

Cryptic Blooms: Are Thin Layers the Missing Connection?

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Abstract Harmful algal blooms (HABs) are common in Monterey Bay, CA, and have resulted in repeated closures of shellfish fisheries and the poisoning and death of marine mammals. In the majority of instances, HAB events in this region are first detected by the presence of sick or dying animals. The phrase “cryptic blooms” was adopted to denote the appearance of poisoning at higher trophic levels with no prior evidence of a large phytoplankton bloom. We hypothesize that the onset of many HAB events goes undetected because the bloom is initially concentrated in discrete thin subsurface layers in the water column that are

easily missed by conventional sampling and monitoring methods. In this paper, we report on the detection and monitoring of a subsurface layer of phytoplankton in northern Monterey Bay, CA, using a high-resolution, autonomous profiler. This ‘thin layer,’ which measured from 10 cm to 3 m in thickness (85% < 2 m; 54% < 1 m), persisted over a 7-day period near the base of the pycnocline. The phytoplankton assemblage in the layer was primarily composed of a multi-species assemblage of *Pseudo-nitzschia* including the toxin-producing species *Pseudo-nitzschia australis*. Concentrations of toxic phytoplankton (*P. australis*), cyanobacteria, and bacteria in the layer were significantly higher than outside the layer ($P < 0.05$). Counts of total *Pseudo-nitzschia* spp. showed similar levels of enrichment in the layer compared to outside the layer. Our findings indicate that, when monitoring for HABs, it is critical to sample at scales appropriate to resolve thin layers. Thin layers have been identified as a common recurrent feature in a variety of coastal systems, suggesting that the use of autonomous high-resolution vertical profilers coupled with targeted sampling, could allow more timely detection of HABs in many coastal environments.

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Introduction

Monterey Bay, located on the central coast of California, is classified as one of the largest National Marine Sanctuaries in the USA. The bay is highly productive and economically important in terms of both fishing and tourism, and it has been identified as a key harmful algal bloom (HAB) monitoring site and “hot spot” for the West Coast (Scholin

et al. 2000; Trainer et al. 2000). Monterey Bay exhibits a wide dynamic range of oceanographic conditions, from vigorous diatom-dominated upwelling to warm, nutrient-depleted oceanic conditions dominated by picoplankton (e.g., Pennington and Chavez 2000). This is one of the most extensively characterized coastal regions in the USA in terms of basic biological and physical oceanographic processes. Despite this wealth of information, HAB events in Monterey Bay are not easily predicted (Kudela et al. 2004). Unfortunately, the majority of HAB events are first detected by the presence of sick or dying animals. This phenomenon has led to the use of the phrase “cryptic blooms,” which refers to the sudden onset of toxic events with no immediately apparent large phytoplankton bloom (Scholin, personal communication).

We propose that unexplained toxicity events might be a consequence of toxic phytoplankton being concentrated in thin layers that escape detection by routine monitoring methods. ‘Thin layers,’ in this paper, refers to subsurface layers of increased plankton biomass that meet specific criteria presented by Deksheniaks et al. (2001). Thin layers have thickness on the order of centimeters to only a few meters (<5 m), may extend horizontally for kilometers, and can persist for days (Deksheniaks et al. 2001; Alldredge et al. 2002; Rines et al. 2002).

The term ‘thin layers’ was coined to specifically distinguish these structures from the commonly recognized “subsurface” or “deep” chlorophyll maximum, which is typically a less intense increase in chlorophyll that is expressed over a depth range of tens of meters. Thin layers are notable for their greatly compressed vertical structure, which makes them difficult to detect by conventional sampling and profiling methods (Cowles et al. 1998). Discrete sampling with relatively tall Niskin bottles, integrated plankton collection by vertical net tows, and rapid-descent CTD casts all tend to average out features with spatial scales less than several meters in the vertical dimension. A greater appreciation of the prevalence and importance of thin layers was made possible recently because of (1) significant advancements in technology that permit observations at fine spatial scales (Holliday et al. 2003) and (2) new sampling techniques that remove the effects of ship motion from oceanographic observations (Donaghay et al. 1992).

Thin layers have been identified as a common, recurrent feature in a variety of coastal systems (Bjornsen and Nielsen 1991; Donaghay et al. 1992; McManus et al. 2003; Koukaras and Nikolaidis 2004). In this paper, we report on the detection and monitoring of a persistent thin layer of phytoplankton in Monterey Bay, CA, using a high-resolution, autonomous profiler. The biological composition of this layer relative to waters above and below was determined from discrete, diver-collected water samples.

Materials and Methods

Study Site

Monterey Bay is located on the central California coast between 36.5 and 37°N. It is an open embayment, measuring roughly 37 km along its north–south axis (i.e., across the mouth) and 19 km along its east–west axis (Breaker and Broenkow 1989). A submarine canyon, which runs along the east–west axis, divides the bay into northern and southern regions (Fig. 1).

Autonomous Profiler

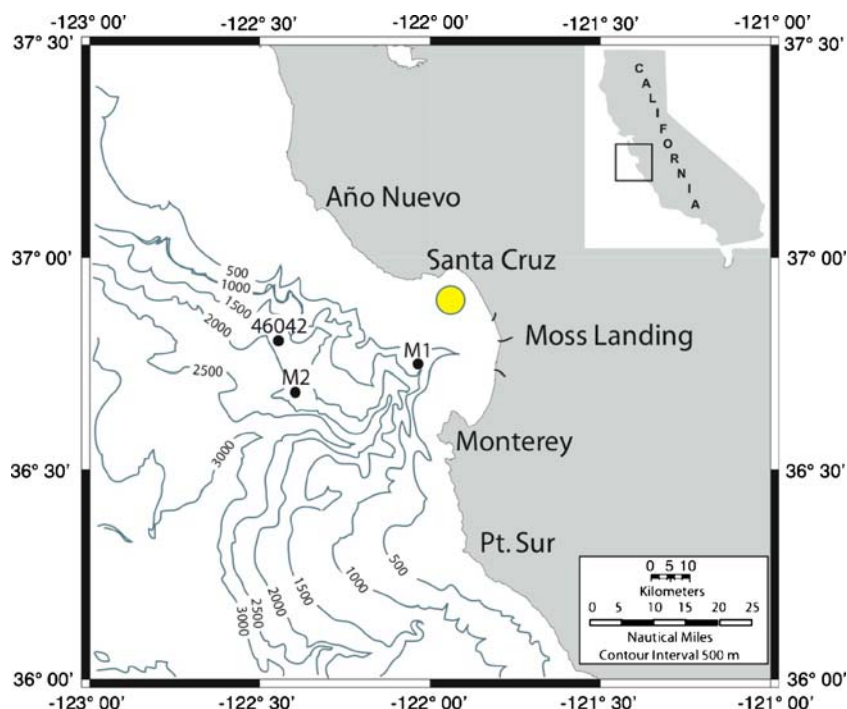
A bottom-mounted autonomous underwater winch profiler (Ocean Response Coastal Analysis System, ORCAS, profiler) was deployed at a site in northern Monterey Bay (36°56.2′N, 121° 55.8′W) with a bottom depth of approximately 22 m in August of 2002. High-resolution vertical profiles were collected hourly between the bottom and the surface at an average ascent rate of 3 cm s⁻¹. Between profiles, the sensor package was held stationary at the bottom until the next sampling interval. A week-long time series of 168 hourly, fine-scale profiles of temperature, salinity, density, spectral absorption and attenuation (at 9 wavelengths), backscatter at 532 nm, chlorophyll fluorescence, bioluminescence, and oxygen concentration were collected. The data from the autonomous profiler were telemetered to shore, processed in near real-time, and visualized, so we could follow the development of thin layers in the system. A detailed description of the ORCAS profiler is given in Sullivan et al. 2005.

Layer Samples

Using the hourly ORCAS profiles as a guide, scuba divers collected water samples on August 13th, 2002 from 5.3 m above the thin layer, within the thin layer, and 4.9 m below the thin layer with small (1.1 l) Aquatic Research Instruments Point Water Sampling bottles triggered by hand. Two bottles were triggered in each location, with a total of six bottles being triggered. While two bottles were collected within the thin layer, it is likely that these bottles were not triggered at the exact maximum of the layer because of isopycnal movement between the time the midpoint of the layer was identified by the ORCAS profiler, and the time of the dive (~20 min).

It should be noted that the original objectives for this study were simply (1) to detect the presence or absence of thin high-frequency optical layers, and (2) to obtain a first-order indication of their frequency of occurrence. As a consequence of our limited original objectives, only a few, conventional water samples (e.g., bottles) were collected.

Fig. 1 Location and bathymetry of Monterey Bay and the adjacent shelf. Bathymetric contours are 500 m. *Yellow circle* Location of autonomous profiler, *M1* MBARI mooring, *M2* MBARI mooring, and *46042* NDBC wind buoy



The purpose of the water samples was to obtain a representative sample of the most abundant phytoplankton present.

Counts

For counts of prokaryotes, water samples were chilled on ice for transport from the field to the lab where they were preserved with formaldehyde (2% final concentration). Sub-samples were then stained with the fluorescent DNA stain 4',6-diamidino-2-phenylindole ($1 \mu\text{g ml}^{-1}$ final concentration) for 10 min then filtered onto 0.2- μm pore-size black polycarbonate track-etched membrane filters (Poretics). Filters were mounted in oil on glass slides with cover slips and examined by epifluorescence microscopy using UV and blue filter sets to count total prokaryotes (4',6-diamidino-2-phenylindole-stained) and cyanobacteria (by autofluorescence), respectively.

Pseudo-nitzschia were viewed and counted on filters using our standard methods (Bargu et al. 2002). Water samples (5–30 ml) were filtered onto 13-mm, 1.2- μm filters treated with species-specific large subunit ribosomal RNA-targeted probes using protocols of Miller and Scholin (1996) for Monterey Bay clones of *P. australis* and *P. multiseries* (the two local species of toxic *Pseudo-nitzschia*). The filters were viewed using epifluorescence microscopy on a Zeiss Standard 18 compound microscope equipped with a fluorescence Illuminator 100 with 480 nm excitation and 520 nm emission filter set (Zeiss, Thornwood, NY, USA). Volume-specific abundance of the two toxic *Pseudo-nitzschia* species

found locally, *P. australis* and *P. multiseries*, were determined from counts of the entire filter surface area and the total counts of *Pseudo-nitzschia* also noted, as they also were readily recognized by the characteristic chain morphology on the filters.

Results

Finescale sampling with the autonomous profiler revealed the existence of an intense thin layer of phytoplankton in northern Monterey Bay. This subsurface layer ranged in vertical dimension from 10 cm to 3 m in thickness (85% < 2 m; 54% < 1 m) and persisted near the base of the pycnocline for a period of 7 days (from August 7th to August 13th, 2002). This thin layer, evident in 168 profiles, increased in intensity by an order of magnitude from initial chlorophyll concentrations of 10 mg m^{-3} on August 7th, to extremely high values of $>150 \text{ mg m}^{-3}$ on August 10th (Fig. 2), later decreasing to 70 mg m^{-3} on August 13th.

On August 13th, divers took water samples from above, within and below the persistent thin layer. The phytoplankton assemblage was primarily composed of a multi-species assemblage of *Pseudo-nitzschia*, including the toxic species *P. australis* (Table 1). Concentrations of toxic phytoplankton (*P. australis*), cyanobacteria, and bacteria in the layer were significantly higher than outside the layer ($P < 0.05$). Counts of total *Pseudo-nitzschia* spp. showed similar levels of enrichment in the layer, compared to outside the layer; however, differences were not considered highly significant

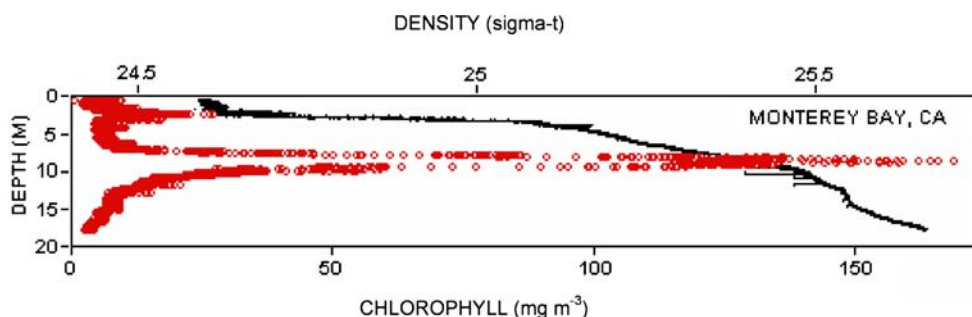


Fig. 2 A thin layer measured by the ORCAS profiler in northern Monterey Bay (36°56.2'N, 121°55.8'W) 10 August 2002. Density (sigma-*t*) is represented by the *black solid line* (top axis); chlorophyll concentration (mg m⁻³) is represented by *red circles* (bottom axis).

The chlorophyll vertical structure is dominated by a thin (<2 m at 1/2 peak height), very intense (>150 mg m⁻³) layer located near the base of the pycnocline

(*P*=0.055). Concentrations of *Pseudo-nitzschia* spp., *P. australis* (toxic), cyanobacteria, and bacteria in the layer were enhanced (2.8, 3.3, 2.4, and 3.5 times, respectively) relative to concentrations outside the layer.

Discussion

Our results demonstrate that intense, persistent thin layers of phytoplankton occur in Monterey Bay, as has been observed in other coastal environments (Donaghay et al. 1992; McManus et al. 2003; Koukaras and Nikolaidis 2004). There is a clear separation in scale between the structure we observed (ranging from 0.1 to 3 m, with 85% of profiles <2 m in thickness) and the more traditional deep chlorophyll a maximum (Anderson 1969; Dekshenieks et al. 2001). The fine vertical scales of thin layers are very difficult if not impossible to sample with traditional sampling methods, which tend to vertically average out features with spatial scales less than several meters in the vertical dimension. The observed enrichment of *Pseudo-nitzschia* spp. within the layer compared to the samples taken above and below the layer in Monterey Bay is similar to observations of populations of *Pseudo-nitzschia* spp. in thin layers in a fjord in the San Juan Islands, WA (Rines et al 2002).

The rarity of very high-resolution vertical sampling could explain why thin layers of toxic phytoplankton have not been previously reported in Monterey Bay despite extensive oceanographic work in the area. The observed elevated concentrations of prokaryotic and eukaryotic phytoplankton, as well as heterotrophic bacteria in the layer we observed, suggest that a generalized physical concentrating mechanism could be responsible for forming the layer. However, it is also possible that physical processes act on select organisms to initiate layer formation, after which intensification and diversification in the layer occurs through in situ growth of a complex plankton community around the seed populations. Regardless of their mechanism of formation, the extreme vertical heterogeneity

in biomass represented by thin layers has important consequences for understanding ecological processes that are dependent on cell concentration (e.g., foraging, predation, mating, chemical signaling, spread of infections, etc.). As many ecological rate processes scale nonlinearly with cell numbers, the vertical averaging of concentration that occurs by standard sampling techniques can result in a disproportionately large miscalculations of those rates. This has particular relevance for understanding the impacts of HABs, as poisoning at higher trophic levels occurs via transfer of the toxin up the grazing food chain.

The presence of thin layers of phytoplankton has been observed to result in the transient and secondary formation of thin layers of vertically migrating zooplankton, presumably congregating in response to high concentrations of food in the layer (Alldredge et al 2002; McManus et al. 2003). Diel formation of thin layers of zooplankton were

Table 1 Average abundance (per milliliter) of selected groups of plankton within the thin layer compared to the averages from samples collected outside (above and below) the layer and the ratio of abundances

Organism	Abundance				Ratio	<i>t</i> test
	In layer (<i>n</i> =2)		Out of layer (<i>n</i> =4)			
	Mean	Range	Mean	Range		<i>P</i> value
<i>Pseudo-nitzschia</i> spp.	3,258 ^a	–	1,148 ^b	934–1,362	2.8	0.055
<i>P. australis</i> (toxic)	65	40–90	20	0–45	3.3	0.048
Cyanobacteria (×10 ³)	6.3	5.9–6.8	2.6	2.0–3.9	2.4	0.003
Bacteria (×10 ⁶)	1.4	1.4–1.4	0.4	0.3–0.6	3.5	0.0004

The ratio of abundance = mean in layer/mean out of layer. Values of *P*, calculated for a one-tailed *t* test, are the probability of falsely rejecting the null hypothesis that abundances outside the layer are greater than or equal to those inside the layer.

^a *n*=1

^b *n*=2

also observed by acoustics at the same location in Monterey Bay over the same period of time as the phytoplankton layers reported in this paper. These thin zooplankton layers ranged in vertical thickness from ~30 cm to 4 m, with an average thickness of 1.01 m. Thin zooplankton layers persisted throughout daylight hours but were observed to dissipate during evening hours and reform at daybreak because of nightly diel migration. These layers persisted from time frames of 1 h 45 min to 15 h 30 min, with an average time of 8 h and 12 min. During this study, all zooplankton layers were located mid-water column, between 5 and 10 m above the bottom – in the vicinity of the thin phytoplankton layers. The occurrence of thin zooplankton layers as they relate to physical processes is summarized in McManus et al. 2005.

Small planktivorous fish (e.g., sardines and anchovies) are also likely to cue into the increased concentrations of phyto- and zooplankton within thin layers, thereby, dramatically increasing their ingestion rate. Likewise, zooplankton and small fish aggregating in areas of high food density would be more efficiently consumed by sea birds and marine mammals. Thus, the concentration of toxic phytoplankton in thin layers is likely to account for unexpected appearance of intoxicification in sea birds and marine mammals.

If toxic algae are commonly found in thin layers, as this study suggests, then the use of surface sampling or standard low vertical resolution sampling methods to monitor for HABs could result in failures to predict the onset of poisoning at higher trophic levels in two ways: first, by reducing the chances that toxic algae are detected and, second, because of underestimates of the local effective concentrations of toxic species and their consumers and, consequently, underestimates of the efficiency with which the toxins are passed through the grazing food chain.

Conclusion and Recommendations for Monitoring

Historically, the majority of HAB events in Monterey Bay have first been detected by the presence of sick or dying animals, indicating that traditional early warning methods such as mussel tissue sampling are inadequate (Scholin et al. 2000). New molecular methods have vastly improved our ability to rapidly detect the presence of toxic phytoplankton species (Scholin et al. 2000), but successful detection of a bloom still requires collection of appropriate samples. Thin layers may provide a mechanism for the long-term maintenance and sudden expression of HAB species in areas like Monterey Bay and may result in more efficient transfer of toxins to higher trophic levels. These cryptic blooms could account for the observed marine animal toxicity in the absence of cells or toxin from monitoring programs. A report summarizing the potential

economic impacts of HABs in the USA estimates the annual cost of HAB events at between \$33–82 million per year, with approximately 4% of that cost related to monitoring and management (Anderson et al. 2000). Our current monitoring efforts are most certainly underestimating both the intensity and abundance of HABs in thin-layer structures. For this reason, it is critical that sampling for HABs be conducted at scales appropriate to resolve these layers. In areas where HABs are of particular concern, we recommend the deployment of autonomous, high-resolution profilers with data telemetry to allow monitoring of water-column optical properties in real time. Relatively, inexpensive instrumentation can reveal the presence of phytoplankton blooms occurring in thin layers and the resulting data can help focus the more laborious processes of field sampling and of positive species identification and toxin assays in the laboratory. A high-resolution sampling design is not only important for Monterey Bay but for all coastal systems where thin layers occur.

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