bound to sodium channel receptors (prepared from rat brains; Pel-Freez Biologicals, Rogers, AR, USA). Standard curves were generated for each assay run using a certified STX standard (National Research Council of Canada, Institute for Marine Biosciences, Halifax, Nova Scotia, Canada).

Shellfish samples were tested routinely for PSP toxins by CDPH using the standardized mouse bioassay (AOAC, 2000). All PSP toxin concentrations are reported in micrograms of STX equivalents per 100 g of tissue ($\mu\text{g STXequiv. } 100\text{ g}^{-1}$). The regulatory limit for PSP toxins in shellfish is set at 80 $\mu\text{g STXequiv. } 100\text{ g}^{-1}$. Because the receptor-binding methods used here are more sensitive than the mouse bioassay methods used by the CDPH, the data obtained from PSP toxin analysis of fish using our receptor assays should not be considered equivalent to CDPH methods used to protect shellfish consumers in the state of California.

2.3. Data analysis

2.3.1. Monterey bay data

Phytoplankton data for Monterey Bay consisted both of cell counts of toxic species of interest but also of more qualitative assessments of community composition from net tow samples. Based on observed changes in patterns of both the toxin-producing species and the phytoplankton community in the summer of 2004 (see Section 3.1), the data from the earlier period (January 2000 to

June 2004) were compared to those collected in the later one (July 2004 to December 2006). Differences in cell concentrations of toxic *Pseudo-nitzschia* species and *A. catenella* between the two time periods were tested for statistical significance using a Wilcoxon–Mann–Whitney Rank Sum test. The phytoplankton community composition for the two periods was similarly compared. The number of times each genus was observed in any relative abundance category (i.e. observed as present, common or dominant vs. not observed) was tallied for the two time periods (January 2001 to June 2004, $n = 177$; July 2004 to December 2006, $n = 133$) and then compared using a two-tailed Fisher's Exact Test (FET).

2.3.2. Statewide shellfish data

In an attempt to gain a broader geographic perspective on the potential temporal shift in phycotoxins that was possibly occurring in Monterey Bay, we analyzed a subset of the statewide shellfish data from CDPH that was available from the period of interest, namely between July 1999 and October 2006. For each week and each of the coastal counties, we selected the data with the highest level of toxin recorded for that week and categorized it as non-detect, below regulatory limit or above regulatory limit. Next, counties were grouped into northern, central and southern California regions. Boundaries were based on the following county lines: the northern counties included Del Norte to Marin (41.99–37.83 N), the central counties San Francisco to San Luis Obispo (37.84–34.97 N), and the southern counties Santa Barbara to San Diego (34.97–32.53 N). As above, the shellfish data was grouped into two time periods (July 1999 to June 2004 and July 2004 to October 2006) and compared using FET to determine whether the three regions were experiencing similar changes to those noted in Monterey Bay.

3. Results

3.1. Changes in the phytoplankton community and toxic species in Monterey Bay

Our assessments of relative abundance for the most common phytoplankton genera at the Santa Cruz Wharf (SCW) show that genera of red tide forming dinoflagellates substantially increased starting in July 2004 (Fig. 1), particularly *Akashiwo*, *Ceratium*, *Cochlodinium*, and *Prorocentrum* ($p \ll 0.01$, FET). Correspondingly, there was a marked decrease in the relative abundance of diatoms. Hereinafter, we refer to this fundamental change in the Monterey Bay phytoplankton community, from diatom-dominated to a dinoflagellate-dominated, as a “floral shift”. The most notable change was in the potentially toxic *Pseudo-nitzschia*, which showed a highly significant 31% decrease in the number of times members of the genus were observed (Fig. 1A; $p \ll 0.01$, FET). Although *Pseudo-nitzschia* spp. were still regularly present in net tow samples (Fig. 1B) the overall dominance of this genus in the phytoplankton community was greatly reduced. Prior to the floral shift these diatoms were recorded as a dominant member of the community in 64 samples (36%), compared to only 3 samples (2%) after the shift. The other genera known in Monterey Bay to include toxin-producing species, *Alexandrium* and *Dinophysis*, showed significant increases in the number of times they were observed ($p < 0.05$, FET), though there was no significant difference between the two periods when considering their “dominance” in net plankton samples, not a surprising result given their relatively low cell numbers even when contaminating mussel samples.

The densities of toxic *Pseudo-nitzschia* and *A. catenella* obtained using species specific probes at SCW and M1 are shown in Fig. 2A and B. The changes in cell densities of both species are highly

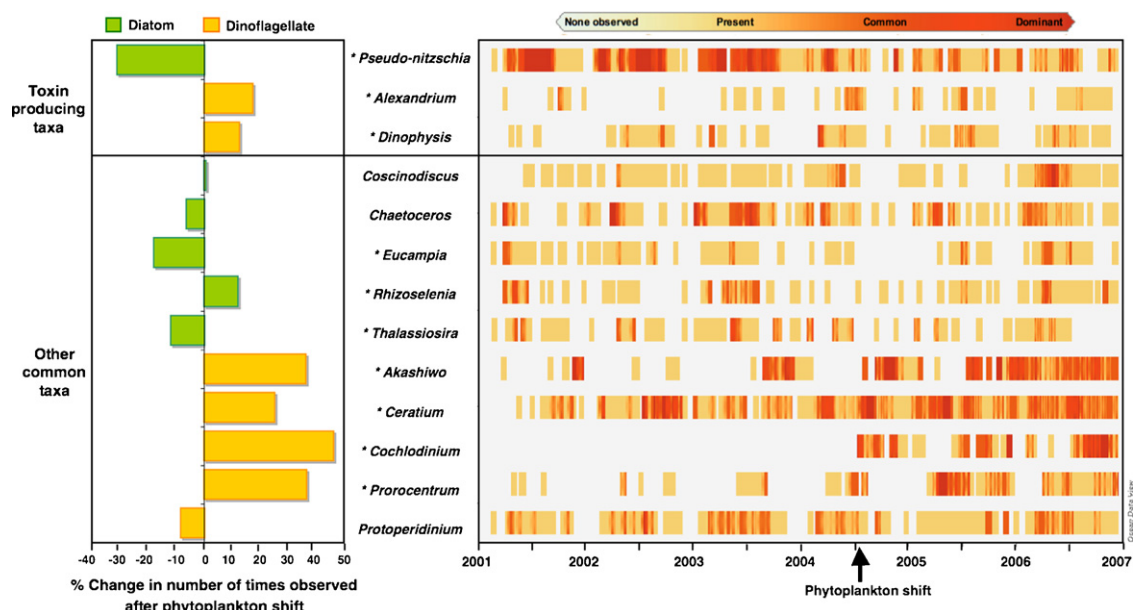


Fig. 1. Relative abundance of diatoms and dinoflagellates common to Monterey Bay, CA: (A) change in the % of samples in which a given genus was observed after the floral shift [diatoms (green) and dinoflagellates (yellow); asterisks indicate a statistically significant difference ($p < 0.05$, FET)]; (B) time series showing the relative abundance of diatom and dinoflagellate genera. Each row represents the change in relative abundance over time for the genus indicated on the y-axis; intensity of color increases with dominance and the arrow indicates the floral shift. Relative abundance ranges from not present to dominant, as shown in the overlying arrow bar.

significant, with toxic *Pseudo-nitzschia* decreasing and *A. catenella* increasing after the floral shift ($p < 0.01$, Wilcoxon–Mann–Whitney Rank Sum Test). Blooms of toxic *Pseudo-nitzschia* spp., i.e. periods when *P. australis* and/or *P. multiseriata* were dominant, virtually disappeared within Monterey Bay after the 2004 shift. In contrast, *A. catenella* not only became more frequent but cell abundance also exceeded “danger” levels more often (Table 1).

3.2. Presence of toxins in shellfish and fish in monterey bay

Both DA and PSP toxins were detected in commercially caught sardines and anchovies and sentinel shellfish collected around the bay (Fig. 2E–G). On a number of dates, both PSP and DA co-occurred in the same samples. Twelve of the 33 fish viscera samples (Fig. 2E and F) and, of the data obtained by CDPH (Fig. 2G and H), 7 of the 141 shellfish samples collected in Santa Cruz County tested positive for both phycotoxins during the study interval. In Fig. 3 we show the occurrence of PSP toxins and DA in sentinel shellfish before and after the floral shift in Monterey Bay. For example, of the 496 samples collected prior to the shift in northern California, PSP toxins were above the regulatory limit in 5% of the samples, present but below regulatory limit in 25%, and not detected in 70% of the samples. There was a statistically significant increase in not only the number of shellfish samples in which PSP toxins were detected, but also in the number that exceeded the regulatory limit in central and southern California ($p < 0.01$, FET), but not in northern California. The region that demonstrated the greatest change for PSP toxins, central California, is also the region where there was a significant decrease in number of shellfish samples that tested positive for DA ($p < 0.01$, FET). In both northern and southern CA there was no significant change in the number of samples in which DA was detected between the two time periods.

4. Discussion

Starting in the summer of 2004, a shift in the ecosystem occurred when the dominant phytoplankton in Monterey Bay changed from diatoms to dinoflagellates (Fig. 1A and B). While

diatoms were still routinely present in our samples after the floral shift, the relative increase in dinoflagellates was quite striking. Blooms of dinoflagellates, or “red tides,” are not uncommon in Monterey Bay, though there are no documented cases in which dinoflagellates dominated the phytoplankton community so frequently as observed in this study. Generally, this region is considered diatom-dominated and red tide events are usually short lived and seasonal (Bigelow and Leslie, 1930; Bolin and Abbott, 1963; Garrison, 1979). A distinctive signal associated with this transition was the presence, and the subsequent frequent overwhelming dominance, of a dinoflagellate previously rarely noted in Monterey Bay, *Cochlodinium* (Fig. 1B). There has not been any confirmed animal mortalities associated with this species in the Bay, yet there is concern that dense red tides of this organism have the potential to negatively impact local wildlife. These concerns may be justified, as blooms of *C. polykrikoides*, *C. catenatum* and *Cochlodinium* sp. have been associated with kills of wild fish populations in central America (Garate-Lizarraga et al., 2004; Lara et al., 2004; Vargas-Montero et al., 2006) and implicated in the mortality of farmed salmon in western Canada (Whyte et al., 2001). Since 2004, *Cochlodinium* sp. has caused visible discoloration in nearshore waters and has been one of the most common dinoflagellates observed at SCW (Curtiss et al., 2008).

An important feature of the shift from diatoms to dinoflagellates was the concurrent shift in the toxic species. Clearly the dominant HAB taxa in the Bay during the early years of our time series (2000–2004), *Pseudo-nitzschia*’s relative importance was significantly reduced during the dinoflagellate-dominated interval. Although *Pseudo-nitzschia* spp. were still present during this period, as shown in the community composition data (Fig. 1), the known toxic species (*P. australis* and *P. multiseriata*) virtually disappeared (Fig. 2A). Not only there was a 31% decrease in number of times *Pseudo-nitzschia* species were found in net tow samples (Fig. 1A), but also a 22% decrease in the number of times toxic *Pseudo-nitzschia* exceeded cell densities we consider “danger levels” (Table 1). Associated with this latter interval was an increase in the presence of two potentially toxic dinoflagellate genera, *Alexandrium* and *Dinophysis*. The human health threat

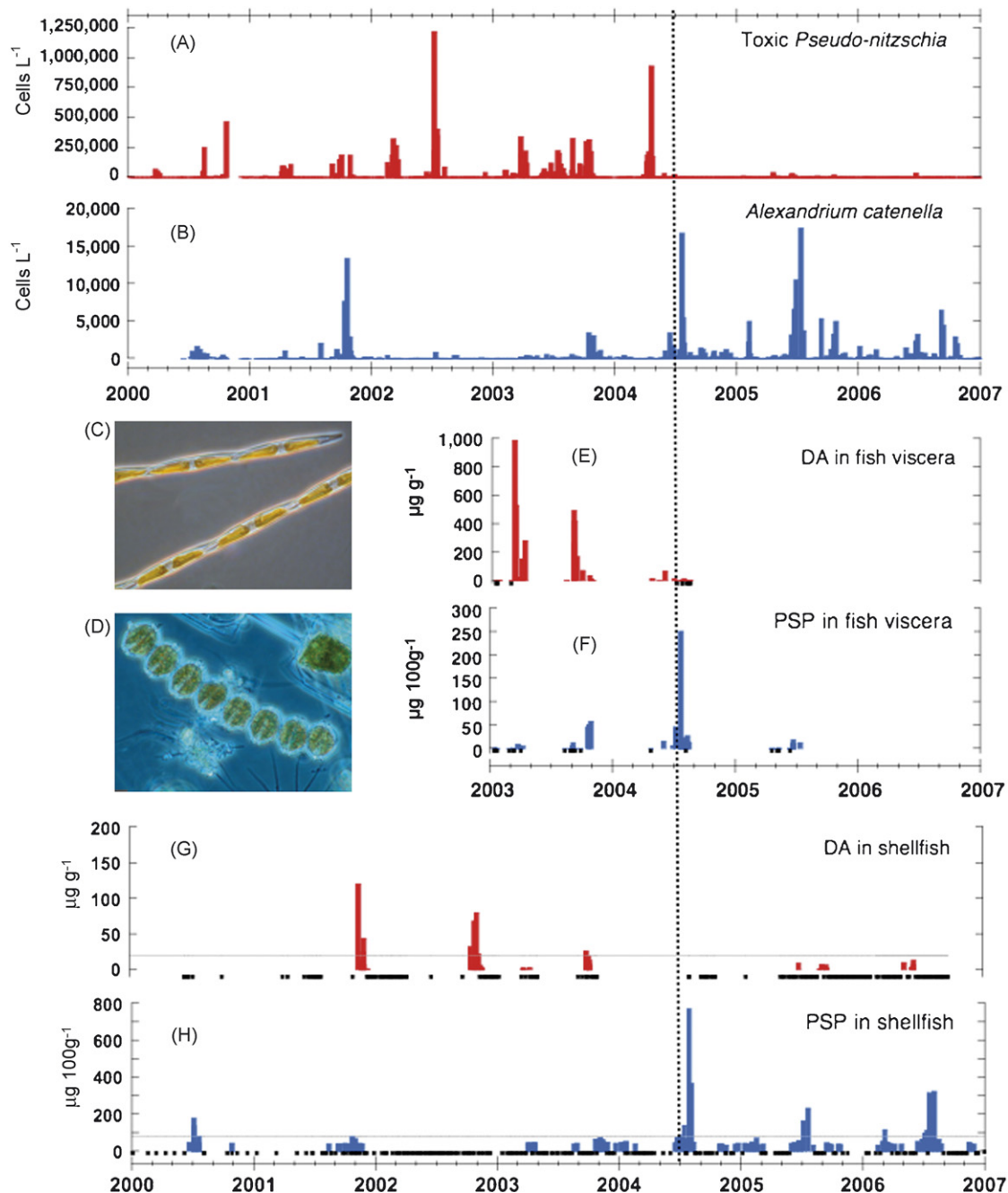


Fig. 2. Abundance of two toxic *Pseudo-nitzschia* species, *Alexandrium catenella* and their respective toxins in planktivorous fish and shellfish within Monterey Bay, CA. Vertical dotted line indicates period of floral shift: (A) density of toxic *Pseudo-nitzschia* species (sum of *P. australis* and *P. multiseries*); (B) density of *A. catenella*; (C) *Pseudo-nitzschia*; (D) *Alexandrium catenella*; (E) concentration of DA in fish ($\mu\text{g g}^{-1}$ viscera); (F) concentration of PSP toxins in fish ($\mu\text{g 100 g}^{-1}$ viscera); (G) concentration of DA in sentinel shellfish ($\mu\text{g g}^{-1}$ total body); (H) concentration of PSP toxins in sentinel shellfish ($\mu\text{g 100 g}^{-1}$ total body). In G and H, the gray line is the federal regulatory limit in shellfish ($20 \mu\text{g g}^{-1}$ for DA and $80 \mu\text{g 100 g}^{-1}$ for PSP toxins); black squares represent samples in which no toxin was detected.

posed by the presence of *Dinophysis* in California is not yet clear, though the recent confirmation of low levels of DSP toxins in shellfish (Sutherland, 2008) warrants consideration of this group as potentially problematic. While these two genera almost never dominated the community (Fig. 1B), human health threats can occur at low cell densities. Coincident with the absence of toxic *Pseudo-nitzschia* after the floral shift was a general increase in the frequency and abundance of *A. catenella* (Fig. 2B), as well as an 8% increase in the number of times this species exceeded “danger levels” (Table 1). Despite the fact that *A. catenella* rarely comprises a major fraction of the community, its highly toxic nature raises concerns about this species’ increasing presence. Furthermore, this

change represents a shift from a genus of toxin producers that typically dominate the community during a toxic event, to taxa that can produce dangerous quantities of toxin even at low cell densities. Such a shift has considerable implications for phytoplankton monitoring and substantiates the importance of shellfish assays, because these toxic events are not dictated by the relative dominance of a species.

From an environmental and human health perspective, it is critical not only to recognize the prevalent toxin in a region but also to understand its possible movement through the food web. Potential dangers to humans are recognized through shellfish monitoring, but additional dangers to humans and marine wildlife

Table 1
Occurrence of toxic *Pseudo-nitzschia* species and *A. catenella* during two intervals

	Number of observations (n)	Toxic species detected (% of n)	Exceeded danger level ^a (% of n)
<i>Toxic Pseudo-nitzschia</i>			
Diatom-dominated interval	409	56	26
Dinoflagellate-dominated interval	263	31	4 ^b
<i>A. catenella</i>			
Diatom-dominated interval	363	31	4
Dinoflagellate-dominated interval	243	62	12 ^c

^a Danger level defined here as cell densities exceeding 10,000 cells l⁻¹ for toxic *Pseudo-nitzschia* (*P. australis* + *P. multiseriata*) and 1000 cells l⁻¹ for *A. catenella*, densities above which toxic events can occur.

^b A significant 22% decrease ($p \ll 0.01$, FET).

^c A significant 8% increase ($p \ll 0.01$, FET).

exist when schooling pelagic filter feeders are in the region where toxic algae are present. Sardines and anchovies are known vectors of DA in Monterey Bay (Lefebvre et al., 1999; Lefebvre et al., 2002), and a shift in the predominant toxic phytoplankton and associated toxins will have important consequences for higher trophic level consumers. During the study period we tested commercially caught sardines and anchovies and sentinel shellfish for both DA and PSP toxins (Fig. 2E–G). The trend in toxins present in the

clupeoids and shellfish tends to mimic the pattern of the cells in the water, though there are some discrepancies in the magnitude of events. We attribute this to the variability in cellular toxin production that can occur in both DA-producing *Pseudo-nitzschia* species (Baugh et al., 2006; Buck et al., 1992; Wells et al., 2005) and PSP toxin-producing *A. catenella* (Anderson, 1990; Poulton et al., 2005).

The floral shift noted here might well result in a change in the toxin potentially transmitted to higher trophic levels, a possibility we were able to test in this study. During periods of toxic *Pseudo-nitzschia* events, which occurred frequently prior to the shift, DA concentrations in fish viscera reached levels that have previously sickened and killed marine birds and mammals (Lefebvre et al., 1999; Scholin et al., 2000; Work et al., 1993), and concentrations in shellfish periodically exceeded the regulatory limit (20 µg g⁻¹) (Fig. 2 E and G). Fish collected after the floral shift were not tested for DA because of the absence of toxic *Pseudo-nitzschia* in the water, and though shellfish were regularly tested their DA levels did not exceed the regulatory limit. Here we report for the first time in California, that planktivorous fish do regularly acquire PSP toxins in the presence of toxin-producing *Alexandrium* (Fig. 2F). Thus, sardines and anchovies can potentially transfer critical levels of PSP toxins, not just DA, to their predators. The pattern of PSP toxins in shellfish also clearly reflects the increase in *A. catenella*; after the floral shift PSP toxins levels exceeded the regulatory limit

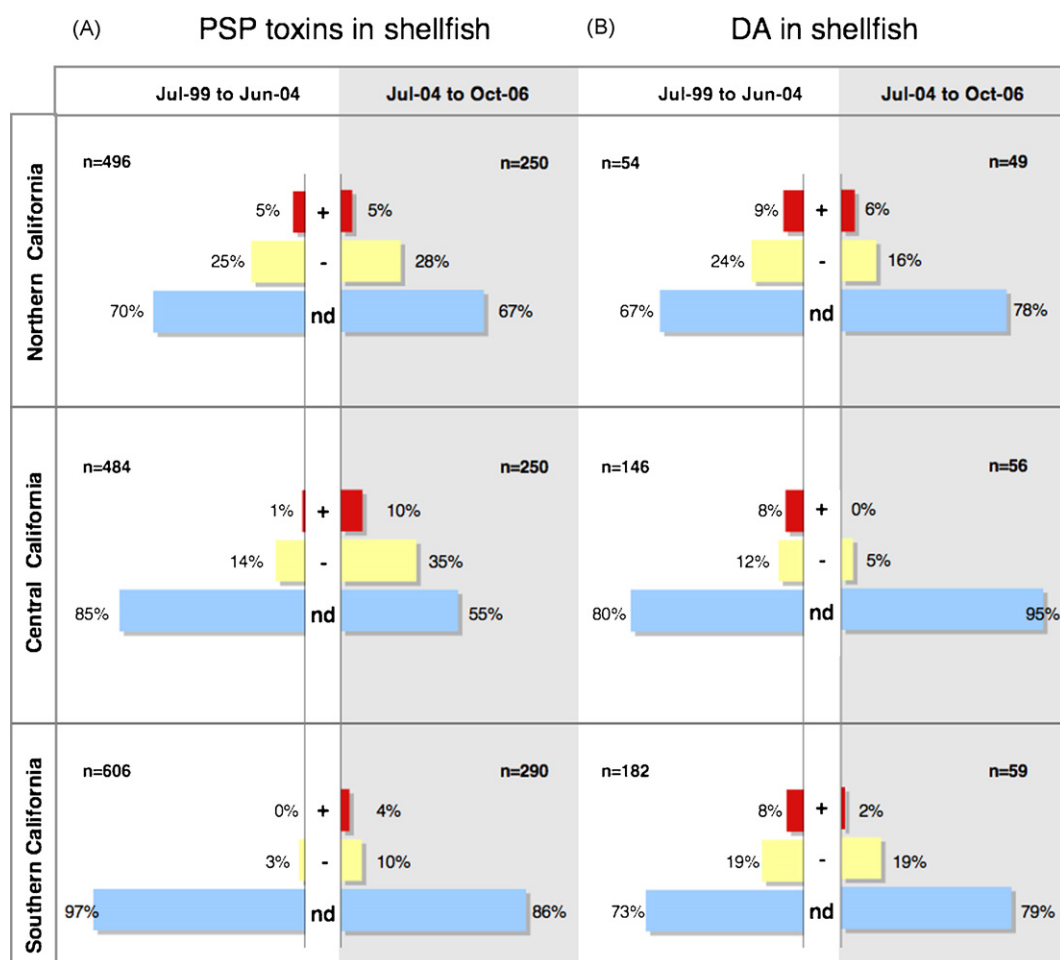


Fig. 3. Regional patterns along the California Coast of PSP and DA toxins in shellfish before and after the floral shift in Monterey Bay: distribution of (A) PSP toxins and (B) DA in shellfish from northern, central and southern California during two intervals; toxin concentration above regulatory limit (+), toxins detected but below regulatory limit (-), or not detected (nd). In both A and B the left panel represents the period when diatoms were dominant in Monterey Bay, the right panel when dinoflagellates were dominant, with the floral shift occurring in July 2004.

(80 $\mu\text{g } 100 \text{ g}^{-1}$) four times, compared to only once in the previous 4 years (Fig. 2H). At times both toxic *Pseudo-nitzschia* and *A. catenella* were simultaneously present in the water, and, correspondingly, fish were simultaneously contaminated with both DA and PSP toxins (12 of 33 samples). While PSP toxin levels in the co-contaminated samples were relatively low, in half of these samples DA concentrations in the viscera were dangerously high (up to 493 $\mu\text{g } \text{g}^{-1}$). This co-contamination also occurred in Santa Cruz County shellfish (7 of 141 samples). Currently it is unknown how simultaneous exposure to two neurotoxins affects predators.

The floral shift, with its corresponding change in the dominant toxin-producing genera in Monterey Bay, raises the possibility of a broader shift along the coastline. To investigate the geographic scale of this shift, we used PSP toxin and DA data from statewide shellfish samples collected between July 1999 and October 2006 as part of the CDPH biotoxin monitoring program. In Fig. 3A, we show the statewide occurrence of PSP toxins in shellfish before and after the period of the floral shift in Monterey Bay. The most striking change was along the central California coast, where there was a notable increase (from 1 to 10%) in the number of shellfish samples in which PSP toxins exceeded the regulatory limit. Furthermore, for the first time during this study period, shellfish samples in southern California exceeded the regulatory limit, a region not often experiencing PSP events since the 1980s (Price et al., 1991). These shellfish data suggest that *Alexandrium* not only increased in frequency and abundance in Monterey Bay, but also along central and southern California. The occurrence of DA in shellfish (Fig. 3B) showed a significant decline in the number of shellfish samples that tested positive for DA in central California before and after the floral shift ($p \ll 0.01$, FET), suggesting toxic *Pseudo-nitzschia* were absent from this region during that time. Overall, these results imply that the change in the dominant toxic species that occurred in Monterey Bay likely occurred over at least most of the central coast where the greatest changes in shellfish toxicity were seen.

Biological transitions in marine ecosystems and sudden shifts in the dominance structure of the plankton community have been linked to interannual and interdecadal basin-scale regime shifts, such as El Niño Southern Oscillation and Pacific Decadal Oscillation (Chavez et al., 2003; McGowan et al., 1998). The shift we observed in Monterey Bay from a diatom-dominated to a dinoflagellate-dominated system may exhibit features that are similar to a “regime shift,” however the temporal and regional scale of our observations limits the conclusions we can make. Interestingly, the dinoflagellate-dominated interval we describe encompassed a period in which anomalous oceanographic conditions were reported in some areas within the California Current System (CCS). Goericke et al. (2005) and Peterson et al. (2006) indicate that in 2004 and 2005 the central CCS experienced a delayed onset of upwelling conditions (weaker than average in the spring/summer and stronger than average in the fall/winter) and was anomalously warm at times of the year. This late transition to upwelling conditions, particularly in 2005, was linked to several unusual and detrimental changes in higher trophic levels. These included reports of low zooplankton biomass, poor recruitment of krill and a variety of fish, reduced seabird fecundity and altered foraging patterns in marine mammals, all of which occurred within central California during this dinoflagellate-dominated period (Peterson et al., 2006; Sydeman et al., 2006; Weise et al., 2006). This is not to say that the cause of these events was related to the prevalence of dinoflagellates, but rather to suggest that the underlying mechanism driving these events may be the same as those responsible for the shift from diatoms to dinoflagellates. Given the importance of physical forcing to phytoplankton growth and, specifically, those conditions that favor proliferation of the dinoflagellate taxa we observed (Kudela et al., 2008; Smayda,

2002), it is likely that the anomalous oceanographic conditions reported in the central CCS played a role in the floral shift we observed. At this time, we still do not have a clear idea of what triggered these anomalies that began in 2004 and that have persisted through at least 2006.

5. Conclusions

The results presented here document a shift in toxin-producing species associated with an overall restructuring of the phytoplankton community. If a shift in the base of the food web, such as the one we describe here, persists for even a few years it can have large-scale consequences for migratory animals that seasonally visit areas such as Monterey Bay (e.g. turtles, birds and whales). Such a change emphasizes the necessity of continuously sampling the full suite of toxic algae, not just one genus or species. Indeed, a recent and unexpected bloom of *Pseudo-nitzschia* occurred in the spring of 2007 after the present study ended, a bloom extending from southern California up to Monterey Bay (pers. obs.). It is not yet clear, given that event, whether the community is returning to its “normal” diatom-dominated state, or if this recent diatom “outbreak” is an anomaly in a presently dinoflagellate-dominated period. Nonetheless, the response of toxin-producing taxa documented here demonstrates that changes in the taxonomic composition of the phytoplankton community alters the algal toxins that move through local food webs to ultimately reach marine animals and humans.

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