# BIOGEOGRAPHY AND REGIONAL EVENTS SESSIONS

# *Pfiesteria* Species Identified in Ships' Ballast Water and Residuals: A Possible Vector for Introductions to Coastal Areas

Martina A. Doblin[1], Lisa A. Drake[1], Kathryn J. Coyne[2], Parke A. Rublee[3], and Fred C. Dobbs[1]
[1]*Department of Ocean, Earth and Atmospheric Sciences, Old Dominion University, Norfolk, Virginia, USA;*
[2]*College of Marine Studies, University of Delaware, Lewes, Delaware, USA;* [3]*Biology Department, University of North Carolina at Greensboro, Greensboro, NC, USA*

## Abstract

Phytoplankton species most likely to survive in ballast water and unpumpable ballast residuals are those that form resistant resting stages, have alternative modes of nutrition, or both. In our field studies of ballasted ships arriving to Chesapeake Bay and NOBOB (No-Ballast-On-Board) vessels arriving to the Great Lakes, we monitored for the HAB species *Pfiesteria piscicida* and *P. shumwayae* using polymerase chain reaction (PCR)-based methods. During 2001, we boarded 10 vessels arriving to Chesapeake Bay, and *P. piscicida* was detected in 1 ship (10%), while *P. shumwayae* was detected in 3 ships (30%). Of the residual NOBOB water samples tested, *P. piscicida* was detected in 2 of 34 samples (6%), and *P. shumwayae* was detected in 1 of 34 samples (3%). *P. piscicida* and *P. shumwayae* each were detected in only 1 of 33 (3%) residual NOBOB sediment samples. Thus, *Pfiesteria* is found in low frequency in ships' ballast tanks. Further investigation is required to determine whether these strains are toxic.

## Introduction

It is well established that cyst-forming HAB species are transported in ships' ballast water (*e.g.,* Hallegraeff and Bolch, 1992). Resting cysts are relatively resistant to adverse environmental conditions and thus represent an effective means of long-term survival for these organisms, also making them primary candidates for ship-assisted dispersal. Sediment accumulates in quiescent parts of ballast tanks, and since cysts have densities similar to silt and clay particles, they also accumulate in these locations.

Previous studies have detected cyst-forming HABs in ballast tanks by examining sediment and water samples using various filtration and concentration steps (*e.g.,* Bolch, 1997), followed by microscopy (light and SEM). Recent development of molecular probes for various HAB species, including *Pfiesteria* (Bowers *et al.,* 2000; Coyne *et al.,* 2001), has enhanced our ability to detect both cyst- and non-cyst-forming HAB species in environmental samples. The potential advantages of molecular probes include a lower detection limit (*e.g.,* approximately 10 cells $L^{-1}$ with fluorescent fragment detection PCR; see Coyne *et al.,* 2001), lower cost per sample, and shorter analysis times.

We focused our ballast-water investigations on *Pfiesteria piscicida* and *P. shumwayae* because these HAB species have characteristics that render them likely to survive ballast transit: a life history that includes cyst stages (Burkholder and Glasgow, 1997; Litaker *et al.,* 2002) and the ability to take up organic substances or feed phagocytotically (Burkholder *et al.,* 1998). Further, the appearance of *Pfiesteria* in disjunct locations such as New Zealand (Rhodes *et al.,* 2002), Australia (CSIRO, 2001), Norway (Jakobsen *et al.,* 2002) and the east coast of the United States (including the lower Chesapeake Bay) (Rublee *et al.,* 2001; Rublee *et al.,* this Proceedings) prompts the question of whether its distribution is historically cosmopolitan or whether anthropogenic activities—such as commercial shipping, eutrophication, or both—have increased its apparent dispersal.

## Materials and Methods

We sampled ballasted bulk carriers arriving to the eastern U.S.A. (Chesapeake Bay), and NOBOB (No Ballast On Board) vessels (20 bulk carriers and 1 chemical tanker) arriving to the North American Great Lakes. NOBOB vessels contain unpumpable ballast materials (water and sediment) that can be resuspended during future vessel operations, thus posing a risk for biotic introductions. In all except one case, the ships' previous ports of call were foreign (United Kingdom, Western Europe, Baltic and Black Seas, Mediterranean, Asia and Caribbean Sea). Further, all ships arriving to Chesapeake Bay contained ballast water that had been exchanged in the open ocean, and 7 of 21 NOBOB vessels sampled had exchanged their ballast water after their last ballast load. Ballast water was sampled using a Niskin bottle or collected by hand into sterile bottles. Residual water and sediments of empty NOBOB tanks were sampled using plastic hand-operated pumps and sterile plastic scoops, respectively.

Between 100 and 300 mL of water was filtered onto glass fiber filters (nominal pore size 0.7 µm) or polycarbonate membranes (0.4 µm pore size). The membrane was folded and submersed in DNA extraction buffer and stored at –80°C (Coyne *et al.,* 2001) or at room temperature for a maximum of 7 days (Bowers *et al.,* 2000) until analysis. Sediments were placed into 6 mL tubes and refrigerated at 4°C until analysis. We assayed independent replicates (*i.e.,* separate water and sediment samples) for *Pfiesteria* using two different molecular methods: Bowers *et al.* (2000) and Coyne *et al.* (2001). In all cases, DNA quality and presence of PCR inhibitors was evaluated by PCR amplification with universal eukaryotic primers. PCR assays were performed with positive and negative (no template added)

Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

**Table 1** Presence of *Pfiesteria* in ships' ballast water arriving to Chesapeake Bay and in residual water and sediments arriving to the Great Lakes; n = number of tanks sampled. Data are compared with presence of *Pfiesteria* at Chesapeake Bay monitoring sites (bay mouth, adjacent to 2 coal piers and Pagan River).

| Location | *P. piscicida* | *P. shumwayae* |
|---|---|---|
| Chesapeake Bay ballast water | 1 (n = 10) | 3 (n = 10) |
| NOBOB residual water | 2 (n = 34) | 1 (n = 34) |
| NOBOB residual sediments | 1 (n = 33) | 1 (n = 33) |
| Chesapeake Bay water | 0 (n = 9) | 3 (n = 9) |
| Chesapeake Bay sediments | 0 (n = 3) | 0 (n = 3) |

controls. Detection of *Pfiesteria* indicates presence of vegetative or cyst forms, since the probes make no distinction between *Pfiesteria* life stages.

## Results and Discussion

During 2001, we found *P. piscicida* in ballast water of 1 of 10 vessels (10%) arriving to Chesapeake Bay and *P. shumwayae* in ballast water of 3 vessels (30%; Table 1). The ships containing *Pfiesteria* arrived from Immingham (U.K.), Antwerp (Belgium), and Amsterdam (The Netherlands). *Pfiesteria* was detected at lower frequency (3–6% of tanks) in residual ballast water, sediment, or both, in 34 NOBOB ballast tanks arriving to the Great Lakes (Table 1). The NOBOB vessels that contained *Pfiesteria* in ballast water were from Western Europe, having taken on ballast water most recently in Antwerp (Belgium), Amsterdam (The Netherlands), or Hull (U.K.), and in 1 of 3 cases, had undergone open-ocean exchange. *Pfiesteria* was detected at a similar frequency in residual NOBOB sediments (Table 1). The NOBOB vessels that contained *Pfiesteria* in ballast sediments originated in China or Western Europe, but had taken on ballast in numerous ports including Inchon (South Korea), Hong Kong, Ghent or Antwerp (Belgium), Sept. Iles or Montreal (Canada) as well as the Mediterranean (Augusta, Sicily and Ravena, Italy). There was no consistent pattern with respect to *Pfiesteria* presence (water or sediment) and ballast origin or whether ballast had been exchanged.

In 10 of 11 cases where NOBOB water samples were analyzed in replicate, results were consistent between subsamples. In the one instance in which *Pfiesteria* was not found in both subsamples, it was not detected in water withdrawn from the top of a sampling container that had been left sitting for approximately 1 hour, but was detected in water withdrawn from the bottom. Further, there were 3 instances where *Pfiesteria* (*P. piscicida* or *P. shumwayae*) was detected in residual water but not in sediments. Likewise, there were 2 instances where *Pfiesteria* was detected in sediments but not in water samples from the same tank. These differences may be the result of encystment and subsequent sedimentation—likely during rapid changes in salinity during ballasting operations. However it could also be related to sampling—puddles can form where there is no accumulated sediment, and water is often collected in different locations than sediment within a tank, to ensure that samples are relatively mud-free (for better DNA extraction efficiency). The disagreement of sediment and water results could also be due to patchy *Pfiesteria* distribution within the tank, as well as molecular probe sensitivity (where relatively small differences in cell abundance can determine whether the sample exceeds the detection limit).

We also monitored for *Pfiesteria* at several lower Chesapeake Bay sites: at the bay mouth adjacent to a sewage outfall (4 sampling events), in the Pagan River, Virginia (3 sampling events), and at nearby coal terminals (2 sampling events). There were 3 samplings in which multiple samples were collected that yielded at least one water sample positive for *Pfiesteria* (33%; Table 1). However, *P. shumwayae* was not detected in sediments collected from two locations (Pagan River upstream and downstream, stations separated by approximately 1.3 km).

There were some differences in results among independent, replicate water samples assayed for *Pfiesteria* using the two different PCR-based methods, with only 20 of 28 samples giving the same result (either positive or undetected). Only 1 sample tested positive using both methods; 8 other samples tested positive with one method only. Both methods are very sensitive, and differences between the assays are likely due to small differences in cell abundance within the samples, sample storage protocols, DNA extraction or amplification efficiencies between replicates, or a combination of these factors. Given such sensitivity, a more rigorous way to compare PCR-based methods would be to assay subsamples of the DNA after extraction, rather than using DNA extracted from independent filters.

It should be noted that methods for toxicity testing have not been developed for *Pfiesteria* present in ballast tanks. Despite the methodological issues associated with molecular detection, however, this study shows that *Pfiesteria* is transported in ships' ballast water and residual sediments. While its distribution within and between ballast tanks is variable, it is clear that commercial shipping could contribute to *Pfiesteria*'s global dispersal and potential for toxic blooms. This finding is particularly relevant in view of *Pfiesteria*'s disjunct global distribution, and the similarity of European *P. piscicida* strains to those in the U.S.A. (Jakobsen *et al.,* 2002; Rublee *et al.,* this Proceedings).

## Acknowledgements

We thank members of the Great Lakes NOBOB sampling team for their efforts (particularly Phil Jenkins), Eric Schaefer and Cory Williams for running the PCR assays, and Leslie Kampschmidt for laboratory assistance. We also thank Rob Brumbaugh of the Chesapeake Bay Foundation for boat time and his assistance in collecting samples from the Pagan River. This work was conducted under the multi-institutional Great Lakes NOBOB Project funded by the Great Lakes Protection Fund, the National Oceanic and Atmospheric Administration (NOAA), the U.S. Environ-

mental Protection, and the U.S. Coast Guard. The project was co-managed by the Cooperative Institute of Limnology and Ecosystems Research and the NOAA Great Lakes Environmental Research Laboratory and sponsored under cooperative agreement NA17RJ1225 from NOAA's Office of Oceanic and Atmospheric Research. Additional funding for this project was derived from NOAA Sea Grant award NA96RG0486 (through Maryland Sea Grant College Program) to F. Dobbs and ECOHAB award NA860P0495 to D. Hutchins and C. Cary, University of Delaware. This is ECOHAB contribution #88.

## References

C. J. S. Bolch, Phycologia 36(6), 472–478 (1997).

H. A. Bowers, T. Tengs, H.B. Glasgow Jr., J.M. Burkholder, P.A. Rublee, and D.W. Oldach, Appl. Environ. Microbiol. 66(11), 4641–4648 (2000).

J. M. Burkholder and H. B. Glasgow, Limnol. Oceanogr. 42(5), 1052–1075 (1997).

J. M. Burkholder, H. B. Glasgow and A. J. Lewitus, in: Physiological Ecology of Harmful Algal Blooms, D. M. Anderson, A. D. Cembella and G. M. Hallegraeff, eds. (Springer-Verlag, Berlin), pp. 175–191 (1998).

K. J. Coyne, D. A. Hutchins, C. E. Hare and S. C. Cary, Aquat. Microb. Ecol. 24, 275–285 (2001).

CSIRO, Division of Marine Research Fact Sheet #47; http://www.marine.csiro.au/LeafletsFolder/index.html (2001).

G. M. Hallegraeff and C. J. Bolch, J. Plankton Res. 14(8), 1067–1084 (1992).

K. S. Jakobsen, T. Tengs, A. Vatne, H. A. Bowers, D. W. Oldach, J. M. Burkholder, H. B. Glasgow Jr., P. A. Rublee, and D. Klaveness, Proc. R. Soc. Lond. B. Biol. Sci. 269, 211–214 (2002).

R. W. Litaker, M. W. Vandersea, S. R. Kibler, V. J. Madden, E. J. Noga, and P. A. Tester, J. Phycol. 38(3), 442–463 (2002).

L. L. Rhodes, J. M. Burkholder, H. Glasgow, P. A. Rublee, C. Allen, and J. E. Adamson, N. Z. J. Mar. Freshwater Res. 36, 621–630 (2002).

P.A. Rublee, J. W. Kempton, E. F. Schaefer, C. Allen, J. Harris, D. W. Oldach, H. Bowers, T. Tengs, J. M. Burkholder, and H.B. Glasgow, Environ. Health Perspect. 109 [Supplement 5], 765–767 (2001).

P. A. Rublee, C. Allen, E. Schaefer, L. Rhodes, J. Adamson, C. Lapworth, J. Burkholder and H.B. Glasgow, this Proceedings.

# Global Distribution of Toxic *Pfiesteria* Complex Species Detected by PCR Assay

Parke A. Rublee[1], Coy Allen[1], Eric Schaefer[1], Lesley Rhodes[2], Janet Adamson[2], Caroline Lapworth[3], JoAnn Burkholder[4], and Howard Glasgow[4]

*[1]Biology Dept., University of North Carolina at Greensboro, Greensboro, NC 27402, USA; [2]Cawthron Institute, Nelson, NZ; [3]University of Tasmania, AU and CSIRO Marine Research, Hobart, Tasmania, AU; [4]Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC 27606, USA*

## Abstract

*Pfiesteria* species were detected by PCR-based methods in 19 of 22 countries (not including the USA), spanning six continents. Neither species appeared to be correlated to salinity or temperature. The widespread distribution suggests that *Pfiesteria* species are endemic and generally benign members of estuarine communities since impacts on fish and human health have not been reported outside of USA coastal waters.

## Introduction

*Pfiesteria piscicida* was discovered as a contaminant of fish cultures in the late 1980s. In the next decade, *P. piscicida* and a second species, *P. shumwayae,* were identified as members of estuarine communities, their life cycles and metabolic capabilities studied, and their role as causative agents of some fish kills and human health problems elucidated (cf. Burkholder *et al.,* 2001a). Additionally, methods were developed to test cultures for toxicity (Burkholder *et al.,* 2001b), characterize toxins (Melo *et al.,* 2001; Moeller *et al.,* 2001), and to probe for the organisms in field samples (*e.g.,* Bowers *et al.,* 2000; Oldach *et al.,* 2000). Through 1999, the distribution of *Pfiesteria* spp. was extended from North Carolina, USA, to the east and gulf coasts of the US (*e.g.,* Rublee *et al.,* 2001; Lewitus *et al.,* 2002; Villareal *et al.,* this Proceedings).

In 1999, we began to assess *Pfiesteria* distribution outside the United States. One of the first sites to test positive for *Pfiesteria* was near Trondheim, Norway. Simultaneously and independently, colleagues at the University of Oslo also found evidence of both species of *Pfiesteria* in Oslo Harbor (Jakobsen *et al.,* 2001). Ribosomal sequence data from cultures confirmed the presence of both *Pfiesteria* species, and bioassays confirmed the toxicity of the *P. shumwayae* isolate (Burkholder *et al.,* 2001b). Similarly, the presence of toxic *Pfiesteria* in New Zealand has been confirmed by PCR probing, ribosomal gene sequencing, and fish bioassay (Rhodes *et al.,* 2002).

## Materials and Methods

Sampling kits (Whatman GFC glass fiber filters and vials with 1 mL CTAB buffer) were sent to colleagues with requests to sample from estuarine sites. Water samples were filtered, the filter placed in the buffer and vials maintained at room temperature until return to our laboratory. Sediment samples were collected occasionally. Upon return to the laboratory, DNA from water samples was extracted and purified using a CTAB protocol (Schaefer, 1997); sediment material was extracted and purified using a commercial kit (MoBio® Soil DNA Extraction Kit). During 2000 and

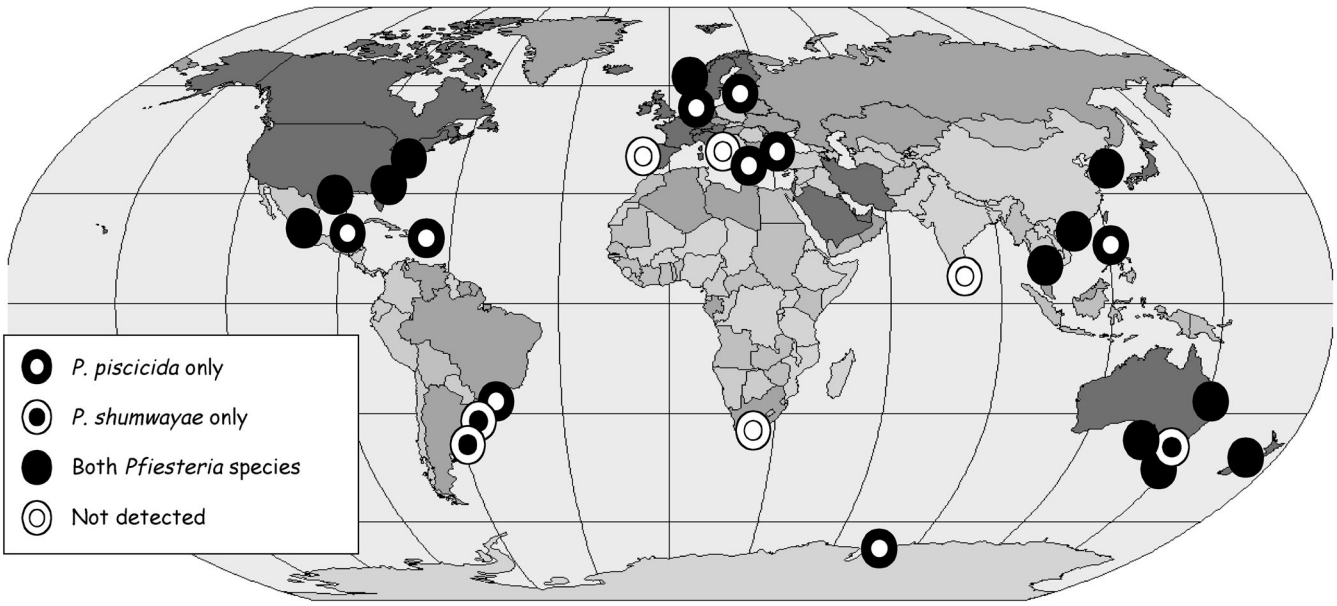


**Figure 1** Current known worldwide distribution of *Pfiesteria* species.



Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

**Table 1** Detection of *Pfiesteria* species in samples by continent (excluding samples from the United States).

| Country or Region | Number of Samples | *P. piscicida* detected | *P. shumwayae* detected | Both *Pfiesteria* species detected |
|---|---|---|---|---|
| Africa | 6 | 0 | 0 | 0 |
| Antarctica | 1 | 1 | 0 | 0 |
| Asia | 66 | 15 | 14 | 5 |
| Australia/NZ | 302 | 31 | 37 | 4 |
| Europe | 88 | 10 | 9 | 0 |
| North America | 18 | 10 | 1 | 1 |
| South America | 19 | 2 | 2 | 0 |

2001, assays were conducted using PCR primers and protocols described previously (Rublee *et al.,* 1999, Oldach *et al.,* 2000); during 2002 we used real-time PCR with Taqman® probes (Bowers *et al.,* 2000), which provides greater sensitivity. Earlier samples were also retested using the real-time assay. In many cases the improved sensitivity resulted in detection of *Pfiesteria* from samples that did not demonstrate the presence of the organisms under the conventional PCR protocols.

## Results and Discussion

We received and assayed 500 samples from twenty-two countries/regions, excluding the United States (Fig. 1, Table 1). Most samples came from Australia (n = 190) and New Zealand (n = 112), and the fewest from Antarctica, Mexico, and Argentina (n = 2). *Pfiesteria piscicida* was found in 15 countries and *P. shumwayae* in 12. Neither species was detected in Portugal, South Africa, or Sri Lanka. The most unique result was detection in a sample from Ace Lake, a saline lake in the Vestfold Hills region of Antarctica, although this has not been confirmed by sequence comparison.

The overall frequency of detection of the two *Pfiesteria* species was similar, about 12%. Both species were present in only 10 samples (2% of the total), and 9 of these were found in countries bordering the Pacific Ocean. These frequencies are similar to the frequency of detection in samples from the United States (Rublee *et al.,* 2001), but may underrepresent the true presence of *Pfiesteria* spp., since the individuals collecting the samples had no prior experience locating this estuarine dinoflagellate. Temperature and salinity did not appear to be correlated with the presence of either *Pfiesteria* species (Fig. 2).

The PCR-based detection methods used in this study do not indicate if samples contain toxic or potentially toxic forms of *Pfiesteria*. No samples were reported to coincide with fish health problems, although toxin production has been demonstrated in isolates from Norway, Australia, and New Zealand, which also killed fish in bioassays.

## Conclusions

*Pfiesteria* species appear to be widely distributed globally. In most cases they appear to be benign members of estuarine communities, but under certain circumstances (including warm temperatures, mid-level salinity, high nutrient concentrations, high fish abundance and low turbulence) they may exhibit toxicity leading to fish, shellfish, or human health problems. To date, such conditions have only been reported in estuaries of the US east coast.

The widespread distribution raises questions about the relatedness of geographically disparate populations and the mechanisms that might have contributed to the distribution. SSU rDNA sequence data from Norway isolates suggested that while there were some sequence differences between Norwegian and US *P. piscicida,* the strains of *P. shumwayae* were virtually identical. Similarly, strains of *P. shumwayae* from New Zealand showed little variation from North American counterparts. Geographic variation among

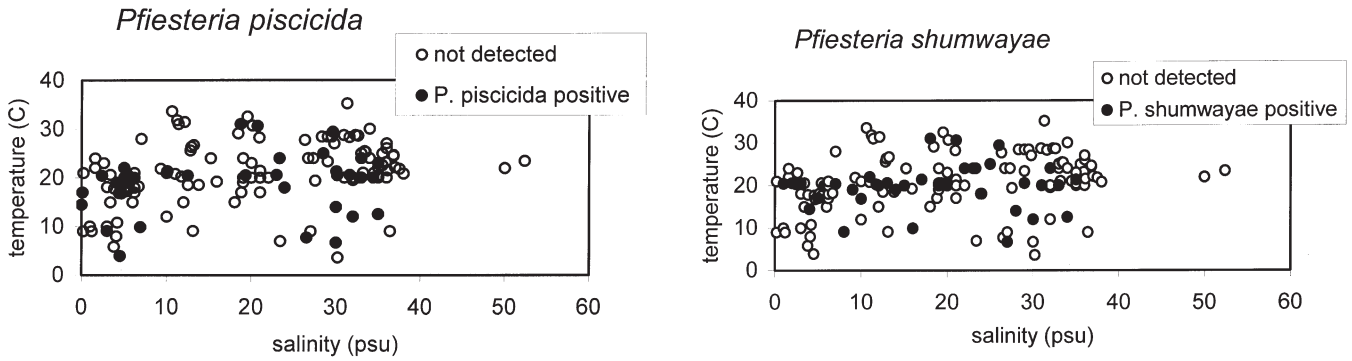


**Figure 2** Salinity and temperature distribution for samples where physical parameters were measured.

strains across both small and large scales is an ongoing effort in collaboration with colleagues. Detection of *Pfiesteria* in ballast water of marine vessels suggests one mechanism by which *Pfiesteria* species may be distributed globally (cf. Doblin *et al.,* this Proceedings).


## Acknowledgements

This study was supported in part by a U.S. EPA–ECOHAB grant and the New Zealand ISAT Linkages Fund. We thank collaborators who provided sample material: Alicia Escalante (Argentina); Pru Bonham, Jenny Keys, Susan Blackburn, Peter Thompson, Gustaaf Hallegraeff, Judith O'Neil, Was Hosja, Jonathan Smith (Australia); Clarisse Odebrecht, Virginia Garcia (Brazil); F. Leonardo Guzman (Chile); Qi Yuzao, Gu Xiaolian (China); Helge Thomson (Denmark); Konstantino Kormas (Greece); Wen-Xiong Wang (Hong Kong); Maria Cabrini (Italy); Anda Ikauneice (Latvia); Carolyn Burns (New Zealand); Matilde Chauton, Kjetill Jakobsen, Dag Klaveness, Torstein Tengs, Andreas Vatne (Norway); Wolfgang Reichardt (Phillipines); Maria Jose Boavida (Portugal); Brian Allanson (South Africa); Jong-Gyu Park (South Korea); Esra Kocum (Turkey); Ajcharaporn Piumsomboon (Thailand); Graciela Ferrari (Uruguay); Holly Bowers, David Oldach, Ted Smayda, Fred Dobbs, Steve Morton (United States).


## References


H. A. Bowers, T. Tengs, H.B. Glasgow, Jr., J.M. Burkholder, P.A. Rublee, and D.W. Oldach, Appl. Environ. Microbiol. 66, 4641–4648 (2000).

J. M. Burkholder, H. B. Glasgow, and N. J. Deamer-Melia, Phycologia 40,186–214. (2001a).

J. M. Burkholder, H. G. Marshall, H. B. Glasgow, D. W. Seaborn, and N. J. Deamer-Melia, Environ. Health Perspect. 109, 745–56 (2001b).

M. A. Doblin, L. A. Drake, K. J. Coyne, P. A. Rublee, and F. C. Dobbs, this Proceedings.

K. S. Jakobsen, T. Tengs, A. Vatne, H. A. Bowers, D. W. Oldach, J. M. Burkholder, H. B. Glasgow Jr, P. A. Rublee, and D. Klaveness, Proc. R. Soc. Lond. B. Biol. Sci. 269, 211–214 (2002).

A. J. Lewitus, K. C. Hayes, B. M. Willis, J. M. Burkholder, H. B. Glasgow, Jr., A. F. Holland, P. Maier, P. A. Rublee and R. Magnien, Estuaries 25(4A), 586–597 (2002).

A. C. Melo, P. D. R. Moeller, H. B. Glasgow, J. M. Burkholder, and J. S. Ramsdell, Environ. Health Perspect. 109, 731–738 (2001).

P. D. R. Moeller, S. L. Morton, B. A. Mitchell, S. K. Sivertsen, E. R. Fairey, T. M. Mikulski, H. B. Glasgow, N. J. Deamer-Melia, J. M. Burkholder, and J. S. Ramsdell, Environ. Health Perspect. 109, 739–744 (2001).

D. W. Oldach, C. F. Delwiche, K. S. Jakobsen, T. Tengs, E. G. Brown, J. W. Kempton, E. F. Schaefer, H. Bowers, K. Steidinger, H. B. Glasgow, Jr., J. M. Burkholder, and P. A. Rublee, Proc. Natl. Acad. Sci. USA 97, 4303–4308 (2000).

L. L. Rhodes, J. M. Burkholder, H. Glasgow, P. A. Rublee, C. Allen, and J. E. Adamson, N. Z. J. Mar Freshwater Res. 36, 621–630 (2002).

P. A. Rublee, J. Kempton, E. Schaefer, J. M. Burkholder, H. B. Glasgow, and D. Oldach, Va. J. Sci. 50, 325–36 (1999).

P. A. Rublee, J. W. Kempton, E. F. Schaefer, C. Allen, J. Harris, D. W. Oldach, H. Bowers, T. Tengs, J. M. Burkholder, and H.B. Glasgow, Environ. Health Perspect. 109 [Suppl. 5], 765–767 (2001).

E. F. Schaefer, MS Thesis, Univ. NC at Greensboro. 1–86 (1997).

T. Villareal, J. D. Simons, and P. Rublee, this Proceedings.

# The Use of Volunteers to Monitor Harmful Phytoplankton

Katherine A. Schaefer, Heather L. Blankenstein, Kimberly J. Nowocin, and Steve L. Morton
*National Oceanic and Atmospheric Administration, National Ocean Service, Marine Biotoxins Program, Hollings Marine Laboratory, 331 Fort Johnson Road, Charleston, SC 29412*

## Abstract

The South Carolina Phytoplankton Monitoring Network comprises over 35 groups monitoring for potential harmful algal species. Volunteer groups are composed of both high school classes and environmental citizen groups. This NOAA-sponsored community program increases awareness of harmful algae to constituent groups and directly involves volunteers in coastal stewardship. Observation and identification of phytoplankton along the South Carolina coast will be used to develop a species list. In the program's first year, volunteers observed several algae taxa not previously known to exist in South Carolina, including *Pseudo-nitzschia, Dinophysis,* and *Prorocentrum lima.*

## Introduction

Phytoplankton monitoring networks exist in nine coastal USA states. These networks are based on volunteer collection and identification of phytoplankton in water samples. One example of a successful program is the Maine Phytoplankton Monitoring Network, which began in 1996. Volunteers were able to identify *Alexandrium* and a number of blooms of *Dinophysis* in Maine waters before potential human health complications arose (Morton *et al.,* 1999).

The South Carolina Phytoplankton Monitoring Network (SCPMN) began in January 2001 as a result of a recommendation by the National Oceanic and Atmospheric Administration (NOAA) Marine Biotoxins Program to the South Carolina Task Group on Harmful Algae. One step in the assessment of harmful algal blooms in South Carolina was to begin a statewide surveillance of South Carolina's marine waters. The goals of SCPMN include promoting education on harmful algae to the general public, monitoring of coastal waters in South Carolina where potentially harmful algae may exist, and the development of a general species list for the state of South Carolina.

Volunteers are currently sampling in six of the eight coastal counties in South Carolina. These counties include Beaufort, Berkeley, Charleston, Colleton, Georgetown, and Horry (Fig. 1). Volunteer groups maintain between one and four sampling sites depending on group size and time availability. Volunteer groups consist of middle and high school biology and marine biology classes or extracurricular organizations, environmental citizen organizations, and state parks.

## Materials and Methods

SCPMN volunteers attend a training session before becoming involved with the program. Background information on SCPMN, volunteer responsibilities, phytoplankton identification, and harmful algal blooms (HABs) are presented in the training session. HABs are defined as "accumulations of microscopic species of algae that cause injury or death to other organisms in the water." Volunteers are trained on sampling techniques and identification methodologies by watching a training identification video, performing a plankton tow, and performing light microscope.

Volunteers are supplied with a 20-µm student plankton net (Sea-Gear Corporation) and a refractometer (VWR Scientific). Plankton tows are performed mainly from floating docks by pulling the net horizontally through the water for three minutes. Samples can be identified on the same day

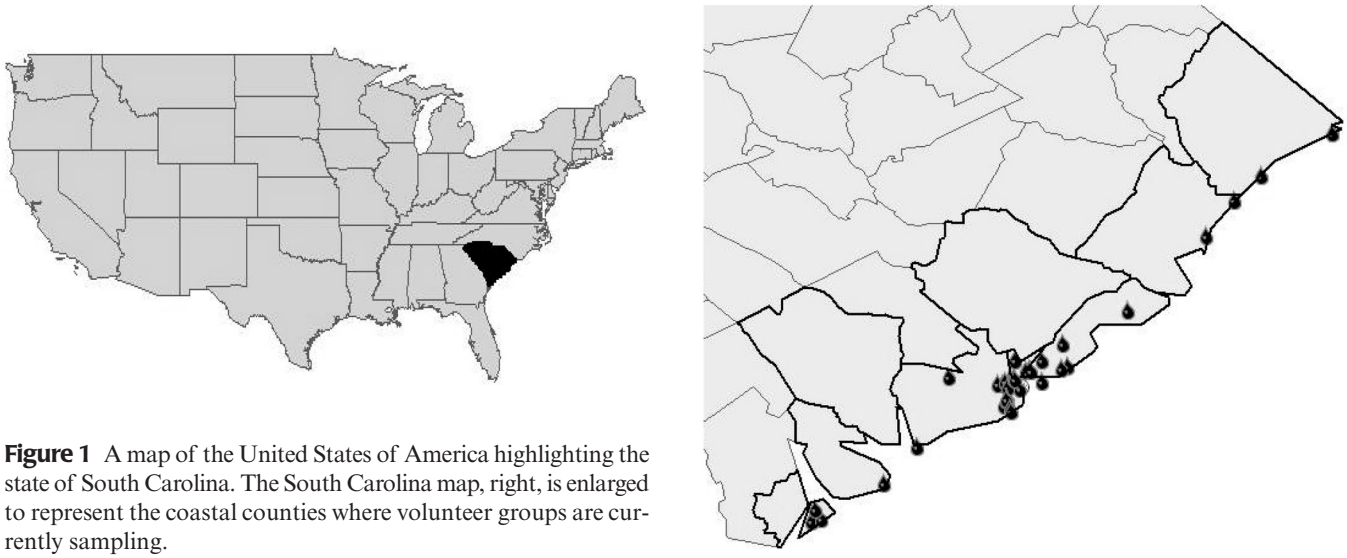

**Figure 1** A map of the United States of America highlighting the state of South Carolina. The South Carolina map, right, is enlarged to represent the coastal counties where volunteer groups are currently sampling.

Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

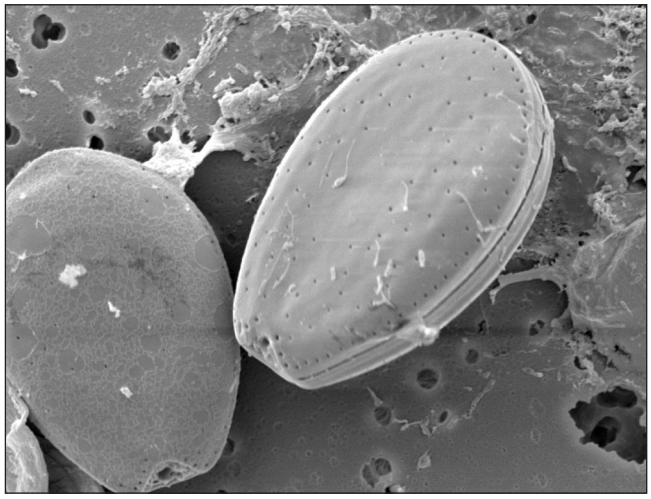

**Figure 2** Scanning electron micrograph of *Prorocentrum lima.*

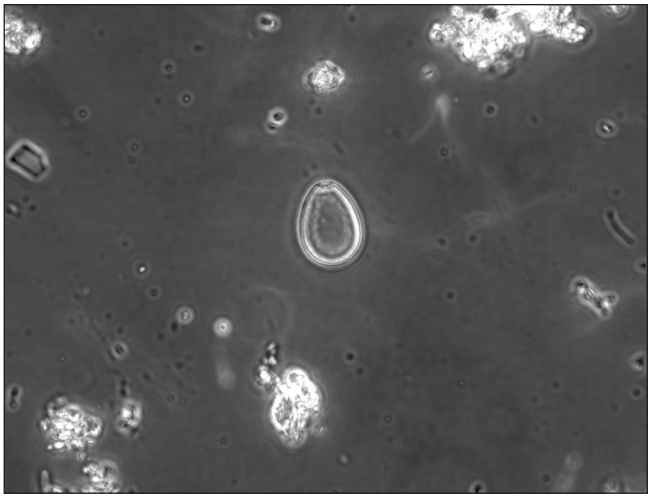

**Figure 3** Light micrograph of *P. lima* theca.

or up to three days after the tow was performed, although older samples can have lyzed, dead phytoplankton. Data sheets are completed at the time of identification and submitted via electronic mail or facsimile.

Volunteers record sample results on the SCPMN data sheet. The species on the SCPMN data sheet include six dinoflagellate taxa (*Akashiwo sanguinea, Ceratium* spp., *Dinophysis* spp., *Karenia brevis, Prorocentrum micans,* and *Protoperidinium* spp.) and six diatom taxa (*Chaetoceros spp., Coscinodiscus* spp., *Ditylum* spp., *Odontella* spp., *Pseudo-nitzschia* spp., and *Rhizosolenia* spp.). Volunteers record qualitative rather than quantitative abundance ratios (Table 1) for these species and submit weekly or biweekly data sheets.

In addition to phytoplankton abundance, volunteers record other ancillary data that include water temperature, salinity, date and time of the plankton tow, and the location of the sampling site.

After volunteer groups perform a plankton tow and submit their data sheet, data is then entered into a Geographic Information Systems (GIS) database to create a spatial interpretation of the collected data. The GIS database will assist scientists in understanding trends of phytoplankton species found by volunteers in South Carolina waters. Maps show species distribution along the coast and the volunteer sampling locations. Map layouts will be placed on the SCPMN web site at http://www.chbr.noaa.gov/CoastalResearch/SCPMN/SCPMNmain.htm. for volunteers and interested visitors to observe.

**Table 1** Abundance ratios represent the percent abundance of each observed species in the sample. Volunteers assign qualitative abundance ratios to species on the SCPMN data sheet.

| | |
|---|---|
| None | 0 |
| Rare | 0.1–1% |
| Present | 1.1–10% |
| Common | 10.1–40% |
| Abundant | 40.1–80% |
| Bloom | 80.1–100% |

## Results and Discussion

The use of volunteers to monitor phytoplankton has proven to be beneficial to scientists in South Carolina. Results from the first year of monitoring (2001) include the discovery of three potentially toxic algae not previously known to exist in the state. These taxa are *Dinophysis* spp., *Prorocentrum lima* (Schaefer and Morton, this Proceedings), and *Pseudo-nitzschia* spp. *Dinophysis* spp. were first observed by volunteers during the spring of 2001 at the Folly Beach Fishing Pier (32°39′14″N, 79°56′28″W), Charleston, South Carolina by the Academic Magnet School. This species was seen in rare abundance. *Dinophysis* spp. was not observed from the spring of 2001 until September 2002 at four different SCPMN sampling sites. *Prorocentrum lima* (Fig. 2) was first observed at the Fort Johnson Road sampling site (32°45′15″N, 79°54′34″W) in May 2001 in rare abundance (Schaefer and Morton, this Proceedings). *Prorocentrum lima* theca (Fig. 3) were found in rare abundance at three additional sampling locations in central coastal South Carolina: Amoco Creek (32°57′56″N, 79°54′28″W), Flagg Creek (32°56′56″N, 79°54′34″W), and Goose Creek (32°58′01″N, 79°56′05″W), South Carolina (Schaefer and Morton, this Proceedings). *Pseudo-nitzschia* spp. were observed the most frequently out of these three species. *Pseudo-nitzschia* spp. ranged from rare to abundant on 185 submitted data sheets covering 33 sampling sites. The period of highest activity was from August to September 2002. All three of these taxa, along with other potentially toxic algae, are continually monitored by SCPMN volunteers.

There are numerous scientific and outreach benefits resulting from volunteers monitoring South Carolina waters. The data collected by volunteers has helped scientists build

a continuing species list for the state, which was one of the initial program goals. The long term benefits of this monitoring program will hopefully lead scientists to new sampling sites for further study, to identify the time and place of blooms, and to eventually predict blooms. The outreach aspect of the SCPMN has increased community awareness of HABs, resulted in increased public awareness of research conducted by federal and state groups, and enabled improved communication between scientists and volunteers. The long-term outreach benefits of the SCPMN will be a stronger interaction between the scientific community and the general public.

In order to obtain a better spatial resolution of monitoring data along the coast, counties will extend sampling for SCPMN into all eight coastal counties of South Carolina. The extended survey area throughout the year will allow for a more thorough investigation of coastal waters, leading to fulfillment of the long-term scientific benefits listed above.

The discovery of three potentially toxic taxa in South Carolina (*Dinophysis* spp., *Prorocentrum lima,* and *Pseudo-nitzschia* spp.) has initiated new research projects conducted by NOAA scientists. Scientists are testing oysters for the presence of okadaic acid from the sampling sites where *Prorocentrum lima* was originally discovered in 2001 (Schaefer and Morton, this Proceedings). The efforts of volunteers are crucial for the continued success of SCPMN and to provide preliminary observations to NOAA scientists on the current status of phytoplankton in South Carolina's waters.

## Acknowledgements

The authors would like to thank NOAA for funding this program and the South Carolina Task Group on Harmful Algae for their assistance.

## References

S.L. Morton, T.A. Leighfield, B.L. Haynes, D.L. Petitpain, M.A. Busman, P.D.R. Moeller, L. Bean, J. McGowan, J.W. Hurst, and F.M. VanDolah, J. Shellfish Res. 18, 681–686 (1999).

K.A. Schaefer and S. L. Morton, this Proceedings.

# Harmful Dinoflagellates in the Gulf Stream and Atlantic Barrier Coral Reef, Belize

Maria A. Faust[1] and Patricia A. Tester[2]
[1]*Department of Systematic Biology–Botany, NMNH, Smithsonian Institution, 4210 Silver Hill Road, Suitland, MD 20746, USA;* [2]*Center for Coastal Fisheries and Habitat Research, National Ocean Service, NOAA, 101 Pivers Island Road, Beaufort, NC 28516, USA*

## Abstract

Dinoflagellates from two locations in Gulf Stream waters of the US South Atlantic Bight are reported and compared with dinoflagellates from the tropical Atlantic Barrier Coral Reef, Belize. There is limited information on HAB species in the two areas. A total of 45 tropical oceanic warm-water species, and 16 benthic, potentially harmful species were present.

## Introduction

This report presents information on the distribution of harmful dinoflagellates in the Gulf Stream along the southeastern coast of the United States where expatriate *Karenia brevis* blooms have occurred (Tester and Steidinger, 1997). The aim of this paper is to report the harmful algal bloom (HAB) species composition and oceanic dinoflagellates in the Gulf Stream and compare these with the species composition found in Belizean coral reef-mangrove habitats (Faust 2000). In the continental shelf waters of the US South Atlantic Bight, phytoplankton distribution is highly variable and dinoflagellates represent 46% of total phytoplankton biomass (Marshall, 1971, 1982). The tropical oceanic dinoflagellates are major components in the plankton. The entry of tropical assemblages of dinoflagellates into the Gulf Stream is from the Gulf of Mexico via the Florida Current (Tester and Steidinger 1997), the Caribbean Sea (Marshall, 1978), or the Sargasso Sea (Parker, 1971). Inshore habitats may also contribute organisms to the Gulf Stream (Richardson, 1976).

In the waters off Carrie Bow Cay and Douglas Cay, Belize, distribution of dinoflagellates revealed countless surprises. This coral reef-mangrove ecosystem is characterized by great diversity, showing distinct characteristics relative to adjacent oceanic water from those in lagoonal shallow mangrove cays (Macintyre and Ruetzler, 2000) where harmful and oceanic dinoflagellate species co-exist (Faust, 2000). HAB dinoflagellates are abundant inside protected cays, maintaining blooms in naturally enriched shallow water (Morton and Villareal, 2000), with different species from those in adjacent pelagic waters (Faust, 2000). Information, however, is limited on the species of HAB dinoflagellates in the swift-moving current of the Gulf Stream compared with the oceanic regime of the fore reef of Carrie Bow Cay and deep channel waters outside of Douglas Cay, Belize.

## Materials and Methods

The samples include eight integrated water column net tows (20 and 35 mm pore size net) in the Gulf Stream and Atlantic Barrier Reef, Belize (Table 1). Sample origin: 1) Gulf Stream—27 nm offshore from Cape Lookout, NC, and 6 km offshore from the Indian River Inlet, FL; and 2) Belize—50 m offshore from Carrie Bow Cay, and in the channel outside Douglas Cay. Cells were preserved in 2% glutaraldehyde final concentration and processed for scanning electron microscopy following Faust (1990). Species identifications were made from 685 SEM micrographs of alike oceanic and HAB dinoflagellate species.

## Results

A total of 53 dinoflagellate species, 45 oceanic, and 17 HAB species were identified from eight plankton collections. The number of oceanic species ranged from 41 to 45 per collection (Table 2). Cell size was generally large (<100 µm). The diversity of species varied in each collection. The worldwide-distributed tropical oceanic species were the most abundant—*e.g., Ceratium trichoceros, C. vultur, C. macroceros,* and *C. concilians, C. horridum, C. tripos, C. massiliense, C. lunula, C. declinatum,* and *C. candelabrum*—whereas *C. furca* was cosmopolitan. The *Protoperidinium* spp. identified were *P. curtipes, P. depressum, P. divergens, P. elegans, P. globulosus, P. grande, P. obtusum,* and *P. steinii.* Frequently the following species were included: *Diplopelta*

**Table 1** Station location, date, sample depth (m), temperature (°C), salinity (psu) of collections.

| Site | Date | Location | Depth (m) | Temperature | Salinity |
|---|---|---|---|---|---|
| Cape Lookout, NC | 4-Jun-02 | 34°23′N 79°56′W | 10 | 28 | 36 |
| | 18-Jun-02 | 34°36′N 76°06′W | 15 | 27 | 36 |
| Indian River Inlet, FL | 2-Feb-02 | 27°30′N 79°56′W | 114 | 22.5 | 36 |
| | 22-Apr-02 | 27°31′N 79°55′W | 100 | 22.5 | 36 |
| Carrie Bow Cay, Belize | 17-May-00 | 16°48′N 88°05′W | 10 | 26 | 35 |
| | 1-Jun-01 | 16°53′N 88°13′W | 10 | 27 | 35 |
| Douglas Cay, Belize | 22-May-01 | 16°43′N 88°13′W | 10 | 31 | 35 |
| | 29-May-01 | 16°43′N 88°10′W | 10 | 29 | 35 |



Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

**Table 2** Number of oceanic and HAB dinoflagellates in collections.

| Dinoflagellates | Cape Lookout | Indian River | Carrie Bow Cay | Douglas Cay |
|---|---|---|---|---|
| Oceanic species | 45 | 41 | 44 | 43 |
| HAB species | 7 | 8 | 9 | 9 |
| Total numbers | 52 | 51 | 53 | 52 |
| % HAB spp. | 13 | 16 | 17 | 17 |

*bomba, Prorocentrum micans, P. compressum,* and *P. gracile, Goniodoma sphaericum,* and *G. polyedricum.* Ornamented oceanic species were *Ornithocercus quadratus, O. magnificus, O. steinii,* and *O. thumii, Ceratocorys horrida,* and *Podolampas bipes,* while the rare species included *Blepharocysta hermosiliai* and *B. splendor-maris, Diplopsalopsis orbicularis, Lissodinium taylori,* and *Spiraulax kofoidii.*

Distribution of HAB species varies from 7 to 9 in the collections (Table 2). Cell size of autotrophic species are relatively small (<100 µm). Red tide-forming HAB species were *Gonyaulax grindleyi, G. polygramma,* and *G. spinifera;* benthic HAB species were *Coolia monotis, Gambierdiscus toxicus, G. pacificus,* and *G. australes, Ostreopsis siamensis, Prorocentrum belizeanum, P. borbonicum, P. emarginatum, P. lima,* and *P. mexicanum;* and planktonic HAB species were *Dinophysis caudata, D. rotundata,* and *D. rapa.* The full listing of the sixteen HAB species representing planktonic and benthic tropical warm-water species is shown in Table 3, below.

**Table 3** HAB dinoflagellate species in collections. Values are yes (present), no (absent).

| Dinoflagellate | Cape Lookout | Indian River | Carrie Bow Cay | Douglas Cay |
|---|---|---|---|---|
| *Coolia monotis* | no | yes | no | no |
| *Dinophysis caudata* | yes | no | yes | yes |
| *Dinophysis rotundata* | no | no | no | no |
| *Dinophysis rapa* | yes | no | yes | no |
| *Gambierdiscus toxicus* | yes | yes | yes | yes |
| *G. australes* | yes | yes | no | no |
| *G. pacificus* | no | no | yes | no |
| *Gonyaulax grindleyi* | no | no | yes | no |
| *G. polygramma* | no | no | yes | yes |
| *G. spinifera* | yes | yes | yes | yes |
| *Ostreopsis siamensis* | no | yes | no | yes |
| *Prorocentrum lima* | yes | yes | no | yes |
| *P. emarginatum* | no | yes | yes | yes |
| *P. mexicanum* | no | yes | no | yes |
| *P. borbonicum* | no | no | yes | no |
| *P. belizeanum* | no | no | no | yes |

## Discussion

Our study reports the distribution of HAB species in the recent collections at four geographically distant sites and locations collected in the Gulf Stream and the Atlantic Barrier Reef, Belize. A total of 53 dinoflagellate species were present in the eight samples, separated into oceanic, benthic HAB and red tide-forming HAB species. Dinoflagellate taxa were representative of tropical oceanic species. Coral reef-mangrove HAB dinoflagellates enter southeastern waters of the United States in Gulf Stream warm-core rings (Gould, 1988). These rings carry water of distinct quality and composition (Parker, 1971, Richardson, 1976) and have a stabilizing influence on planktonic organisms of the Gulf Stream (Wiebe, 1976). The Gulf Stream, with its eddy formation, provides the entry of the tropical and subtropical species into waters north of Cape Hatteras (Marshall, 1978). Warm-core ring protection and survival offers a possible explanation to findings of 45 warm water oceanic and 16 HAB species as far north as Cape Lookout, NC (Marshall, 1978). The dinoflagellate assemblages are diverse in both shelf and coral reef waters, in association with chain-forming and planktonic diatoms. Marshall (1982) examined dinoflagellate distribution in southeastern shelf waters south of Cape Lookout, NC. He found that of the 72 specimens, 46% of the species were present at far-off shelf stations, where highest species concentrations of *Ceratium furca, C. lineatum,* and *C. trichoceros, Dinophysis caudata,* and *Prorocentrum micans* occurred. Comparison of our studies with those of Marshall (1982) is not possible. He did not report any HAB dinoflagellate species in his collections south of Cape Lookout. In our studies, four genera represent the highest number of tropical oceanic species: 33% *Ceratium,* 8% *Dinophysis,* 19% *Protoperidinium,* and 19% *Prorocentrum.* Similarly, four genera depicted 16% benthic HAB taxa: *Dinophysis, Gonyaulax, Gambierdiscus,* and *Prorocentrum.*

The study provides new knowledge on tropical benthic HAB species associations and distributions within tropical oceanic taxa in Gulf Stream and Belizean reef-mangrove habitats. The discovery of the presence of tropical benthic HAB species in Gulf Stream waters in the South Atlantic Bight is considered significant new information. We report benthic HAB dinoflagellate assemblages in Gulf Stream water that represent potentially harmful species distributed worldwide (Steidinger and Tangen, 1997) and HAB dinoflagellate species described from the Belizean coral reef mangroves (Faust 1996, 2000). The distribution pattern of tropical HAB species exhibited a long geographical distance from Cape Lookout, NC, to Douglas Cay, Belize. Our data showing HAB specimens in the Gulf Stream's fast-moving current suggests that this is a possible mechanism for the dispersal of HAB species. However, the relationship of broad hydrographic events, and dispersal of HAB species, and phytoplankton dynamics in the Gulf Stream need further investigation.

## Acknowledgements

We thank Dr. Klaus Ruetzler for the use of the facilities at

Carrie Bow Cay, Belize, and Dr. Mary Rice, Smithsonian Marine Station at Fort Pierce, FL, and Sabrina Varnam National Ocean Service, NOAA, Beaufort, NC for collecting Gulf Stream samples. This paper is a contribution from the Smithsonian Institution's CCRE No. 649 and SMSFP No. 557 Programs.

## References

M. A. Faust, J. Phycol. 26, 548–558 (1990).
M. A. Faust, Atoll Res. Bull. No. 473, 133–150 (2000).
R. W. Gould Jr., Deep-Sea Res. 35, 1595–1614 (1988).
I. G. Macintyre and K. Ruetzler, Atoll Res. Bull. Nos. 466–480, pp. 1–333 (2000).
H. G. Marshall, Bull. Mar. Sci. 21, 806–825 (1971).
H. G. Marshall, Mar. Biol. 45, 203–208 (1978).
H. G. Marshall, Proc. Biol. Soc. Wash. 95, 99–113 (1982).
S. L. Morton and T. A. Villareal, Bull. Mar. Sci. 63, 1–4 (2000).
C. E. Parker, Deep-Sea Res. 18, 981–993 (1971).
P. Richardson, Oceanus 19, 65–69 (1976).
K. A. Steidinger and K. Tangen, in: Identifying Marine Phytoplankton, C. R. Tomas, ed. (Academic Press, San Diego), pp. 387–584 (1997 ).
P. A. Tester and K. A Steidinger, Limnol. Oceanogr. 42, 1039–1051 (1997).
P. Wiebe, Oceanus 19, 69–79 (1976).

# The Geographical Distribution of *Alexandrium catenella* is Extending to Italy! First Evidence from the Tyrrhenian Sea

Antonella Luglié[1], Mariagrazia Giacobbe[2], Fatima Fiocca[1], Antonietta Sannio[1], and Nicola Sechi[1]
[1]*DBEV, Dipartimento di Botanica ed Ecologia Vegetale, Università di Sassari, Via Muroni 25–07100 Sassari, Italy;*
[2]*CNR Istituto Ambiente Marino Costiero, Sezione Talassografico di Messina, Italy*

## Abstract

The extension of the geographical range of *Alexandrium catenella* within the Mediterranean region, from the northwestern area to the Italian coastline, in the Gulf of Olbia, northeastern Sardinia (Tyrrhenian Sea), is reported. This locality is characterized by an important commercial harbour and the largest site of shellfish farming of the island of Sardinia. A recent introduction of *A. catenella* through ship ballast waters or mussel stocks from other countries is hypothesized and supported by no evidence of this dinoflagellate before 1999. Morphological traits of *A. catenella* from Tyrrhenian waters were consistent with other descriptions of the species. Although in Sardinia *A. catenella* displayed a limited growth, PSP toxins were recently detected (May 2002). This implies a real risk of further toxic events and resultant public health and economic problems linked to mussel farming in the area.

## Introduction

The global increase in HABs, including blooms of PSP-producing dinoflagellates such as various species of *Alexandrium,* is of recent interest in the Mediterranean Sea. However, only a few records of *A. catenella* (Whedon et Kofoid) Balech from the Mediterranean can be found in the scientific literature, limited to the Balearic Basin, the Spanish coastline, and the Thau Lagoon in France (Margalef and Estrada, 1987; Gomis *et al.,* 1996; Masselin *et al.,* 2001; Vila, 2001; Vila *et al.,* 2001, 2002; Lilly *et al.,* 2002). In 1999, *A. catenella* was reported for the first time along the Italian coastline (Lugliè *et al.,* 2000), in the Gulf of Olbia, northeastern Sardinia (Tyrrhenian Sea). This area has one of the most important commercial harbours and the largest site of shellfish farming on the island, yielding about 4000 t of mussels per year. Mariculture activities also involve an import-trade of shellfish from European localities, especially in summer when the market demand increases. Here, we confirm the extension of the geographical range of *A. catenella* to Sardinia, through observations in 1999 and 2001, and further support the spreading of this dinoflagellate in the Mediterranean region, possibly in relation to human activities. The risk of future toxic events and their spread to new Italian localities are discussed.

## Materials and Methods

Since 1992, the Gulf of Olbia–Sardinia (40°55,356N, 009° 32,279E, Fig. 1) has being monitored continuously (except for a gap in summer 2000) for phytoplankton and HAB species. Water samples were taken fortnightly to monthly at 2 stations (0.5 m depth) located within the gulf (Stns a and b). This site, previously described as eutrophic (Sechi *et al.,* 1987; Sannio *et al.,* 1996, 1997), is characterized by the presence of two municipal sewers and slow water exchange. Its inner harbor holds an important commercial port with urban, tourist and industrial activities, as well as the largest area of mussel and clam farming of Sardinia.

Phytoplankton was analysed in Utermöhl chambers using neutralised formaldehyde fixed samples. Fixed specimens of *A. catenella* were stained with Calcofluor White M2R (Fritz and Triemer, 1985) and examined by light microscopy using epifluorescence. 4% glutaraldehyde fixed cells were treated for SEM analyses (Giacobbe and Yang, 1999). Plate tabulation formulae and morphological features of the thecal plates were studied following Balech's criteria (1995). Live specimens of *A. catenella* were isolated from plankton assemblages and kept in culture. Parameters determined in conjunction with *A. catenella* abundance were temperature, salinity, fluorometric chlorophyll *a* (multiparameter probe Idromar), dissolved inorganic nitrogen (DIN) and total phosphorus (TP), according to Strickland and Parsons (1972).

## Results

Specimens of *A. catenella* exhibited cell size and pattern of

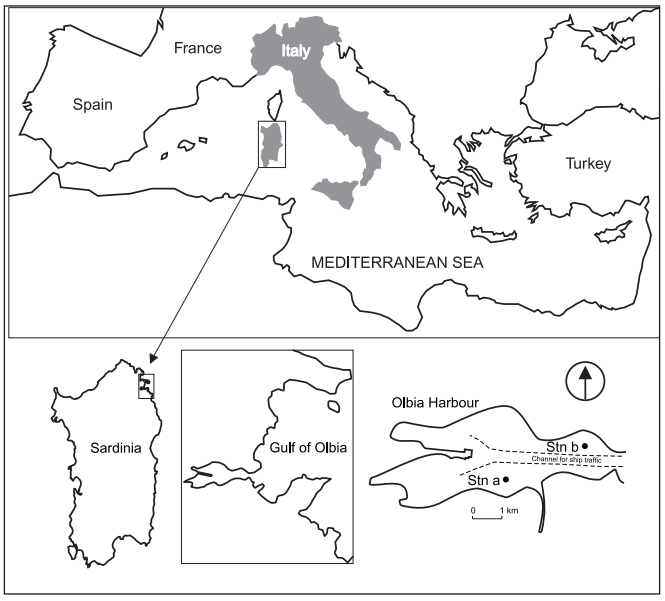


**Figure 1** Map of the Italian area affected by *A. catenella* (Gulf of Olbia, Sardinia) and sampling points. Extension = 6.5 km², mean depth = 5 m, max. depth = 10 m along the channel of ship traffic.

Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

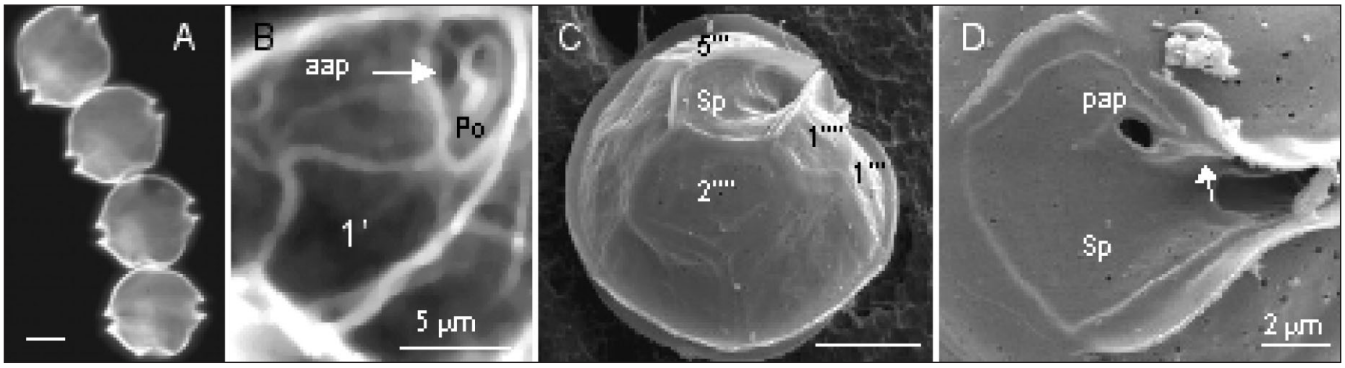


**Figure 2** *A. catenella* from the Tyrrhenian Sea (Sardinia, Italy). **A** and **B**: LM. Specimens stained with Calcofluor. Chain of 4 cells and epitheca: 1' plate lacking a v.p., Po plate with an anterior attachment pore (arrow); **C** and **D**: SEM. Antapical views with detail of Sp plate. Note the posterior attachment pore and connecting channel (arrow). Scale bars = 10 µm, unless indicated.

thecal plates (Fig. 2) comparable with those described from other localities: cell size 25–34.4 µm long, 28.8–36.9 µm wide (n = 30 field specimens), 6" medium-sized, 1' without ventral pore with Po-direct contact, Po plate triangular, aap present, Sp with a large, round pap linked to the Sp margin by a small channel, cingulum displaced; 2–4 celled chains were common in natural assemblages, 6–8 celled chains were occasionally observed, 2–8 celled chains were frequent in culture (exponential growth phase).

At Olbia, the presence of *A. catenella* appeared to be limited both in temporal and spatial terms, with a single detection of the species in August 1999 (Stn b, $2.2 \times 10^3$ cells $L^{-1}$). In June-August 2001, although the cell densities of *A. catenella* were still lower than in 1999, ranging between 100 and 200 cells $L^{-1}$ (Fig. 3), the species was found to be spread over a number of points inside the gulf, at locations in addition to the two routine sampling stations. The environmental conditions associated with *A. catenella* were: temperature higher than 20°C, with maximum cell densities at 26°C, salinity between 37.5 and 38.5 psu, DIN < 2 µM with a significant incidence of NH4-N, chlorophyll *a* not exceeding 9 µg $L^{-1}$, TP < 2 µM and phytoplankton density < $2 \times 10^6$ cells $L^{-1}$.

## Discussion

The records of *A. catenella* in the Tyrrhenian area, although at very low densities, suggest a progressive areal expansion of the distribution of this dinoflagellate within the Mediterranean region. Its occurrence at the Italian site could be explained by a recent, accidental introduction of the species through either ballast waters discharged from foreign ships or mussel stocks imported from other countries. The topographic features of the Olbia Gulf, especially the inner, confined part with reduced water exchange might then have favoured the colonization of *A. catenella* in this site, as observed in other Mediterranean localities (Vila *et al.,* 2001). On the other hand, phytoplankton data since 1992 have never shown any suspected cells in the area, although they could have been present in the waters at such a low density to escape detection. Alternatively, *A. catenella* could have been encysted in the surface sediments as resting stages that later encountered favourable environmental conditions, permissive for germination and growth. The absence in recent years of significant variations in trends in summer seawater temperatures and other environmental conditions, however, supports the idea of an introduction. Preliminary genetic studies involving sequence analyses of the 5.8S rDNA-ITS regions from Sardinian clones and other Mediterranean isolates (Catalonia, Spain) of *A. catenella* showed their complete alignment (Penna *et al.,* 2003). Phylogenetic analyses by these authors, including sequences from GenBank of isolates from other geographical areas such as Chile (ACC01) and Japan (M17), evidenced a grouping of Mediterranean and Japanese populations that could suggest an Asiatic origin, in agreement to Lilly *et al.* (2002). Further studies and comparisons are in progress to know and confirm the exact origin and ways of introduction of this species in Sardinia. More recent HAB surveys, including analyses of shellfish by mouse bioassay and HPLC, evidenced PSP toxicity in mussels farmed at Olbia (May 2002) during the simultaneous occurrence in the waters of *A. catenella* (max. $40 \times 10^3$ cells $L^{-1}$) and *A. minutum.* The HPLC analyses of *A. catenella* clones from the same site also confirmed the production of STX and GTXs (data in preparation by the Laboratory of National Reference for Biotoxins, Cesenatico, Italy). Despite the reduced growth of *A. catenella* in Sardinia not reaching bloom proportions, there is a real risk of further toxic events, serious sanitary (public health) and economic problems linked to the important activity of mussel farming in the area. The potential for spreading to new Mediterranean localities and the resulting constraints to the sustainable development of coastal areas, and other possible recent "aliens" as well (Wyatt and Carlton, 2002), indicates greater attention may be required to manage coastal areas where introductions are more likely to occur (harbors, farming areas, etc.).

## Acknowledgements

This study was supported by the European project STRATEGY (EVK3-CT-2001-00046). STRATEGY Coordinator:

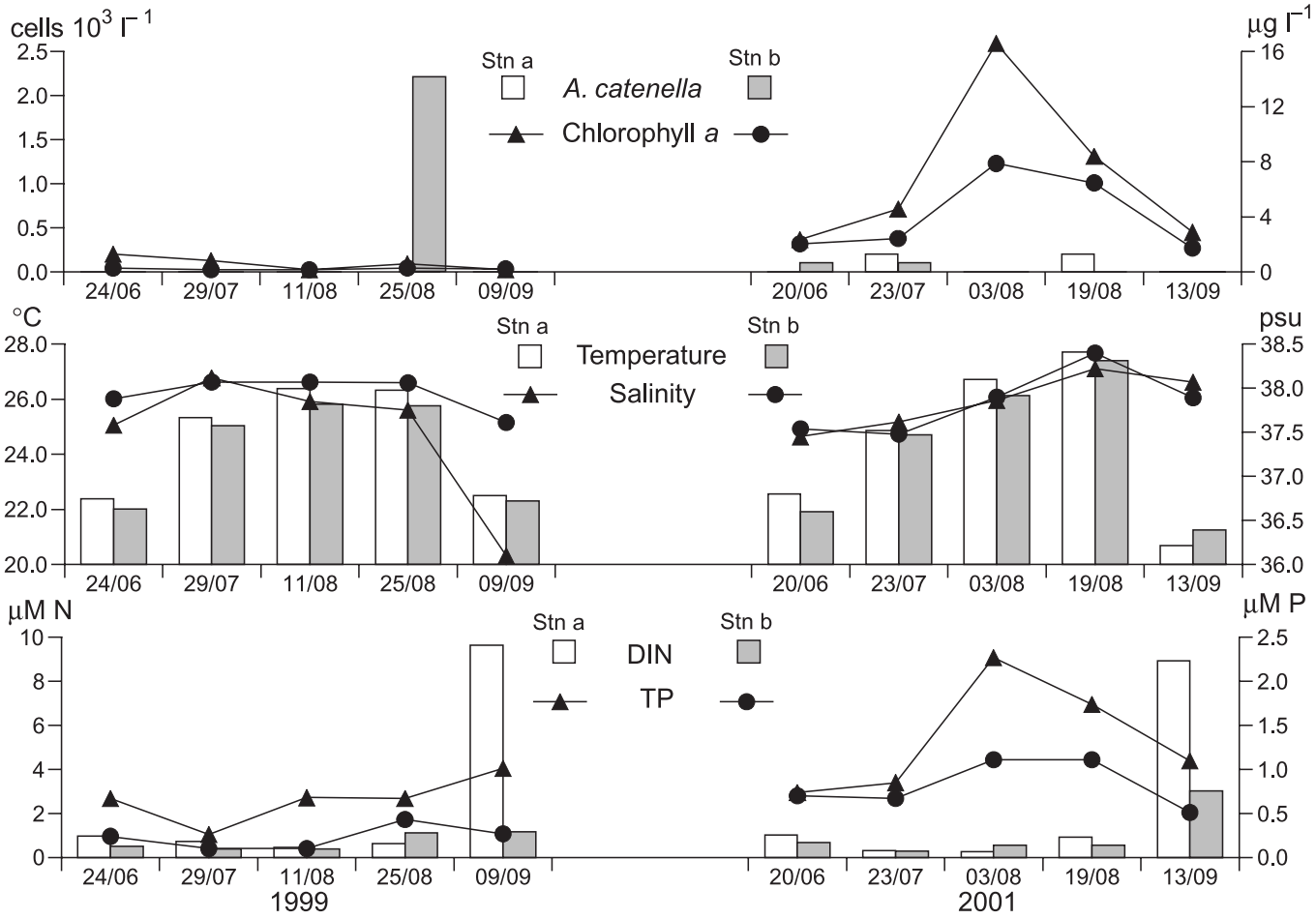


**Figure 3** Cell densities and environmental data from the Olbia Gulf (Italy).

Dr. Mercedes Masó, CSIC-ICM, Barcelona (Spain). www.icm.csic.es/bio/projects/strategy.

## References


E. Balech, The genus *Alexandrium* Halim (Dinoflagellata), Sherkin Island Marine Station (Sherkin Island Press, Ireland), 1–151 (1995).

L. Fritz and R. E. Triemer, J. Phycol. 21, 662–664 (1985).

M. G. Giacobbe and X. Yang, J. Phycol. 35, 331–338 (1999).

C. Gomis, J. Alcober and A. Bernabeu, in: IV Reunion Ibérica de Fitoplancton Toxico y Biotoxinas St. Carles de la Ràpita (Tarragona): Generalitat de Catalunya, E. Matamoros and M. Delgado, eds. (Departament d'Agricultura, Ramaderia i Pesca), pp. 29–38 (1996).

E. L. Lilly, D. M. Kulis, P. Gentien and D. M. Anderson, J. Plankton Res. 24, 443–452 (2002).

A. Lugliè, M. G. Giacobbe, A. Sannio, F. Fiocca and N. Sechi, X OPTIMA 2000 Meeting (Organization for the Phyto-Taxonomic Investigation of the Mediterranean Area) Proceedings, in press.

R. Margalef and M. Estrada, Inv. Pesq. 51, 121–140 (1987).

P. Masselin, Z. Amzil, E. Abadie, E. Nézan, C. Le Bec, A. Carreras, C. Chiantella and P. Truquet, in: Harmful Algal Blooms 2000, G. M. Hallegraeff, S. Blackburn, C. J. Bolch and J. L. Lewis, eds. (UNESCO, Paris), pp. 26–29 (2001).

A. Penna, M. Magnani, E. Bertozzini, F. Andreoni, M. G. Giacobbe, E. Garcès, M. Vila, I. Bravo, S. Fraga and A. Lugliè, EUROHAB Cluster Workshop, Amsterdam, 17–18 March 2003.

A. Sannio, A. Lugliè and N. Sechi, Giorn. Bot. Ital. 130, 1037–1050 (1996).

A. Sannio, A. Lugliè and N. Sechi, Plant Biosystems 131, 73–78 (1997).

N. Sechi, L. Volterra, F. A. Aulicino, L. Bonadonna, G. Bagella, P. D'Amaddio, M. C. Muresu and G. Soggia, Igiene Moderna 88, 126–136 (1987).

J. D. H. Strickland and T. R. Parsons, Ver. int. Ver. theor. Angew. Limol. 9, 1–38 (1972).

M. Vila, Ph.D. Thesis, University of Barcelona, 1–179 (2001).

M. Vila, M. Delgado and J. Camp, in: Harmful Algal Blooms 2000, G. M. Hallegraeff, S. Blackburn, C. J. Bolch and J. L. Lewis, eds. (UNESCO, Paris), pp. 8–11 (2001).

M. Vila, E. Garcés, M. Masó and J. Camp, Mar. Ecol. Prog. Ser. 222, 73–78 (2001).

T. Wyatt and J. T. Carlton, in: Alien marine organisms introduced by ships in the Mediterranean and Black Sea, CIESM Workshop Monogr. 20, 41–46 (2002).

# Potentially Toxic Thecate Dinoflagellates of Middle Tyrrhenian Coastal Waters (Mediterranean Sea)

Roberta Congestri, Ilen Bianco, and Patrizia Albertano
*Department of Biology, University of Rome "Tor Vergata",*
*Via della Ricerca Scientifica, 00133 Rome, Italy*

## Abstract

The occurrence of 7 potentially toxic thecate dinoflagellates was detected during a monitoring program on phytoplankton community along the Middle Tyrrhenian coast (Latium, Italy). Various PSP and DSP toxin producers were identified in net and water samples from April 2000 to April 2001. Critical densities of *Dinophysis sacculus* were recorded in spring 2000. Observations also revealed the presence of *Alexandrium insuetum,* the first Italian report of this species, whose toxicity is still unknown.

## Introduction

Several HAB events have been reported for Italian coasts in recent decades, originally concentrated in northern Adriatic areas and then spread south and west (Honsell, 1999; Giacobbe *et al.,* 2000). Monitoring programs on marine phytoplankton were thus undertaken along Italian coasts with the aim of identifying and assessing abundance and distribution of harmful species. The present study was initiated after bloom events of the potentially toxic Raphidophycean *Fibrocapsa japonica* Toriumi et Takano occurred in summer 1999 along Latium southern coastal waters with the consequent closure of recreational and fishing activities for several weeks (Congestri *et al.,* 2000). Results are reported here on the temporal and spatial distribution of dinoflagellates in this area over a one-year period, focusing on potentially toxic thecate species, the identification of which was assessed both with light and electron microscopy.

## Materials and Methods

Samples were collected monthly at three coastal stations, Stn. 1 (41°18′N, 13°00′E), Stn. 2 (41°16′N, 13°15′E) and Stn. 3 (41°14′N, 13°44′E), located 300 m from the coastline, from April 2000 to April 2001. Surface (0.5 m depth) water samples were taken using a 2-L Niskin bottle. Using 20 µm mesh net, samples were horizontally collected and stored in 2.5% glutaraldehyde. Quantitative analysis was performed on 25 mL subsamples, preserved with Lugol's iodine solution, according to the Utermöhl method with an inverted microscope at 40× magnification. Qualitative analyses were conducted with a light microscope, equipped with differential interference contrast (DIC), and epifluorescence after 0.02% Calcofluor White staining of samples. Scanning electron microscopy (SEM) was performed on critical-point dried, gold-coated material. Species identification followed Schiller (1931–1937), Rampi and Bernhard (1980), Sournia (1986) and Steidinger and Tangen (1987).

## Results and Discussion

Dinoflagellate species composition was assessed in both water and net samples. A total of 83 taxa were identified, 67 at the species level. Past qualitative-quantitative analyses of phytoplankton along the study area are scanty and date back to 1980 (Massera Bottazzi *et al.,* 1980) so our qualitative data and estimates represent a first update. 7 planktonic species of potentially toxic thecate dinoflagellates were present in the samples, 3 of them (*Alexandrium minutum, Dinophysis fortii, Prorocentrum minimum*) detected for the first time. *Alexandrium minutum* Halim, a small PSP (Paralytic Shellfish Poison) producer widely distributed in the Mediterranean basin and responsible for PSP contamination in the northern Adriatic coastal waters (Honsell *et al.,* 1996), showed typical APC and ventral pore position at SEM. It was recorded from April to October, with peak density of 16,287 cell/L in August at Stn. 3, when it accounted for 25% of dinoflagellate community. Spring-summer occurrence in restricted embayments or in areas influenced by Garigliano River runoff reflects the typical seasonal dynamics of this species along Italian coasts (Honsell, 1999). DSP (Diarrhetic Shellfish Poison) producers, *Dinophysis caudata* Saville-Kent, *D. fortii* Pavillard, *D. sacculus* Stein and *Phalacroma rotundatum* (Claparède and Lachman) Kofoid and Michener, characterised by laterally compressed thecae, were identified with light microscopy on the basis of relative thecal size, shape and ornamentation. *Dinophysis sacculus* showed large morphological variability and was present with two distinct morphotypes in the samples: the sac-like and the dorsally concave type, namely *Dinophysis sacculus* f. *sacculus* (Fig.1a) and *D. sacculus* f. *reniformis* (Fig.1b), respectively (Zingone *et al.,* 1998), the former being present more frequently. *Dinophysis* spp. are common components of phytoplankton along Italian coasts (Honsell, 1999; Caroppo *et al.,* 2001) and are responsible for DSP outbreaks in the northern Adriatic and southern Tyrrhenian areas (Honsell, 1999; Giacobbe *et al.,* 2000). *Dinophysis sacculus, D. fortii* and *Phalacroma rotundatum* showed a winter-spring distribution, while *D. caudata* was present throughout the year. Association of the growth with winter environmental conditions was also observed for *Dinophysis sensu latu* populations of southern Adriatic coasts (Caroppo *et al.,* 2001). The negligible abundance of all species but *D. sacculus* in water samples could be due to the tendency of these dinoflagellates to accumulate in subsurface thin layers that might be overlooked during sampling. *Dinophysis sacculus* reached the highest density, 5,837 cell/L, in spring (May 2000) at Stn. 2, representing only 2% of dinoflagellate community, which



Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

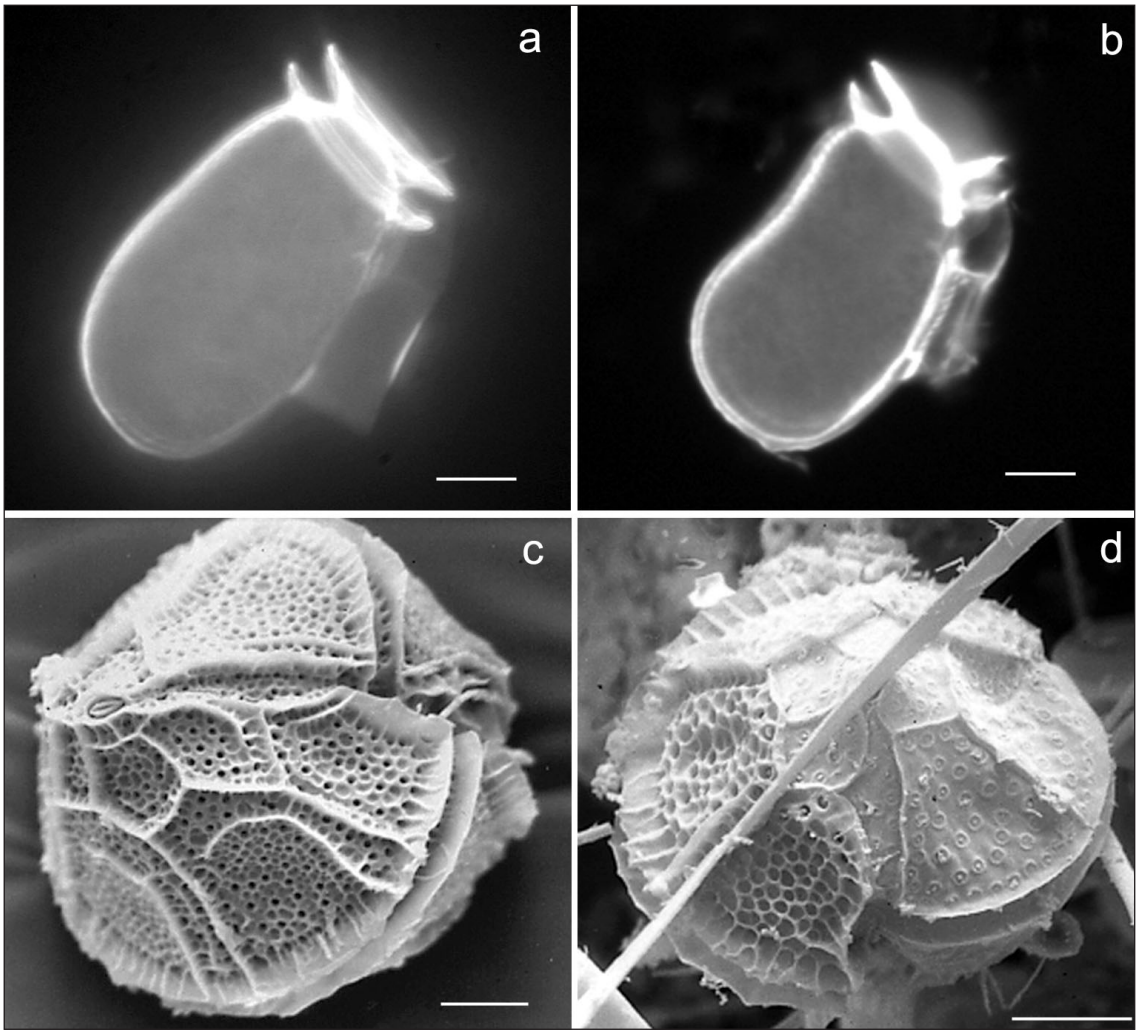


**Figure 1** Right lateral view of *Dinophysis sacculus* f. *sacculus* (**a**) and *D. sacculus* f. *reniformis* (**b**) seen under epifluorescence microscopy after Calcofluor White staining. SEM micrographs of *Lingulodinium polyedrum* epitheca showing the conspicuous reticulation, growth zones at sutures (**c**, right lateral ventral view) and recently divided cells from autumn samples (**d**, ventral view).

in turn was dominated by *Prorocentrum micans.* That density value was above the concentration limit of Italian guidelines, although no toxicity was detected by the local health authorities either in concentrated water samples or mussels. Among the Gonyaulacales, *Lingulodinium polyedrum* Stein showed markedly polyhedral, highly ornamented thecae in light microscopy. SEM observation allowed specific attribution to be confirmed on the basis of the tabulation pattern (Fig.1c). In addition, examination of autumn material revealed the presence of cells with less-developed parts of the thecae, probably indicating that desmoschisis had occurred (Fig.1d). This bloom-forming and widely distributed species has been recently associated with yessotoxin production, a DSP-like toxin extracted from northern Adriatic mussels (Draisci *et al.,* 1999). It was present throughout the year along the entire study area in low numbers, never exceeding 784 cell/L, detected in April 2000 at Stn. 2. *Prorocentrum minimum* (Pavillard) Schiller, distinguished from *P. balticum* by the papillae on thecal surface visible in SEM, was observed from November to April with a peak of 17,736 cell/L in November at Stn 1. The potential toxicity of this species is still under debate; some authors indicate this desmokont was responsible for a human syndrome caused by venerupin toxin, and others report its involvement in fish kill events and shellfish contamination (Landsberg, 2002).

Finally, small-sized (25 μm in width), oval thecae with reticulated surface pattern were abundantly observed in spring net samples at Stn. 1 (Fig. 2a). SEM observation allowed attribution of this dinoflagellate characterised by conic-convex epitheca, well-excavated, descending cingulum and 1' disconnected from Po plate (Fig. 2 b) to the species *Alexandrium insuetum* Balech, originally described from Korean and Japanese waters (Balech, 1995). In addition, the centrally located foramen appeared in SEM characteristically delimited by alveoli (Fig. 2c), while the thecal reticulation was visibly interrupted at all sulcal plates but the S.p. (Fig. 2d). Although included in a genus encom-

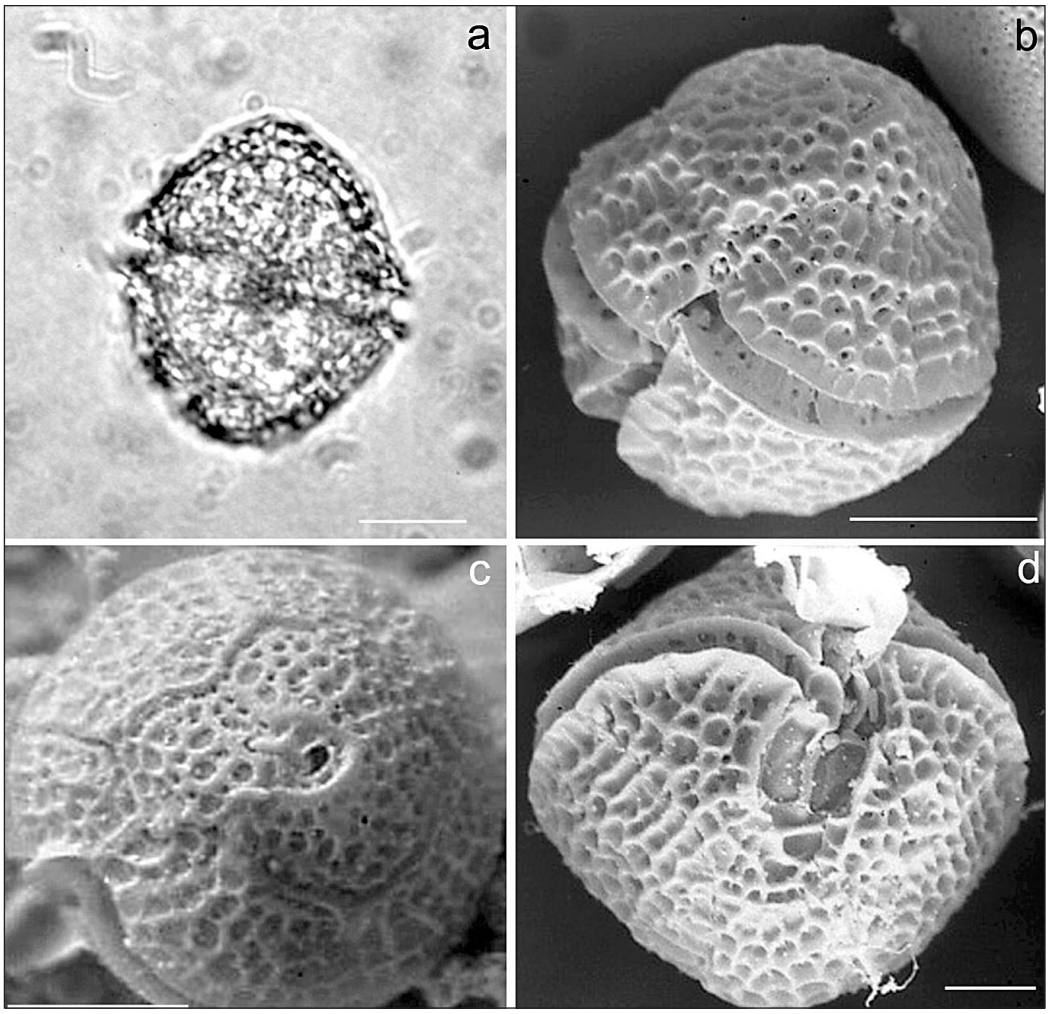


**Figure 2** *Alexandrium insuetum* light (**a**) and SEM micrographs showing highly reticulated oval theca, with characteristic 1' plate and well-excavated descending cingulum (**b**). Foramen ultrastructure is visible in (**c**, apical view) and smooth sulcal plates (S.d.p. and S.s.p.) in ventral hypothecal view (**d**).

passing several toxic species, the toxicity of *Alexandrium insuetum* is still unknown. It has previously been observed only twice in the Mediterranean basin, namely in southern French and northern African, Tunis Bay, waters (Daly Yahia-Kefi *et al.,* 2000), thus this represents the first report of *Alexandrium insuetum* for Italian seas.

## Acknowledgements

We acknowledge Dr. E. Zaottini and his team, ARPALazio-Department of Latina, for sample collection.

## References

E. Balech, The genus *Alexandrium* Halim (Dinoflagellata), Sherkin Island Marine Station (Sherkin Island Press, Ireland), pp. 1–151 (1995).

C. Caroppo, R. Congestri and M. Bruno, Cont. Shelf Research, 21, 1839–1854 (2001).

R. Congestri, P. Albertano, P. Ravizza, M. Le Foche, J. Caldarini and E. Zaottini, International Meeting of American Society of Limnology and Oceanography, Copenhagen, Denmark, SS21-P11 (2000).

O. Daly Yahia-Kefi, É. Nézan and M. N. Daly Yahia, Oceanol. Acta 24 (Suppl.), 17–25 (2000).

R. Draisci, E. Ferretti, L. Palleschi, C. Marchiafava, R. Poletti, A. Milandri, A. Ceredi and M. Pompei, Toxicon 37, 1187–1193 (1999).

M. G. Giacobbe, A. Penna, A. Ceredi, A. Milandri, R. Poletti and X. Yang, Phycologia 39(3), 177–182 (2000).

G. Honsell, Rapporti ISTISAN 99/8, 55–61 (1999).

J. H. Landsberg, Rev. Fish. Sci. 10(2), 113–390 (2002).

E. Massera Bottazzi, M.G. Andreoli and C. Andreoli, L'Ateneo Parmense, Acta Naturalia 16(4), 235–284 (1980).

L. Rampi and M. Bernhard, Chiave per la Determinazione delle Peridinee Pelagiche Mediterranee, C.N.E.N. Roma RT/B10 (80)8, pp. 1–193 (1980).

J. Schiller, Dinoflagellateae, Rabenhorst's Kriptogamen Flora Teil 1, Akademische Verlag, Leipzig, pp. 617 (1931–1937).

A. Sournia, in Atlas du Phytoplancton Marin, A. Sournia ed., Edition du CNRS (Paris), pp. 26–99 (1986).

K. A. Steidinger and K. Tangen, in: Identifying Marine Phytoplankton, C. R. Tomas ed. (Academic Press, San Diego), pp. 387–554 (1997).

Zingone, M. Montresor and D. Marino, Eur. J. Phycol 33, 259–273 (1998).

# Potentially Toxic and Harmful Phytoplankton Species Along the Coast of the Turkish Seas

Tufan Koray
*Marine Biology Section, Ege University, 35100, Bornova, Izmir, Turkey*

**Abstract**

Potentially toxic and harmful phytoplankton species along the Turkish coasts of the southern Black Sea, Sea of Marmara, eastern Aegean Sea, and the northeastern Mediterranean were detected between 1980–2002. Twenty-one potentially toxic microalgae were recorded: three cyanophytes that can cause hepatotoxic effects; fourteen dinoflagellates with a potential to cause Diarrhetic Shellfish Poisoning, Paralytic Shellfish Poisoning, Azaspiracid Poisoning, and yessotoxin-like poisoning symptoms; three diatoms, which can cause Amnesic Shellfish Poisoning; and a raphidophyte species. Harmful but non-toxic blooms were very frequent in the eutrophic Black Sea, Sea of Marmara, and the eastern Aegean coasts.

## Introduction

Microalgal blooms have been responsible for toxic or harmful events along the coasts of Middle Eastern countries since biblical times (Hallegraeff, 1995). Since early centuries to the present, human activities in the Mediterranean Sea have produced a measurable increase in anthropogenic inputs to the neritic waters. Since the Second World War, there has been an increase in the frequency of blooms reported due to eutrophication, with toxic and harmful algal blooms more widespread in the eastern Mediterranean, Aegean, and Black seas (Nümann, 1955). Until recently, only a few species of dinoflagellates were thought to produce red tides and harmful effects (Acara and Nalbantoglu, 1960). Studies on phytoplankton community structure have shown that more taxa might be responsible for blooms along the Turkish coasts of the Mediterranean and Black Sea (Bodeanu and Usurelu, 1979; Koray and Buyukisik, 1988; Friligos and Gotsis-Skretas, 1989; Koray 1990, 1992; Koray *et al.,* 1996; Fevzioğlu and Boran 1997; Fevzioğlu *et al.,* 2000; Polat *et al.,* 2000). The main goals of this paper are to (1)

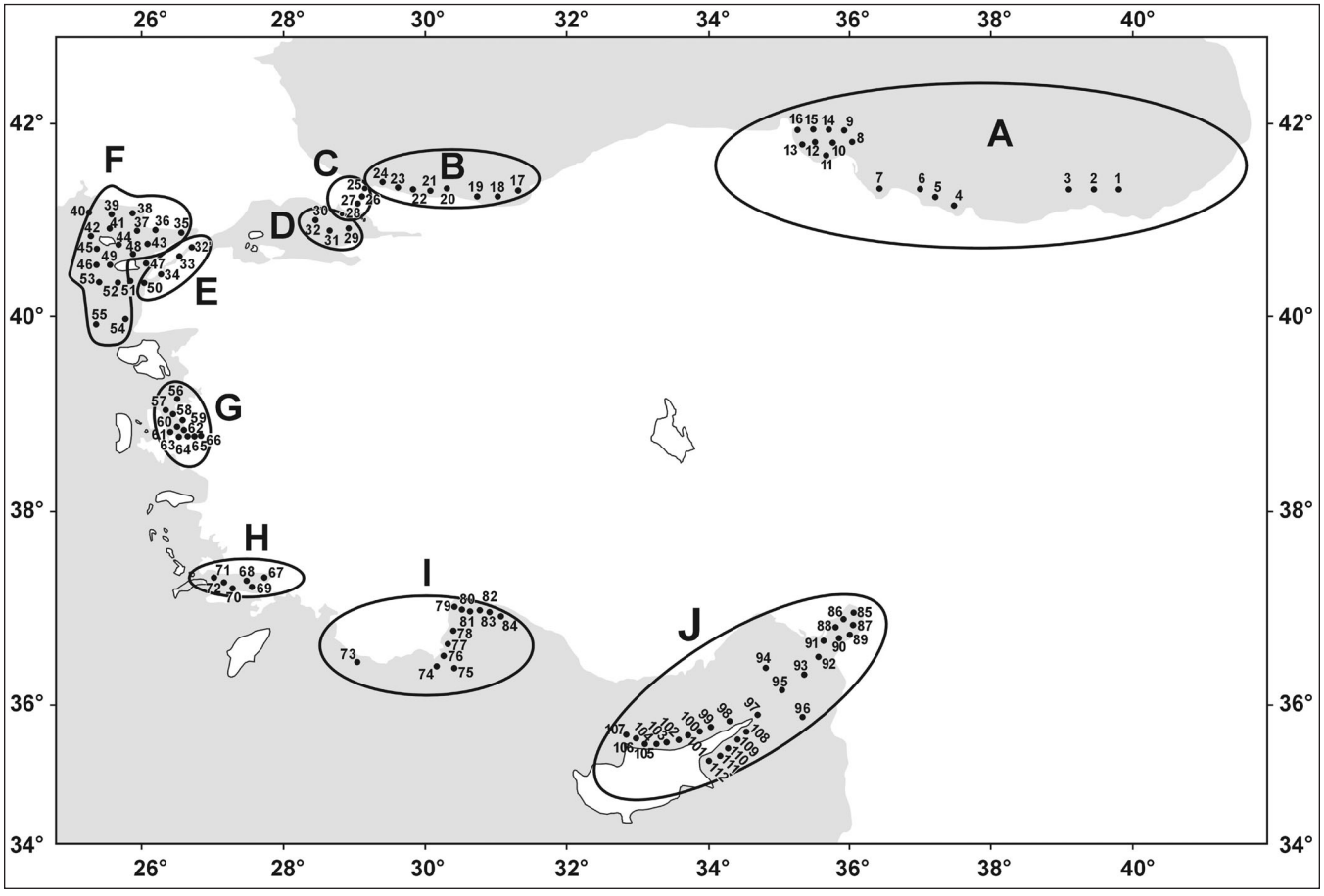


**Figure 1** Sampling stations (numbers) and localities (A, B, southern Black Sea; C–E; Turkish Straits [Sea of Marmara]; F–H, eastern Aegean Sea; I, J, northeastern Mediterranean). Sampling periods: A, 1993–1994; B–F, 1999–2003; G, 1980–2003; H, 1990–1991; I, 1999–2001; J, 1996–2003.

Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

**Table 1** Toxic and harmful microalgae from the Turkish coasts (abbreviations: mc–mean cell concentration observed (cells $L^{-1}$); T–potential toxicity; D–distribution, indicates the localities marked in Fig. 1; *–common; HT, hepatotoxic; MC, microcystin; PSP, Paralytic Shellfish Poisoning; DSP, Diarrhetic Shellfish Poisoning; ASP, Amnesic Shellfish Poisoning; AZP, Azaspiracid Poisoning; YTX, Yessotoxin; ho, Hyperoxia; ao, Anoxia; $NH_3$, ammonia; ?, unknown.

| Species | mc | T | D |
|---|---|---|---|
| CYANOPHYCEAE | | | |
| *Anabaena spiroides* Klebahn | ? | HT | H |
| *Anabaena variabilis* Kützing | $2.0 \times 10^5$ | HT | H |
| *Microcystis aeruginosa* Kützing em. Elenkin | ? | MC | C,D |
| DINOPHYCEAE | | | |
| *Alexandrium minutum* Halim | $10^7$ | PSP | G |
| *Dinophysis acuminata* Claperède and Lachmann | 26 | DSP | E,F |
| *Dinophysis acuta* Ehrenberg | 35 | DSP | E,F |
| *Dinophysis caudata* Saville-Kent | 50 | DSP | * |
| *Dinophysis fortii* Pavillard | 1 | DSP | * |
| *Dinophysis mitra* (Schütt) Abé vel Balech | 1 | DSP | D+H |
| *Dinophysis rotundata* Claperède and Lachmann | 32 | DSP | E+G |
| *Dinophysis sacculus* Stein | 24 | DSP | E+J |
| *Dinophysis tripos* Gourret | 5 | DSP | * |
| *Gonyaulax grindleyi* Rein | ? | YTX | G |
| *Lingulodinium polyedrum* (Stein) Dodge | $5.0 \times 10^4$ | YTX | G |
| *Noctiluca scintillans* (Macartney) Kofoid | $2.0 \times 10^4$ | NH3 | * |
| *Prorocentrum balticum* (Lohmann) Loeblich III | $9.0 \times 10^6$ | ho | A,B |
| *Prorocentrum cassubicum* (Woloszynska) Dodge | ? | DSP | G |
| *Prorocentrum dentatum* Stein | $6.0 \times 10^6$ | ho-ao | E+H |
| *Prorocentrum lima* (Ehrenberg) Stein | 10 | DSP | G |
| *Prorocentrum micans* Ehrenberg | $9.0 \times 10^7$ | ho-ao | * |
| *Prorocentrum minimum* Pavillard (Schiller) | ? | ? | E, H |
| *Protoperidinium crassipes* (Kofoid) Balech | ? | AZP? | E+H |
| *Scrippsiella trochoidea* (Stein) Loeblich III | $6.0 \times 10^6$ | ? | E+I |
| PRYMNESIOPHYCEAE | | | |
| *Emiliania huxleyi* (Lohmann) Hay and Möller | $10^6$ | ? | E+H |
| BACILLARIOPHYCEAE | | | |
| Biddulphiales | | | |
| *Rhizosolenia alata* f. *gracillima* (Cleve) Gran | $2.7 \times 10^6$ | ho-ao | C+J |
| *Rhizosolenia calcar-avis* Schultze | $1.7 \times 10^6$ | ho-ao | * |
| *Thalassiosira allenii* Takano | $10^6$ | ho-ao | A+G |
| *Thalassiosira anguste-lineata* (A. Schmidt) G. Fryxell and Hasle | $10^5$ | ho-ao | E+G |
| *Thalassiosira rotula* Meunier | $2.0 \times 10^4$ | ho-ao | E+G |
| Bacillariales | | | |
| *Pseudo-nitzschia delicatissima* (P. T. Cleve) Heiden | ? | ASP | * |
| *Pseudo-nitzschia pseudodelicatissima* (Hasle) Hasle | $2.0 \times 10^4$ | ASP | A+C |
| *Pseudo-nitzschia pungens* (Grunow ex P. T. Cleve) Hasle | $8.0 \times 10^6$ | ASP | * |
| RAPHIDOPHYCEAE | | | |
| *Heterosigma akashiwo* (Hada) Hada ex Hara (Chihara) | $2.0 \times 10^5$ | ? | G |
| EUGLENOPHYCEAE | | | |
| *Eutreptiella gymnastica* Throndsen | $7.0 \times 10^5$ | ho-ao | C+G |
| PRASINOPHYCEAE | | | |
| *Pyramimonas orientalis* McFadden, Hill and Wetherbee | $10^5$ | ho-ao | A |
| *Pyramimonas propulsa* Moestrup et Hill | $3.7 \times 10^7$ | ho-ao | G |

summarize the algal blooms along the Turkish coastline (northeastern Mediterranean, eastern Aegean, Marmara and southern Black Seas), (2) prepare a species list of potentially toxic and harmful microalgae and (3) present their distributions.

## Material and Methods

Samples were collected monthly or seasonally from 127 sampling points all along the Turkish coast between the years 1980–2002 during local research programs (Fig. 1).

Standard plankton nets with 55–60 μm mesh size were used for qualitative sampling, and samples were fixed with 40%, 1:9 neutral formaldehyde. For quantitative sampling, a 5-liter-capacity Hydro-Bios universal water sampler was preferred. The quantitative samples were fixed with standard Lugol's solution, 10 mL:1 L, and after a one-week sedimentation period they were concentrated gradually from one liter to one mL by reverse filtration with a Masterflex pump, and then transferred to one-mL capacity Eppendorf vessels. For the plate tabulation of armored dinoflagellates, hypochlorite solution, chloral hydrate and iodine-HI staining methods were preferred. The rapid acid cleaning method (1:4, $HNO_3 : H_2SO_4$) was used for frustule observations of diatoms using phase contrast microscopy and transmission electron microscopy. Live specimens were also investigated during the blooms, and identifications of small unarmored species of some of the classes, such as the Prasinophyceae, Raphidophyceae, and Euglenophyceae, were determined according to Balech (1995), Hasle and Syvertsen (1996), Steidinger (1996), and Throndsen (1996). Nomenclature was updated according to Moestrup *et al.* (2004).

## Results and Discussion

A total of 2,164 phytoplankton samples were collected from the Turkish coast during the last two decades. Of

these, 857 were net samples which could be preserved and examined. Potentially toxic or harmful species identified belong to six classes: Dinophyceae (20 species), Bacillariophyceae (8 species), Cyanophyceae (3 species), Prasinophyceae (2 species), Raphidophyceae (1 species), Prymnesiophyceae (1 species) and Euglenophyceae (1 species) (Table 1).

At least eight species of *Dinophysis* and two species of *Prorocentrum* are currently known to be toxic, however, these species have never caused fish kills on the Turkish coasts. There is little information on the effect of okadaic acid on aquatic animals along the Turkish coasts. Occasionally blooms of *Pseudo-nitzschia delicatissima* together with *P. pseudodelicatissima* have been reported from the southern Black Sea (Turkoğlu and Koray, 2002). Widespread *P. pungens* blooms have been observed along the eastern Aegean Sea and northeastern Mediterranean since 1980, while rarely, mass mortalities were noted in the Aegean Sea in 1991 (pers. comm. with local aquaculturists, the species was detected by TEM in fixed samples). There is no evidence for toxicity by yessotoxins and azaspiracid although the responsible species have been recorded in the area. Mass mortalities of aquatic organisms related to anoxic and hyperoxic conditions as a result of other harmful bloom species (Table 1) have been frequently observed in eutrophic zones along the Turkish coastline. The hyperoxia (17–20 ppm oxygen) at the surface water in the daytime and anoxia (down to zero ppm oxygen) during the night caused severe gas bubbling and oxygen depletion, respectively. The mass mortality during these blooms was attributed to high cell biomass, subsequent decaying of sedimented organic material, and the production of hydrogen sulfide. Bloom events have clearly increased during the last two decades in the Aegean Sea, Dardanelles, and Black Sea (Koray, 1987; Fevzioğlu and Tuncer, 1994). HAB species associated elsewhere with DSP and PSP are most prominent in these areas, but they were very rarely associated with harmful events and their cell concentrations were unknown. Harmful or toxic algal blooms are rare and very localized in the oligotrophic eastern Mediterranean where ASP is a more important risk factor than DSP and PSP. Biomonitoring studies for harmful and toxic species should be organized and continued routinely by responsible experts to prevent undesired effects on public health, tourism and aquacultural activities.

## References


A. Acara and U. Nalbantoglu, Rapp. P.-v. Reun. Cons. Int. Explor. Scient. Mer. Mèdit 15, 33–38 (1960).

E. Balech, Sherkin Island Press, Ireland, 151 pp. (1995).

N. Bodeanu and M. I. Usurelu, In: Toxic Dinoflagellate Blooms, D. L. Taylor and H. H. Seliger, eds. (Elsevier, New York), pp. 151–154 (1979).

A. M. Fevzioğlu and M. Boran, Turk. J. Biol. 21, 49–54 (1997).

A. M. Fevzioğlu, M.Boran and N. Sivri, Proceedings of First National Marine Science Conference, Z.Uysal and I. Salihoglu, eds. (Ankara), pp. 121–125 (2000).

A. M. Fevzioğlu and S. Tuncer, Turk. J. Biol. 18, 161–171 (1994).

N. Friligos and O. Gotsis-Skretas, Toxicol. Environ. Chem. 24, 171–180 (1989).

G. M. Hallegraeff, in: Manual on Harmful Marine Microalgae, G. M. Hallegraeff, D. M. Anderson and A. D. Cembella, eds., IOC Manual and Guides, No.3 (UNESCO, Paris), pp. 1–22 (1995).

G. Hasle and E. E. Syvertsen. in: Identifying Marine Diatoms and Dinoflagellates, C. Tomas, ed. (San Diego, Academic Press), pp. 5–385 (1996).

T. Koray, Turk J. Biol. 11, 130–146 (1987).

T. Koray, Rapp. Comm. Int. Mer Médit. 32, 212 (1990).

T. Koray, Harmful Algae News 2, 1–2 (1992).

T. Koray and B. Buyukisik, Rev. Int. Oceanogr. Med. 141/142, 25–43 (1988).

T. Koray, B. Buyukisik, H. Parlak, and S. Gokpinar, UNEP-MAP Tech. Rep. Ser. No. 104, 1–26 (1996).

Ø. Moestrup, G.A. Codd, M. Elbrächter, M.A. Faust, S. Fraga, Y. Fukuyo, G. Cronberg, Y. Halim, F.J.R. Taylor, and A. Zingone, IOC Taxonomic Reference List of Toxic Algae, Intergovernmental Oceanographic Commission of UNESCO; www.ioc.unesco.org/hab/data.htm, 91 pp. (2004).

W. Nümann, Hidrobiyoloji Mec. 3A, 2, 90–93 (1955).

S. Polat, E. Sarıhan, and T. Koray, Turk. J. Bot. 24, 1–12 (2000).

K. A. Steidinger, in: Identifying Marine Diatoms and Dinoflagellates, C. Tomas, ed. (San Diego, Academic Press), pp. 387–598 (1996).

J. Throndsen, in: Identifying Marine Diatoms and Dinoflagellates, C.Tomas, ed. (San Diego, Academic Press), pp. 7–146 (1996).

M. Türkoğlu and T. Koray, Turk. J. Bot. 26, 235–252 (2002).

# Distribution of *Prorocentrum lima* Epiphytic on Macroalgae in Patagonian Gulfs (Argentina)

Ana María Gayoso[1], Vanesa K. Fulco[1], and Cecilia I. Muglia[2]
[1]*Centro Nacional Patagónico, Consejo Nacional de Investigaciones Científicas y Técnicas, Boulevard Brown s/n. Casilla de Correo 128, 9120 Puerto Madryn, Chubut, Argentina;* [2]*Cátedra de Bioquímica Patológica, Facultad de Cencias Exactas, Universidad Nacional de La Plata*

## Abstract

An intensive spatial survey was carried out at five sites in the gulfs of San José and Nuevo, Patagonia, Argentina. Macroalgae and associated epibiota were collected by diving at depths ranging from 8 to 15 meters. Preliminary results indicated that *Prorocentrum lima* is widespread and abundant in the area. Higher densities (up to $3.4 \times 10^3$ *P. lima* cells/g wet weight of macroalgae) were observed on *Ceramium rubrum* (Rhodophyta) and on *Dictyota dichotoma* (Phaeophyta) at two Golfo Nuevo stations characterized by a weak slope and relatively calm waters. Lower densities (from detectable to <500 cells/g wet weight) were found on *Codium vermicularum, C. decorticatum* (Chlorophyta), and *Undaria pinnatifida* (Phaeophyta) at sites with a strong slope and more turbulent waters. Cell extracts were positive for DSP-like activity using the colorimetric protein phosphatase inhibition assay.

## Introduction

In March 1999, an episode of shellfish-associated gastroenteritis affecting at least forty people occurred in the city of Puerto Madryn (Chubut), Argentina. *Prorocentrum lima,* a globally distributed benthic dinoflagellate associated with the synthesis of diarrhetic shellfish poisons (Steidinger, 1983), was identified as the most probable causative organism. Dinophysistoxin-1 (DTX-1) was previously detected in hydrolyzed extracts from two mussels, yielding 21.2 ng DTX-1/g and 94.0 ng DTX-1/g whole tissue (Gayoso *et al.,* 2002). Following the incident, an intensive spatial survey was carried out to determine the distribution and abundance of *P. lima* in the gulfs of San José and Nuevo, Patagonia. In addition, a protein phosphatase inhibition assay for DSP toxins (okadaic acid and derivatives) was performed in some samples that were characterized by high densities of *P. lima.*

## Materials and Methods

Dominant macroalgae were collected bimonthly from February through October 2001 by diving (8–15 meters depth) at several stations around Golfo Nuevo and Golfo San José (Fig. 1) to examine their epiphytic communities and to quantify *P. lima* cells.

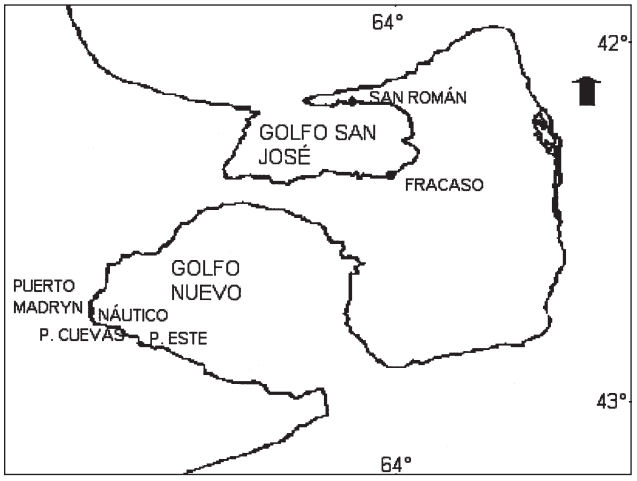


**Figure 1** Map of the North Patagonian gulfs showing sampling sites.

The macroalgae species were analyzed separately. Subsamples (2–6 g wet weight) were placed in a tube containing a solution of formaldehyde-filtered seawater. The suspended epiphytic dinoflagellate populations were examined,and their abundance [cells per gram wet weight of macroalga (FW)] was estimated from counts using a 1-mL Sedgwick Rafter chamber (Morton *et al.,* 1999).

The colorimetric protein phosphatase inhibition assay, which tests the ability of okadaic acid standard or an unknown sample to inhibit activity of purified protein phosphatase (Morton *et al.,* 1999), was carried out on eight samples (five from Náutico and three from Cuevas) characterized by high *P. lima* densities.

## Results

Eleven different species of macroalgae from three divisions—Rhodophyta, Phaeophyta and Chlorophyta (Table 1)—and two hundred samples were examined. Important differences were found between sample stations; the highest abundance of *P. lima* was found at station Náutico, situated on the west coast of Golfo Nuevo, while lower densities were found at the stations Fracaso (Golfo San José) and Punta Este (Golfo Nuevo).

At the station Náutico in April 2001, the maximum cell density of *P. lima* recorded was 3,433 cells/g FW macroalga on *Ceramium rubrum,* followed by *Cladophora* sp. (2,523 cells/g FW), *Anotrichium furcellatum* (1,892 cells/g FW) and *Dictyota dichotoma* (1,677 cells/g FW) (Table 1).

The analyzed samples displayed protein phosphatase inhibitory activity using the colorimetric assay. The extract concentration ranged from 23.7–143.3 pg OA equivalent/cell (Table 1). In a clonal culture of *P. lima* isolated from Golfo Nuevo, toxin concentration was 36.5 pg OA equivalent/cell.

High *P. lima* densities were found during late summer and fall (March–May), and densities declined during the winter (Fig. 2).



Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

**Table 1** *Prorocentrum lima* on dominant macroalgal species at the sampling stations Náutico and Cuevas in Golfo Nuevo from February–October 2001. Average *P. lima* concentration on macroalgae, average abundance, maximum abundance and toxicity by protetin phosphatase assay.

| Macroalgae | Average (cells/g FW) | Max. Abundance (cells/g FW) | OA Equivalent (pg/cell) |
|---|---|---|---|
| **Station Náutico** | | | |
| *Ceramium rubrum* (Hudson) C.Agardh | 754.7 | 3,433.3 | 90.40 |
| *Dictyota* aff. *dichotoma* (Hudson) Lamouroux | 498.9 | 1,677.3 | 143.30 |
| *Ulva rigida* (C.Agardh) Thuret | 41.1 | 157.8 | |
| *Undaria pinnatifida* (Harvey) Suringar | 51.4 | 236.3 | |
| *Codium vermilara* (Olivi) Delle Chiaje | 347.6 | 1,546.0 | 23.70 |
| *Codium decorticatum* | 290.9 | 451.4 | |
| *Polysiphonia argentinica* Taylor | 32.6 | 65.2 | |
| *Cladophora* sp. | 1,221.6 | 2,522.7 | 50.43 |
| *Anotrichium furcellatum* (J.Agardh) Baldock | 1,240.9 | 1,892.0 | 36.50 |
| *Lomentaria* sp. | 24.9 | 40.0 | |
| *Callithamnion* sp. | 11.5 | 11.5 | |
| **Station Cuevas** | | | |
| *Ceramium rubrum* (Hudson) C.Agardh | 273.3 | 1,165.0 | 30.50 |
| *Dictyota* aff. *dichotoma* (Hudson) Lamouroux | 540.5 | 3,116.0 | 90.45 |
| *Ulva rigida* (C.Agardh) Thuret | 5.3 | 16.1 | |
| *Undaria pinnatifida* (Harvey) Suringar | 14.4 | 79.5 | |
| *Codium vermilara* (Olivi) Delle Chiaje | 73.3 | 290.9 | |
| *Polysiphonia argentinica* Taylor | 90.9 | 283.3 | |
| *Cladophora* sp. | 33.3 | 33.3 | |
| *Anotrichium furcellatum* (J.Agardh) Baldock | 438.3 | 1,625.0 | 24.10 |
| *Lomentaria* sp. | 0.8 | 2.4 | |

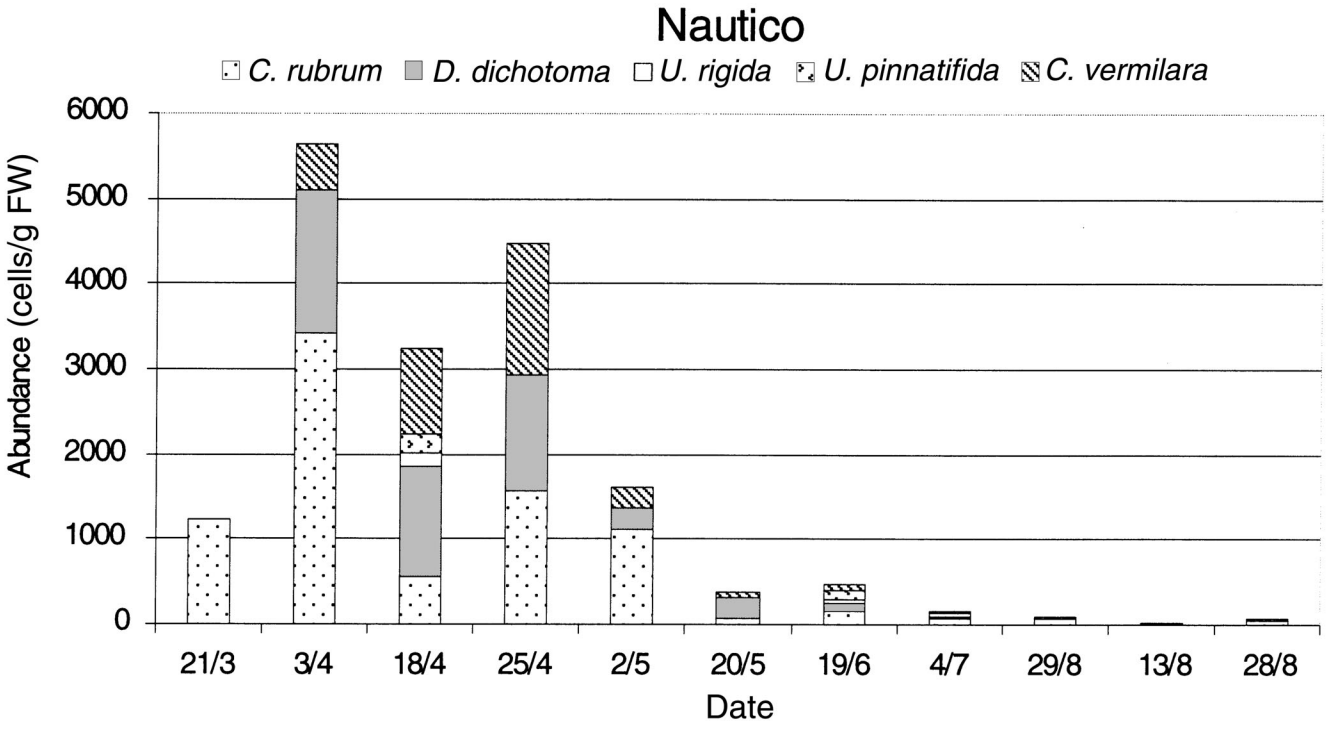


**Figure 2** Relative abundance of *Prorocentrum lima* on dominant macroalgal species at the Station Náutico.

## Discussion

Cell concentrations of *P. lima* found in the Patagonian gulfs were higher than those found along the coast of Maine (Hurst and Van Dolah, 1999) and were similar to densities reported by Vila *et al.* (2001) in Costa Brava, NW Mediterranean.

Epiphytic dinoflagellates are not host-specific (Bomber *et al.,* 1989). *Prorocentrum lima,* a dinoflagellate considered to be epiphytic (Faust, 1995), was found in the Golfo Nuevo and Golfo San José in close association with a variety of macroalgae including Chlorophyta, Phaeophyta and Rhodophyta. Surface area is an important factor in determining the abundance of ciguatera dinoflagellates (Bomber *et al.,* 1989). In this study, *P. lima* tended to be more abundant on the surface of filamentous seaweed, while smaller densities were observed on laminar seaweed, confirming that the epiphytic dinoflagellates prefer high-surface area algae rather than a particular macroalgal species (Vila *et al.,* 2001). Other sources of variability were the hydrographic features of the sampling sites; a maximum abundance of the species was found in the Golfo Nuevo at St. Nautico, a place characterized by a weak slope and relatively calm waters, whereas Punta Este, a station characterized by a strong slope and turbulent waters, had lower densities of *P. lima.*

The toxin responsible for the March 1999 diarrhetic poisoning episode following shellfish consumption was established to be DTX-1, and the potential source of the toxin was *P. lima* (Gayoso *et al.,* 2002). The high density of the species associated with macroalgae in the Patagonian gulfs, and the positive results presented here for DSP-like activity of the cell extracts, also corroborate the association of DSP with *P. lima* in the area.

## Acknowledgments

This study was supported by funds from ANPCyT, Argentina, PICT/98 0103709. M. L. Piriz made the identification of macroalgal species.

## References

J.W. Bomber, M.G. Rubio and D.R. Norris, Phycologia 28, 360–368 (1989).

M.A. Faust, in: Harmful Marine Algal Blooms, P. Lassus, G. Arzul, Erard-Le Denn, P. Gentien and C. Marcaillou-Le Baut, eds., (Lavoisier, Paris), pp. 847–854 (1995).

A.M. Gayoso, S. Dover, S. Morton, M. Busman, P. Moeller, V.K. Fulco and L. Maranda, J. Shellfish Res. 21, 461–463 (2002).

J.W. Hurst, Jr. and F.M. Van Dolah, J. Shellfish Res. 18, 681–686 (1999).

S.L. Morton, T.A. Leighfield, B.L. Haynes, D.L. Petitpain, M.A. Busman, P.D.R. Moeller, L. Bean, J. McGowan, K. Steidinger, in: Progress in Phycological Research, F. E. Round and D. J. Chapman, eds. (Elsevier, New York), pp. 147–188 (1983).

M. Vila, E. Garcés and M. Masó, Aquat. Microb. Ecol. 26, 51–60 (2001).

# Twenty-Three Years of Red Tide Monitoring at Fixed Stations Along the Coast of Uruguay

Silvia M. Méndez and Dinorah Medina
*National Direction of Aquatic Resources (DINARA), Constituyente 1497, 11200 Montevideo, Uruguay*

## Abstract

In 1980, the National Harmful Phytoplankton and Mussels Toxicity Monitoring Program in Uruguay began to monitor toxic phytoplankton and Paralytic Shellfish Poisoning (PSP) toxins in wild molluscs. It is the oldest red tide monitoring program in South America. The coastal stations have been the same from the beginning, but the toxin determination methodology has been improved and complemented. Diarrhetic Shellfish Poisoning (DSP) was confirmed in 1992, and the HPLC technique for detection of Amnesic Shellfish Poisoning (ASP) was implemented in 2000. During cyanobacteria blooms along the Rio de la Plata coast, some microcystin determinations were done by mouse bioassay. The highest PSP toxicity values were associated with *Alexandrium tamarense* blooms in the winter–spring and with *Gymnodinium catenatum* blooms in the summer–fall. These species were responsible for shellfish bed closures during 1980, 1991–1994, 1996, 1998, and 2001 when cell concentrations reached a maximum of 80,000 cells/L and 278,000 cells/L, respectively. Highest PSP toxicities were observed in the blue mussel, *Mytilus edulis,* recorded from Punta del Este beach in 1991 (8,285 µg STX equiv/100 g), and in cockles, *Donax hanleyanus,* from La Paloma in 1992 (1,478 µg STX equiv/100 g). Several reported DSP events were associated with *Dinophysis* species between 1992–1996. The first ASP detection occurred in December 2001.

## Introduction

The National Direction of Aquatic Resources (DINARA) is the responsible institution for sanitary emergencies that could affect human health, including the appearance of a red tide, other blooms or pathogenic organisms, contaminants in water, or from the human consumption of aquatic species. DINARA can adopt emergency measures for public health safety and communicates these afterward to the Executive Power and to other public organizations such as the Public Health and Toxicology Centre. When toxicity in molluscs exceeds the accepted international limits, a harvesting ban is immediately established to prohibit shellfish commercialisation and to avoid human intoxication from their consumption.

In 1980, the National Harmful Phytoplankton and Mussels Toxicity Monitoring Program in Uruguay began to monitor harmful phytoplankton and toxicity in wild molluscs.

## Materials and Methods

The coastal stations are located in Piriápolis, Punta del Este, La Paloma, and Punta del Diablo (close to natural beds of *Mytilus edulis* and *Donax hanleyanus*), while the offshore stations are in areas where the Patagonian scallop, *Zigochlamys patagonica* (V), and clams *Pitar rostrata* (A1-A3, B1-B3 and C1-C3) are harvested (Fig. 1).

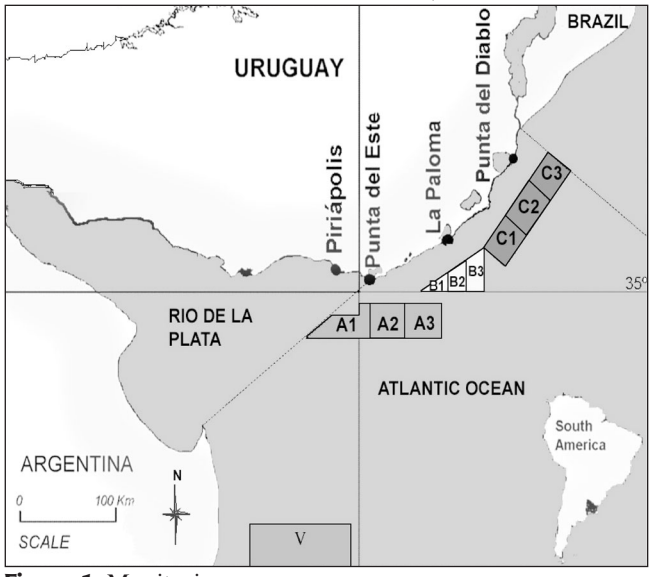


**Figure 1** Monitoring areas.

Harmful phytoplankton is quantified weekly according to the Utermöhl method (1958), whereas the toxicity in molluscs is determined weekly in the spring–summer and every fifteen days in the fall–winter. PSP and DSP are determined by mouse bioassay, and ASP is determined by HPLC following international recommendations. When water discoloration is detected close to the mollusc exploitation area by the local population or from aerial observations by the air force, then DINARA technical assistants collect additional samples.

## Results and Discussion

PSP toxins were not detected between 1980 and 1991. Fig. 2 shows the change in the abundance of *Alexandrium tamarense* since 1991.

During spring 1991, the first *A. tamarense* bloom was reported at the Uruguayan coast with a maximum of 31,000 cell/L and 8,285 µg STX/100 g in shellfish. Since that time, in 1992, 1993, and 1996, several toxic blooms of *A. tamarense* have been reported (Brazeiro *et al.,* 1997; Méndez and Ferrari, 2002) (Table 1).

*Alexandrium tamarense* has produced toxicity along the Argentinean coast since 1980. It is thought that this species reaches high concentrations in the western Atlantic associated with subantarctic waters of the Buenos Aires platform

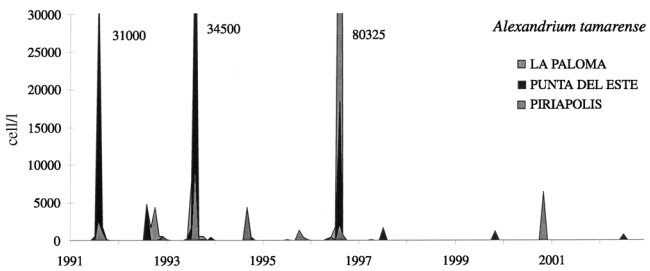


**Figure 2** *Alexandrium tamarense* blooms at three locations in Uruguay from 1991 to 2002.

Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

**Table 1** PSP outbreaks associated with *A. tamarense* in winter–spring 1980–2002. (nd = no data)

| Year | Maximum PSP toxicity winter–spring (μg STX/100 g) | Maximum bloom density cell/L | Salinity ppt | Temp. °C |
|---|---|---|---|---|
| 1980 | 123.0 | nd | – | – |
| 1991 | 8285.0 | 31,000 | 25.2 | 14 |
| 1992 | 97.6 | 4,300 | 24.2 | 16 |
| 1993 | 858.0 | 10,000 | – | 11 |
| 1994 | nd | – | – | – |
| 1995 | nd | – | – | – |
| 1996 | 2300.0 | 80,000 | 27 | 12.6 |
| 1997 | 56.7 | 160 | 24 | 13 |
| 1998 | nd | – | – | – |
| 1999 | 133.0 | nd | – | – |
| 2000 | 51.4 | nd | – | – |
| 2001 | 54.9 | nd | – | – |

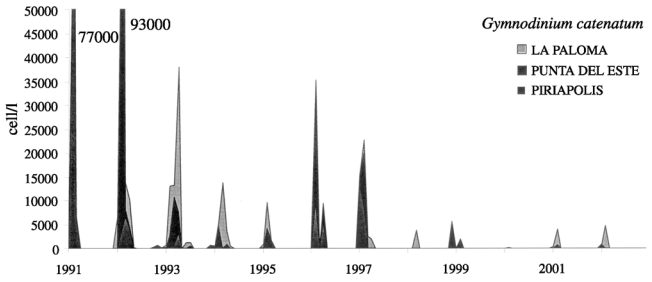


**Figure 3** *Gymnodinium catenatum* blooms at three locations in Uruguay from 1991 to 2002

and is then transported north to Uruguay (Brazeiro *et al.,* 1997; Carreto *et al.,* 1998). The occurrence of *A. tamarense* on the Uruguayan coast depends upon oceanographic factors and upon discharge from the Río de la Plata. In winter, as a consequence of the subtropical convergence north–south oscillation, subantarctic waters reach Uruguayan latitudes. When the river discharge decreases, oceanic waters are transported into the estuarine system of the Río de la Plata where *A. tamarense* blooms come into contact with the natural mollusc beds (Méndez *et al.,* 1996).

In the summer of 1992, the first toxic *G. catenatum* bloom was reported in Uruguayan waters, when *Donax hanleyanus* reached toxicity levels of 1,478 μg STX/100 g and *Mytilus edulis* 387 μg STX/100 g. This was the first toxic bloom of *G. catenatum* in the southwestern Atlantic, although this species was previously reported in the region by Balech (1964). The abundance of *G. catenatum* from 1991–2002 is shown in Fig. 3.

Table 2 shows that all PSP outbreaks in summer–fall of 1993, 1994, 1996, and 1998 were associated with this species. *Gymnodinium catenatum* reached the highest abundance at water temperatures between 22° and 24°C, characteristic of the summer–fall oceanographic conditions at this latitude. This species is absent in the plankton during the rest of the year when the temperature decreases. The presence of resting cysts of *G. catenatum* in the sediments could be the source for future blooms (Méndez *et al.,* 2003).

*Dinophysis acuminata* is a common species all year; higher abundances are reported at the oceanic locations of Punta del Este and La Paloma (Ferrari *et al.,* 2000) (Fig. 4).

Some DSP-positive results were reported in 1992, 1994, and 1996 (Table 3). Although some impressive blooms of this species were reported in September 2002 (112,000 cell/L), no DSP toxicity was associated with this event.

Cyanobacteria blooms are very frequent at the Río de

**Table 2** PSP outbreaks associated with *G. catenatum* in summer–fall 1980–2002. (nd = no data)

| Year | Maximum PSP toxicity summer–fall (μg STX/100 g) | Maximum bloom density (cell/L) | Salinity ppt | Temp °C |
|---|---|---|---|---|
| 1980 | 1251.0 | nd | | |
| 1991 | 42.0 | 77,000 | 16.5 | 23.5 |
| 1992 | 1478.0 | 146,000 | 31.4 | 23 |
| 1993 | 396.0 | 10,400 | 29.6 | 24 |
| 1994 | 867.0 | 63,500 | 31.2 | 22.5 |
| 1995 | 126.0 | 8,000 | 29.3 | 22 |
| 1996 | 73.0 | 24,000 | 32 | 23 |
| 1997 | nd | – | – | – |
| 1998 | 167.0 | 278,000 | 19 | 23 |
| 1999 | 69.9 | – | – | – |
| 2000 | nd | – | – | – |
| 2001 | 154.0 | nd | – | – |
| 2002 | 60.3 | 160 | 30.5 | 23 |

**Table 3** DSP reports 1992–1996.

| Date | Location | DSP-positive molluscs | Associated *Dinophysis* sp. | Density (cell/L) | Salinity ppt | Temp. ºC |
|---|---|---|---|---|---|---|
| 07/02/92 | Piriápolis | *M. edulis* | *D. acuminata* | 1,500 | 31.3 | 25.0 |
| 07/02/92 | P. del Este | *M. edulis* | *D. caudata* | 40 | 30.2 | 26.0 |
| 07/02/92 | La Paloma | *M. edulis* | *D. acuminata* | 2,000 | 26.8 | 25.0 |
| 21/01/92–07/02/92 | P. del Diablo | *M. edulis* | *D. acuminata* | 4,500 | 31.5 | 22.0 |
| 13/12/94 | Chuy | *Mesoderma mactroides* | *D. caudata* | 4,600 | 26.8 | 22.0 |
| 16/02/96–08/03/96 | La Paloma | *D. hanleyanus* | *D. acuminata* | 80 | 31.4 | 22.7 |
| 18/02/96 | La Paloma | *D. hanleyanus* | *D. acuminata* | 80 | 32.3 | 24.0 |

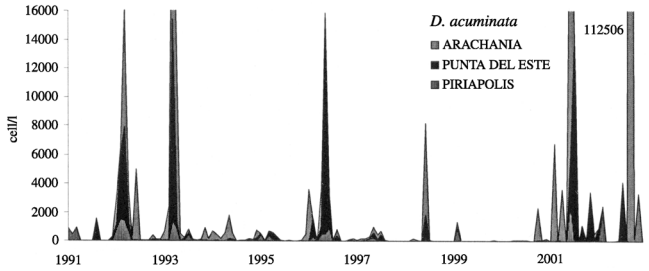


**Figure 4** Blooms of *D. acuminata* in Uruguay.

la Plata, especially in protected areas, where a bright green scum of *Microcystis aeruginosa* can be seen in the summer. Toxicity of this species was reported in the summer of 1997 when the lethal dose (LD50) was 233 and 203 mg/Kg mouse as determined by the mouse bioassay (done with the collaboration of J. Yunes, FURG Brazil). Another toxic *M. aeruginosa* bloom was reported in February 1999 at the Río de la Plata river coast (Department of Colonia). The microcystin level was estimated to be 100–1,000 µg/L by the Faculty of Sciences of Uruguay (De León, 1999). *Trichodesmium erythraeum* is a globally distributed neurotoxin producer that bloomed in the Río de la Plata with brown water discoloration in March 1993 and at La Paloma beach in February 2002. During the last event, red water discoloration was detected with a maximum of 60,000 trichomes/L, but no toxicity was detected in mussels, nor were humans swimming in the bloom affected (Table 4).

*Pseudo-nitzschia multiseries* was associated with the first ASP event at Punta del Este beach in December 2001when low levels of domoic acid were detected. Salinity was 25.5 ppt and the temperature 20.5°C.

These are the main results from the Uruguayan monitoring program for harmful algae and toxicity in mussels. The program has a preventive role. The development of complementary research has allowed us to confirm some toxic species (Méndez *et al.,* 2002), and to know the high-risk periods for these toxic blooms. However, it is recommended that decision-makers promote new avenues for research and invest in new, advanced techniques to continue providing safe seafood to the consumer population.

## References


E. Balech, in: Bol. Inst. Biol. Mar. 4, 1–49 (1964).

A. Brazeiro, S. M. Méndez and G. Ferrari, Rev. Atlántica 19, 19–29 (1997).

J. I. Carreto, N. Montoya, A.D. Cucchi Colleoni and R Akselman, in: Harmful Algae, B. Reguera, J. Blanco, M.L. Fernandez and T. Wyatt, eds. (UNESCO, Paris), pp. 131–134 (1998).

L. De León, Res. XIV Sim. Cien. Tec. Com. Tec. Mix. Fr. Marit. 61–62 (1999).

G. Ferrari, S. M. Méndez, and A. Brazeiro, Pub. Com Tec. Mix. Fr. Marít. 18, 91–95 (2000).

S. Méndez and G. Ferrari, in: Floraciones Algales Nocivas en el Cono Sur Americano, E.A. Sar, M. E. Ferrario and B. Reguera, eds. (IEO,Vigo), pp. 269–289 (2002).

S. M. Méndez, G. Ferrari and S. Svensson, Pub.Com Tec. Mix. Fr. Marít. 19, 103–110 (2003).

S. M. Méndez, D. Kulis and D. M. Anderson, in: Harmful Algal Blooms, G. M. Hallegraeff, S. Blackburn, C. J. Bolch and R. Lewis, eds. (UNESCO, Paris), pp. 352–355 (2002).

S. Méndez, D. Severov, G. Ferrari and C. Mesones, in: Harmful and Toxic Algal Blooms, T. Yasumoto, Y. Oshima and Y. Fukuyo, eds. (UNESCO, Paris), pp. 113–116 (1996).

H. Utermöhl, Limnol. 9, 1–38 (1958).


**Table 4** Cyanobacteria blooms in coastal waters of Uruguay. (nd = no data)

| Date | Location | Species | Toxicity | Salinity ppt | Temp. °C |
|---|---|---|---|---|---|
| 3/1990 | Laguna de Castillos | *Nodularia baltica-spumigena* | nd | nd | nd |
| 16/03/92 | Montevideo | *Microcystis aeruginosa* | nd | nd | nd |
| 27/01/94 | M'deo-P. del Este | *Microcystis aeruginosa* | nd | 2.0 | 23 |
| 30/01/95 | La Paloma | *Trichodesmium erythraeum* | nd | 29.2 | 23.5 |
| 30/01/96 | 35°40′S, 54°26′W | *Nodularia baltica-spumigena* | nd | nd | 18.0 |
| 30/01/97 | Colonia-P. del Este | *Microcystis aeruginosa* | $LD_{50}$ 233.3 mg Kg/mouse | nd | nd |
| 29/03/97 | Portezuelo (P. del Este) | *Microcystis aeruginosa* | $LD_{50}$ 203.1 mg Kg/mouse | | |
| 11/01/00 | P. del Este-C. Polonio | *Trichodesmium erythraeum* | nd | 32.3 | 22.0 |
| 24/01/01 | Mercedes, Soriano | *Microcystis aeruginosa* | | | |
| 14/02/02 | La Paloma | *Trichodesmium erythraeum* | nd | 30.6 | 26.5 |

# Dominance and Permanence of Species of Harmful Algae Forming Blooms in Mazatlán Bay, Mexico (1979–2002)

Roberto Cortés Altamirano[1], Arturo P. Sierra-Beltrán[2], and Maria del Carmen Cortés Lara[3]
[1]*Unidad Académica Mazatlán, Instituto de Ciencias del Mar y Limnología-Universidad Nacional Autónoma de México (ICMyL-UNAM). Apdo. Postal 811, 82040 Mazatlán, Sin. México;* [2]*Laboratorio de Genética Molecular, Centro de Investigaciones Biológicas del Noroeste, S.C. (CIBNOR), Apdo. Postal 128, 23000 La Paz, B.C.S., México;* [3]*Centro Universitario de la Costa, Campus Puerto Vallarta-Universidad de Guadalajara (U de G), 48280, Jal., México*

## Abstract

Discoloration events caused by harmful algal blooms (HABs) in marine surface waters have been recorded during the last 23 years in Mazatlán Bay, Mexico. Observation of 104 HAB discoloration events with 590 days of discoloration were caused by 12 dominant species: six toxic or harmful species—*Gymnodinium catenatum, Akashiwo sanguinea, Cochlodinium catenatum, Noctiluca scintillans, Ceratium dens* and *C. furca* and six benign species—*Mesodinium rubrum, Scrippsiella trochoidea, Protoperidinium quinquecorne, Prorocentrum balticum, P. dentatum* and *P. triestinum.* With the exception of *M. rubrum,* discoloration events have not been observed during "El Niño." However, the number of HABs as well as the number of causative species increased after each major "El Niño" event. *Gymnodinium catenatum* is common during March and April, *A. sanguinea* during May and June, and *Cochlodinium catenatum* during September and October. Seasonality has not been observed in any other species. *Mesodinium rubrum* and *S. trochoidea* do not have a marked seasonality, whereas some species such as *Prorocentrum triestinum* and *Protoperidinium quinquecorne* have been observed only once. Historically, only 1985, 1997 and 2000 recorded more than 50 days of discoloration. The occurrence of events lasting more than one month seems to be a common phenomenon since 2000. The number of discoloration days per year increased two to three times during the last five years, reaching 273 days or 46% of those observed in 23 years. Until 1988, discoloration events were uncommon during summer and autumn, but now this is not the case. Results suggest that in this region HABs have increased both in the number of species involved as well as in the duration of the events.

## Introduction

Previous contributions have remarked on the importance of gaining knowledge about the variability of discoloration events, including their permanence, as a basis for understanding and predicting their trends (Cortés-Altamirano and Nuñez-Pasten, 1992; Cortés-Altamirano *et al.,* 1999). The data presented here, gathered by a long-term project currently ongoing in Mazatlán Bay, México, allowed for determination of the timing of HAB events caused by several species, their permanence and frequency and, more importantly, allowed for decision making to prevent human poisoning.

## Materials and Methods

Data were collected by daily observations and by surface sampling of discolorations in the coastal waters from 1979 to 2002 (Cortes-Altamirano, 1998). Blooms that did not discolor the waters or that did not change its ecological properties in any obvious way were not recorded; for example, diatom blooms were not detected. Continuous days of discoloration were registered as a permanent red tide. When the blooms were observed on some days and not others, and were absent for less than a week, they were registered as an intermittent red tide. When surface discoloration was absent for more than a week, they were considered to be different events. Blooms with high densities of organisms by visual inspection were sampled on the surface of the area and samples were fixed with Lugol's-acetate (1:100). All of the sampling stations were localized in the Mazatlán Bay area (Cortés-Altamirano, 1987; Cortés-Altamirano and Nuñez-Pasten, 1991), mainly in the Dos Hermanos islands region (Fig.1), near where the submarine sewage pipeline is operating. Phytoplankters were identified and quantified after sedimentation for 24 hours of 1, 10, or 50 mL fixed samples (Hasle, 1978), depending on the density of the organisms (detailed data not presented here).

## Results and Discussion

During the study it was found that 12 species were

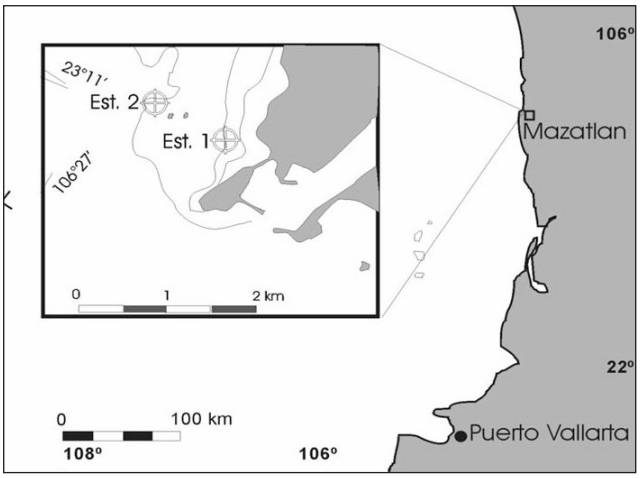


**Figure 1** Study area where the inset shows the region of major HAB events and the two sampling stations.



Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

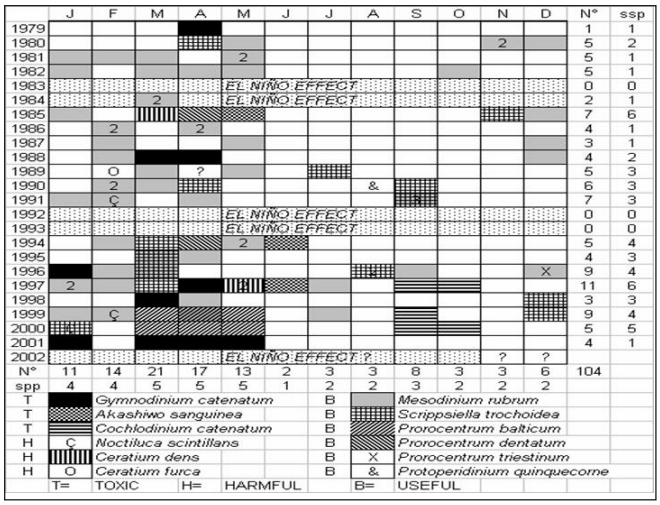


**Table 1** Occurence of HABs in Mazatlán Bay. The right margin has two columns, one with the number of events in that year and the other, the number of species blooming that year. The bottom margin depicts the same parameters but in relation to the month of occurrence.

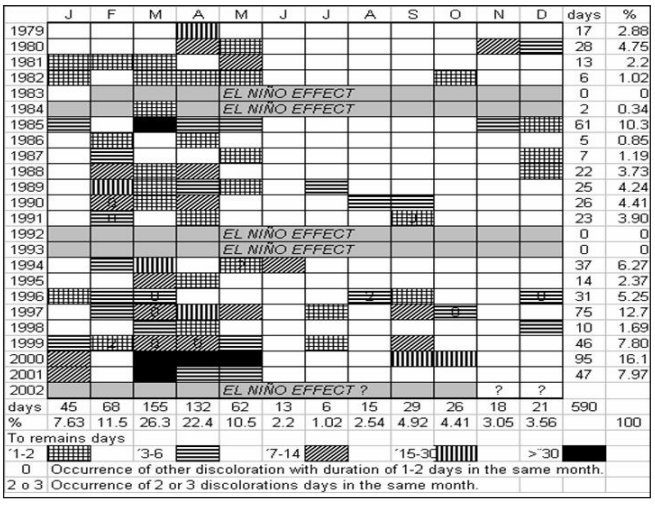


**Table 2** Annual duration of discoloration days from HAB events in Mazatlán Bay.

responsible for the major blooms. Six were toxic or noxious species and six were benign species. When *Cochlodinium* cf. *catenatum* (= *C. polykrikoides*) bloomed, it represented up to 90% of the total biomass while the rest of the bloom species only reached ca. 70% of the total biomass. Table 1 shows the bloom species during the reported events. From 1979 to 1988 there were no blooms during summer and autumn.

During average years, HAB events were caused by only one or two species. In 1985, after a strong ENSO event, the number of discolorations as well as the number of causative species increased. In the decade 1979–1988, *Mesodinium rubrum* was the most abundant species. In the next decade (1989–1998), three major changes occurred: an increase in the average number of events per year, an increase in the number of causative species (3–5), and the gradual but constant occurrence of blooms during the warmer months (summer and autumn). There is strong evidence that the presence of *C. catenatum* during autumn is a recent appearance since it has not been observed before in the area or even on the Pacific coast of Mexico (Cortés-Lara and Cortés-Altamirano, 2002). It is noteworthy that under the influence of ENSO events there was a complete absence of blooms. This phenomenon, whose effects span one or two years, seems to be recurrent each 7–8 years, and as previously mentioned, the number of causative species as well as the number of events and their duration seems to increase. March and April are the months with maximum occurrences involving five species and, recently, September had a maximum peak caused by three species.

The species *Ceratium dens* and *C. furca* often compete to dominate the blooms, with *C. dens* having a preference for areas of high productivity (Cortés-Altamirano and Nuñez-Pasten, 2000). Two other species that compete in a similar fashion are *Prorocentrum balticum* and *P. dentatum,* usually with the former being better adapted to dominate. These two species bloomed during three months in 1999 and 2000. *Protoperidinium quinquecorne* and *Prorocentrum triestinum* have been observed only once as dominant taxa. The only species in the area that can be related to human PSP poisoning is *Gymnodinium catenatum.* It is able to bloom from January to May, but has a maximum abundance during March and April (Cortés-Altamirano and Nuñez-Pasten, 1999). Table 2 depicts the duration of the bloom events with their percentage by year and by month. Five categories were identified: 1–2 days (ephemeral); 3–6 days; 1–2 weeks; 15–30 days and >30 days. The tally reached 590 days of discoloration in 23 years. It seems that each year is different but when analyzed by 5 or 10 year clusters, it is possible to highlight trends as previously suggested by Cortés-Altamirano and Sierra-Beltrán (2001). In general ENSO years showed no discolorations while in the years following, the blooms flourish.

The absence of events during summer from 1978 to 1988, 1992 to 1995, 1998, and 2001 should be noticed. Blooms during March and April represented 48.7% of the discoloration days for the whole period studied.

One of the principal problems of using discoloration as a trend indicator for blooms is that not all blooms discolor the water nor can they be perceived by a toxic effect (Sournia, 1995). Also green discolorations, often caused by diatoms, cannot be easily distinguished. More than 99.9% of the diatoms are benign and of the 0.01% remaining, only *Pseudo-nitzschia* belongs to the toxic group. Other diatoms can be regarded as noxious: *Coscinodiscus* sp. produces mucus, and *Chaetoceros convolutus* and *C. concavicornis* have thick, hooked spines that may damage fish branchia (Rensel, 1993). In the Mazatlán Bay area, red tides caused by *Pseudo-nitzschia* sp. as a dominant species have not been yet detected. However, poisoning events caused by do-

moic acid have been reported in the Gulf of California (Sierra-Beltrán *et al.,* 1997, 1999).

It is very important to note the scale of the observed events. The reported discolorations being observed from elevations closer to the coasts may be only local processes. The influence of ENSO on the entire Gulf of California is evident in the net primary productivity, the fisheries production, and the absence of bloom events (López-Martínez, 2000). Usually, developing countries are not able to financially support costly monitoring activities with many sampling stations for long periods of time. The method depicted here is an economical alternative, and provides valuable data that may contribute to the prevention of the damaging effects of HABs.


## Acknowledgements

Arturo Nuñez-Pasten assistance with field sampling and images by Carlos Suarez Gutiérrez and Adriana Landa are highly appreciated.


## References


Cortés-Altamirano, R. and A. Nuñez-Pasten. Rev. Biol. Trop. 48, 305–311 (2000).

Cortés-Altamirano, R., Las Mareas Rojas, AGT Editor, S.A. (México, D.F.), pp. 14–15. (1998).

Cortés-Altamirano, R. and A. Nuñez-Pasten, An. Inst. Cienc. del Mar y Limnol., Univ. Nal. Autón. de México 19, 113–121 (1992).

Cortés-Altamirano, R. and A. Nuñez-Pasten, Ciencia y Mar 7 (UMAR), 50–56 (1999).

Cortés-Altamirano, R. and A. Nuñez-Pasten. Revista de Investigación Científica, UABCS, 2, 44–55 (1991).

Cortés-Altamirano, R., S. Licea D. and S. Gómez A., in: Memorias VIII Congreso Latinoamericano sobre Ciencias del Mar VIII COLACMAR, Tresierra A.A.E. and Z.G. Culquichicón M., eds. (Trujillo, Perú), pp. 343–345 (1999).

Cortés-Altamirano, R., Revist. Cienc. Mar., UBCN, 13, 1–19 (1987).

Cortés-Altamirano, R. and A. Sierra-Beltrán, in: Memorias 1er Foro Estatal de Ciencia y Tecnología, Universidad Autónoma de Sinaloa y Consejo Estatal de Ciencia y Tecnología, eds., 1, pp. 209–228 (2001).

Cortés-Lara Ma. del Carmen and R. Cortés-Altamirano, Rev. Biol. Trop. In press (2002).

Hasle,G.R, in: Phytoplankton Manual, Sournia A., ed. (UNESCO, Paris), pp. 191–197 (1978).

López-Martínez, J. Tésis doctoral en Ciencias Marinas, Centro Interdisciplinario de Ciencias Marinas, (IPN-México), 1–175 (2000).

Rensel, J.E., in: Toxic Phytoplankton Blooms in the Sea, T.J. Smayda and Y. Shimizu, eds. (Elsevier, Amsterdam), pp. 613–618 (1993).

Sierra-Beltrán, A., Ochoa, J.L., Lluch-Cota, S., Cruz-Villacorta, A., Rosiles, R., López-Valenzuela, M., del Villar-Ponce, L. M. and Cerecero-Gutierrez, J., in: Memorias VIII Congreso Latinoamericano sobre Ciencias del Mar VIII COLACMAR, Tresierra-Aguilar AE, and Culquichicón-Malpica ZG, eds. (Trujillo, Perú), pp. 886–887 (1999).

Sierra-Beltrán, A., Palafox-Uribe, M., Grajales-Montiel, J. Cruz-Villacorta, A. and Ochoa, J.L., Toxicon, 35, 447–454 (1997).

Sournia A., in: Harmful Marine Algal Blooms, P. Lassus, G. Arsul, E. Erard, P. Gentien and C. Marcaillou, eds. (Lavoisier, Paris), pp. 103–112 (1995).

# Spatial and Temporal Patterns of *Pseudo-nitzschia* Species in Central California Related to Regional Oceanography

Raphael Kudela[1], William Cochlan[2], and Alice Roberts[1]
[1]*Department of Ocean Sciences, University of California Santa Cruz, 1156 High St. Santa Cruz, CA 95064, USA;*
[2]*Romberg Tiburon Center for Environmental Studies, San Francisco State University, 3152 Paradise Dr., Tiburon, CA 94920-1205, USA*

## Abstract

We compare similarities and differences in oceanographic and environmental conditions during major HAB bloom events off the California coast, focusing on the Monterey Bay region from 1990–2002. During this period, there were five major events. In 1991, domoic acid was first reported in Monterey Bay. In 1995, a red tide (*Lingulodinium polyedrum*) extended from Baja to Monterey, CA. In 1998, 2000, and 2002, there were a series of widespread *Pseudo-nitzschia* blooms along the California coast. In many cases, these events were initially reported in the south, and appeared to propagate northward. Here we evaluate whether there are consistent environmental patterns associated with these events.

## Introduction.

Coastal California is typically viewed as an upwelling-dominated system, with strong equatorward and Ekman-dominated offshore flows, bounded to the west by the broad, meandering California Current. This implies that (1) biological and physical processes propagate predominantly southward, (2) coastal runoff has negligible impacts on the near-shore oceanographic conditions and (3) much of the biological activity is driven by seasonally intense spring upwelling. Recent observations, however, suggest that this view is misleading, and that the occurrence of infrequent but high-impact events such as precipitation-driven coastal runoff may dominate the biological signal over large spatial and temporal scales (*e.g.,* Friederich *et al.,* 2002). These events can "fertilize" the coastal ocean with anthropogenically derived nutrients and may catalyze or exacerbate HAB conditions in the coastal ocean. Three past HAB events occurring in 1995, 1998 and 2000 exemplify the difficulties associated with monitoring, predicting, and understanding the origins and fate of HABs. In 1995, a massive red tide of the non-toxic dinoflagellate *Lingulodinium polyedrum* occurred off the coast of California, extending from the upper Baja peninsula to Monterey Bay, representing the largest and most widespread red tide observed off California since 1902. In spring 1998, field sampling identified toxic *Pseudo-nitzschia* species along much of the California coastline, at relatively low abundance. A series of bloom events again occurred in 2000 and 2002, extending along much of the California coastline. There were no consistent links to known or potential environmental triggers during the domoic acid events, but in all three cases (1995, 1998, and 2000), there was an apparent northward propagation of plankton assemblages. While intriguing, the possibility of predictable northward transport or of northward propagation of environmental conditions conducive to HABs has not been directly tested.

Here we address several specific questions. (1) Are mesoscale physical (*e.g.,* upwelling, relaxation), or environmental (*e.g.,* rainfall, runoff) events responsible for the large spatial/temporal toxigenic events in California? *Pseudo-nitzschia* spp. are cosmopolitan, and are always present in central California waters (based on long-term monitoring), but major toxin events often occur over large spatial scales (*e.g.,* Southern California Bight to Monterey Bay, Trainer *et al.,* 2000). This suggests that there must be large-scale forcing responsible for the otherwise coincidental timing of these major events. (2) Do nutrient concentrations trigger toxin production? If so, which nutrients? Various macro- and micro-nutrients have been implicated in the onset and maintenance of toxicity, primarily from laboratory studies. A partial listing includes Si, P, Fe, Cu, Li, and N. Other hypothesized mechanisms include temperature, irradiance, and nutrient ratios (*e.g.,* review by Bates, 1998). Despite these laboratory (and field) observations, no consistent pattern has been identified. (3) Will regional, high-resolution monitoring programs provide predictive capabilities for larger scale (West Coast) monitoring programs? Recent evidence suggests that toxigenic events may be associated with persistent mesoscale physical features (*e.g.,* Trainer *et al.,* 2002, the Juan de Fuca Eddy) and "cryptic" blooms (*e.g.,* Rines *et al.,* 2002, thin layers). If the presence/absence of *Pseudo-nitzschia* is not a good indicator of toxigenic events, are there other measurements that can be incorporated into monitoring programs, and will they capture the scales of variability?

## Materials and Methods

Time-series data for physical, biological, and chemical conditions in California (primarily Monterey Bay) were obtained from the Pacific Fisheries Environmental Laboratory (NOAA) database (upwelling indices), the USGS database (river flow), the Monterey Bay Aquarium Research Institute (MBARI; temperature, salinity, nutrients) and the TOPEX/Poseidon satellite system (sea surface height). For analysis of the 1998 bloom event, whole-cell hybridization techniques developed by Scholin *et al.* (1997) were used on samples collected from the Santa Cruz Wharf from Year Day 78-200, 1998. Nutrient values were determined by MBARI using standard colorimetric techniques. Remote sensing data were obtained from the NOAA CoastWatch program (sea surface temperature) and the NASA SeaWiFS program (ocean color).

During the 2000 Monterey Bay field experiment, water was collected from a nearly unialgal near-surface bloom of

Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

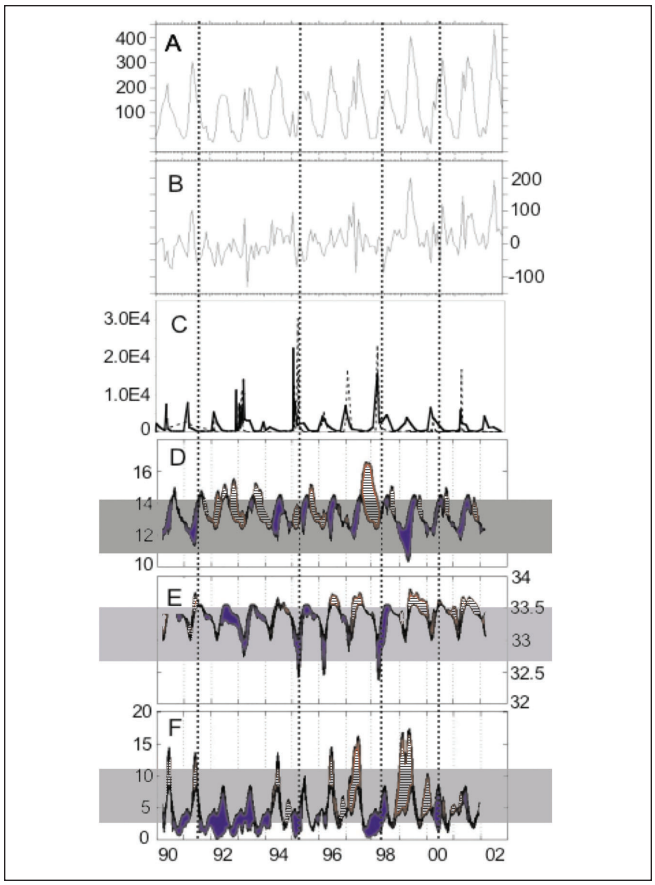


**Figure 1** Time-series plots for **A**: upwelling index ($m^3$/s/100 m coastline); **B**: upwelling anomaly; **C**: Riverflow from San Francisco (solid line) and Salinas River (dashed line; cfs); **D**: temperature anomaly (deg C); **E**: Salinity anomaly (pss); **F**: nitrate anomaly (μM). For **D**–**F**, solid line is the running mean, striped areas are positive anomalies, dark shaded areas are negative anomalies. Data are for Monterey Bay. The dashed vertical lines indicate major bloom events.

*Pseudo-nitzschia australis* (identified using microscopy and molecular probes) and enriched with either 20 μM nitrate, 20 μM silicate, 100 nM Desferol, which is an iron chelator (*e.g.,* Wells, 1999), and no treatment (control) in 9 liter polycarbonate carboys. Water was collected using a trace-metal clean pumping system. The carboys were then maintained under simulated *in situ* conditions for six days, and changes in biomass were monitored using chlorophyll, biogenic silica, or *P. australis*-specific probes. Spatial variable fluorescence data were collected simultaneously (Chelsea FRR using night-time data).

## Results and Discussion.

Time-series plots for oceanographic and environmental parameters centered on Monterey Bay are presented in Fig. 1. Although the red tide event of 1995 was clearly linked to a heavy runoff event (*e.g.,* Kudela and Cochlan, 2000), the trends for major *Pseudo-nitzschia* toxic blooms are less evident. In general, blooms occur during anomalously weak (but not absent) upwelling conditions, typically during a transition from excess to negative macronutrients relative to the climatological mean (black line). There is no evidence that *Pseudo-nitzschia* blooms are strongly correlated to runoff events. The observed south to north trend in bloom events (1991, 1995, 1998, 2000, 2002) is consistent with large-scale physical forcing, as evidenced by changes in sea surface height, which occur south to north at the same time scales (weeks to months), suggesting that the spatial pattern is indicative of a change in environmental conditions, rather than actual transport of seed populations.

During early summer 1998, a widespread DA event occurred along the entire West Coast, and has been well described elsewhere (Scholin *et al.,* 2000, Trainer *et al.,* 2000). Observations from this bloom include its apparent propagation from south to north, starting in the Southern California Bight and extending to the Monterey region, with additional blooms occurring in the Pacific northwest. Toxic blooms were generally associated with headlands and upwelling centers, as reported by Trainer *et al.* (2000). There is some evidence (inconsistent) for bloom initiation to be associated with terrestrial runoff (Scholin *et al.,* 2000). Macronutrients were generally elevated, but lower than the climatological mean (Fig. 1). Conditions preceding and during the toxic bloom were generally indicative of weak upwelling (see SST, Fig. 2), but highly productive (chl *a*, Fig. 2). However, prior to the bloom (March), the concentration of colored dissolved organic matter (CDOM) was very high, indicating substantial terrestrial input (runoff), particularly in central California off San Francisco and Monterey Bay, and in the Channel Islands. These areas were reported to be two bloom "hot spots" (Trainer *et al.,* 2000), suggesting a correlation, although these regions are also associated with headlands, and therefore upwelled nutrients. During the peak toxicity in Monterey Bay (May), CDOM levels were still very elevated in these regions, suggesting continued influence from runoff (although CDOM is also produced by phytoplankton).

Based on possible nutrient-control of toxin production during 1998, we evaluated the importance of macro- and micronutrients during another widespread bloom in August 2000 in the Monterey Bay region. Large-volume grow-out experiments with amended macro- and micronutrients (Si, N, Fe), showed no evidence for iron limitation, and *Pseudo-nitzschia* biomass was nitrogen (not silicon) limited (Fig. 3). Spatial surveys of surface variable fluorescence similarly suggested that there was no widespread iron stress during the bloom. A series of AUV and shipboard observations were also conducted (Ryan *et al.,* 2002), providing a 3-D view of the water column. A subsurface bloom of *Pseudo-nitzschia australis* was mapped, and was associated with a density gradient. This bloom extended spatially for several kilometers, with very little surface expression. A 2-D slice of optical backscatter (data not shown) suggested that the phytoplankton bloom was being "fed" by a subsurface resuspension event, possibly providing a source of bioavailable iron. During this period, DA values in both the particulate and dissolved phase were extremely high (>20 pmol/cell particulate DA and μM concentrations of dis-

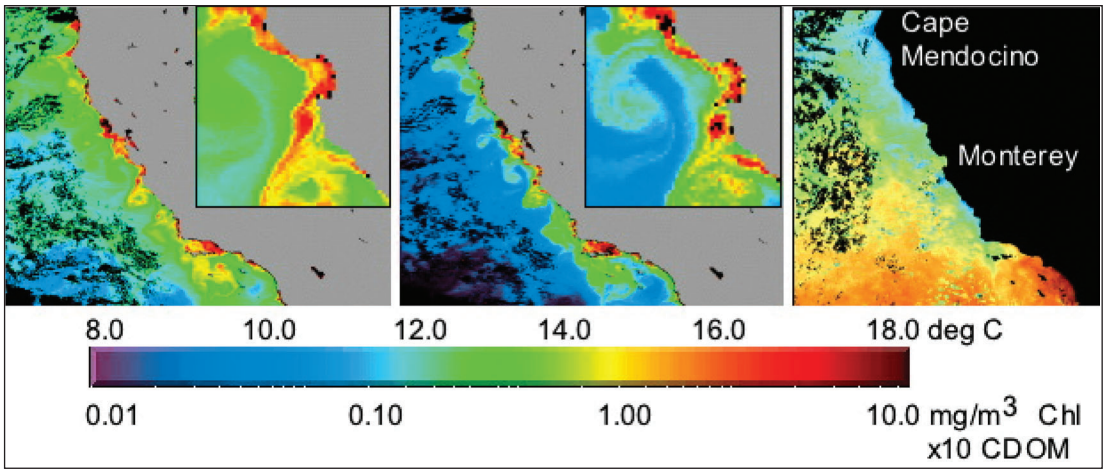


**Figure 2** Chlorophyll (left), colored dissolved material (CDOM, middle) and sea surface temperature (right) for the west coast and for Monterey Bay (insets) during the 1998 toxigenic bloom event. Values for chl (mg/m$^3$), SST (deg. C), and CDOM (dimensionless) scaled by a factor of 10 are given.

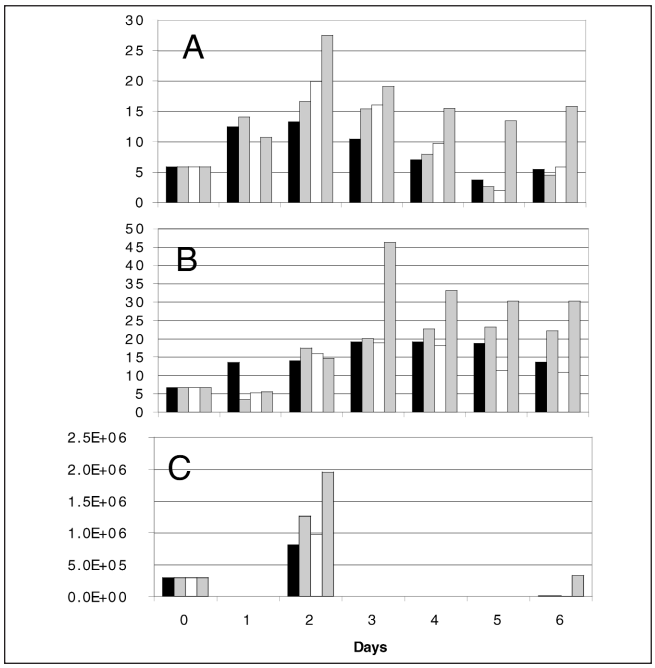


**Figure 3** Large-volume grow-outs of whole water from a *P. australis* dominated bloom with nutrient amendments, Monterey Bay 2000. **A**: chl *a* (mg/m$^3$). **B**: biogenic silica (μM). **C**: species-specific molecular probes for *P. australis* (cells/L). Solid fill: control. Horizontal fill: plus nitrate. White fill: plus silicate. Diagonal fill: plus Desferol.

solved DA; G. Doucette, pers. comm.). The presence of extremely high DA levels during this period, despite a lack of Si-limitation of biomass accumulation or iron stress, suggests that neither macro- nor micronutrient limitation were the primary cause of this toxigenic event.

## Conclusions

Large-scale HAB events in California are inconsistent in their oceanographic and environmental conditions. Although extensive runoff and weak upwelling conditions were clearly responsible for the 1995 red tide (*Lingulodinium*) event, rainfall is not a good indicator of DA production. Bloom conditions are generally associated with weak (but not absent) upwelling, fresher water, transitional periods between anomalously warm and cool waters, and generally low ambient concentrations of macronutrients. There is no consistent evidence for macronutrient or iron concentrations to be directly attributable to toxic events; when tested directly, *Pseudo-nitzschia* was generally nitrogen limited, with little or no iron stress (although it is possible that DA production and lack of Fe stress are correlated, if DA is a chelator (Rue and Bruland, 2001). *Pseudo-nitzschia* is likely often associated with thin-layers, as has been observed by others (*e.g.,* Rines *et al.,* 2002), which may make detection difficult prior to a major toxic event. Latitudinal changes in environmental conditions associated with seasonality are the likely cause for the apparent northward propagation of HAB events along the west coast.

## Acknowledgements

This work was supported by ECOHAB grant C7-94085, NSF grant OCE-9912361, and NASA grant NAG5-8855. Time-series and cruise data were collected by MBARI, supported by the David and Lucile Packard Foundation. We thank J. Ryan, F. Chavez and C. Scholin for data access.

## References

S.S. Bates, in: The Physiological Ecology of Harmful Algal Blooms, D. M. Anderson, A. E. Cembella, and G.M. Hallegraeff, eds. (Springer Verlag, Heidelberg), pp. 405–426 (1998).

G. E. Friederich, P. M. Walz, M. G. Burczynski, and F. P. Chavez, Prog. Oceanogr. 54, 185–204 (2002).

R. M. Kudela and W. P. Cochlan, Aquatic Microb. Ecol. 21, 31–47 (2000).

J. E. B. Rines, P. L. Donaghay, M. M. Dekshenieks, J. M. Sullivan and M. S. Twardowski, Mar. Ecol. Prog. Ser. 225, 123–127 (2002).

E. L. Rue and K. W. Bruland, Mar. Chem. 76, 127–134 (2001).

J. Ryan, F. Chavez, J. Bellingham, E. Rienecker, R. Kudela, A. Vander Woude, R. Maffione, and A. Fisher, in: AVIRIS Airborne Geoscience Workshop Proceedings (Jet Propulsion Laboratory, Pasadena, CA) (2002).

C. Scholin, F. Guillard, G. J. Doucette *et al.,* Nature 403, 80–84 (2000).

V. L. Trainer, N. G. Adams, B. D. Bill, C. M. Stehr, J. C. Wekell, P. Moeller, M. Busman, and D. Woodruff, Limnol. Oceanogr. 45, 1818–1833 (2000).

V. L. Trainer, B. M. Hickey, and R.A. Horner, Limnol. Oceanogr. 47, 1438–1446 (2002).

M.L. Wells, Limnol. Oceanogr. 44, 1002–1008 (1999).

# Prevalence of Raphidophyte Blooms in South Carolina Brackish Ponds Associated with Housing and Golf Courses

Alan J. Lewitus[1,2], Kenneth C. Hayes[1,3], Jason W. Kempton[2], Larissa J. Mason[2], Susan B. Wilde[1], Barry J. Williams[2], and Jennifer L. Wolny[2]

*[1]Belle W. Baruch Institute for Marine and Coastal Sciences, University of South Carolina, P.O. Box 1630, Georgetown, SC 29442, USA; [2]South Carolina Department of Natural Resources, Marine Resources Research Institute, Hollings Marine Laboratory, 331 Fort Johnson Rd., Charleston, SC 29412, USA; [3]South Carolina Department of Health and Environmental Control, Columbia, SC 29208, USA*

## Abstract

The South Carolina (SC) coastal zone is among the fastest growing areas in the U.S., and population centers such as Myrtle Beach, Charleston, Kiawah Island, and Hilton Head Island are marked by numerous brackish water ponds (lagoons) associated with housing complexes and golf courses. These manmade detention/retention ponds were constructed to serve as buffers between developed areas and open estuaries, and for aesthetic reasons. However, the combination of restricted tidal flow and nutrient loading creates an environment conducive to HAB formation. Surveillance efforts in 2001–2002 documented the widespread and common occurrence of several types of potentially or measurably toxic HABs in these ponds. Raphidophytes (*Heterosigma akashiwo, Chattonella subsalsa, C.* cf. *verruculosa, Fibrocapsa japonica*) were exceptionally prevalent, and were observed in 27 of the 40 brackish ponds sampled. Abundances >$10^3$ cell $mL^{-1}$ were observed in 12 of these ponds. In addition to the potential environmental and human health effects of these algae within the ponds, tidal transport of harmful algae, cysts, or toxins may adversely affect fish and shellfish in adjacent tidal creeks or open estuaries. Here, we document the distribution of raphidophytes within brackish ponds in South Carolina sampled during monitoring efforts, including fish kill incidents.

## Introduction

Raphidophyte blooms occur worldwide throughout temperate coastal waters, including eastern and western areas of North America (Tomas 1998). Raphidophytes are well known for causing fish kills, especially those associated with fish aquaculture operations (Landsberg 2002). Over the last 2 years, routine monitoring and fish kill response efforts under the South Carolina Harmful Algal Bloom Program (SCHABP) have revealed a widespread distribution of raphidophytes in South Carolina (SC) brackish lagoonal ponds. The first discovery of a raphidophyte bloom in SC occurred on 11 April 2001, when a bloom of *Heterosigma akashiwo* of $2 \times 10^5$ cell $mL^{-1}$ was observed in a 7-acre pond in Hilton Head (Lewitus and Holland 2003). Triggered by the discovery of these blooms, SCHABP extended its routine monitoring to include several of the >1500 brackish

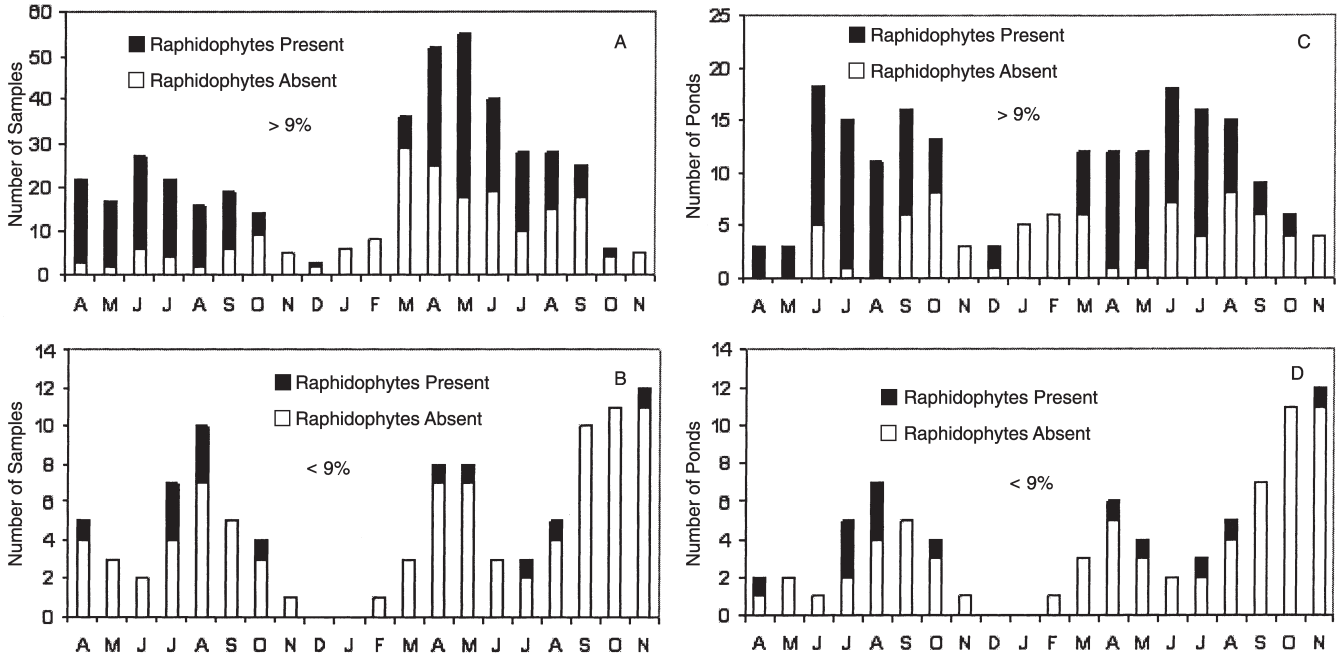


**Figure 1** Number of samples with vs. without raphidophytes when salinity was (**A**) >9‰ or (**B**) <9‰, and number of ponds in a given month with vs. without raphidophytes when salinity was (**C**) >9‰ or (**D**) <9‰. From April 2001 to November 2002.



Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

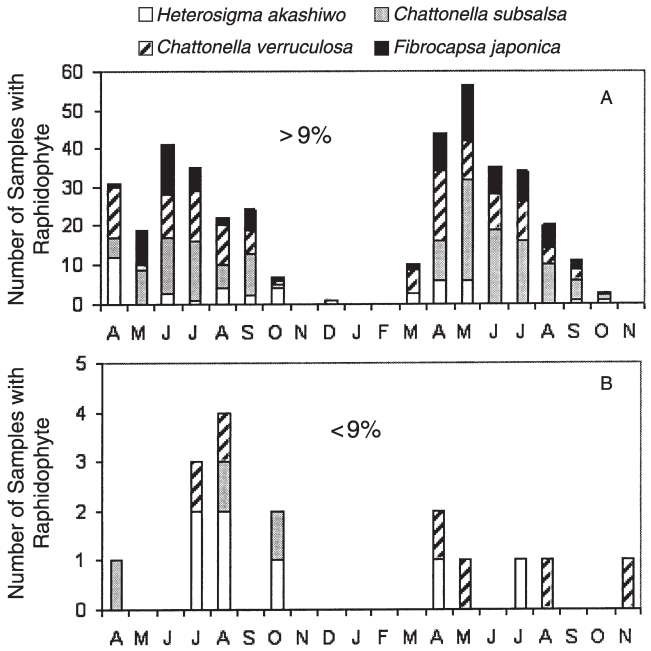


**Figure 2** Number of pond samples with raphidophyte species when salinity was **A** >9‰ or **B** <9‰. From April 2001 to November 2002.

ponds along the SC coast (Lewitus *et al.,* 2003). Of the 9 HAB species found to form blooms in these ponds, the 4 raphidophytes (*H. akashiwo, Chattonella subsalsa, C.* cf. *verruculosa,* and *Fibrocapsa japonica*) were relatively common and in some cases associated with fish kills. Here, we present further documentation of distribution and abundance of raphidophytes in SC brackish lagoonal ponds.

## Materials and Methods

From April 2001 to November 2002, 40 brackish to marine ponds were sampled during monitoring efforts associated with SCHABP. The ponds were primarily on Kiawah Island, Hilton Head Island, and the Charleston area. All of the ponds were associated with housing developments and/or golf courses. A total of 538 samples were collected and analyzed for the presence and/or abundance of HAB species. YSI meters were used to measure temperature, salinity, and dissolved oxygen concentration. Bottle samples were collected in triplicate from 0.5 m below the surface, kept at ambient temperature, and transported to the laboratory for processing. Inorganic nutrients ($NH_4$, $NO_3$ and $NO_2$, $PO_4$, Si) were determined with autoanalyzers, dissolved organic carbon (DOC) was measured using a carbon analyzer, and dissolved organic nitrogen (DON) was estimated by subtracting total inorganic from total dissolved N, measured by the persulfate oxidation technique. Raphidophyte species were identified by microscopic inspection of live (*i.e.,* unpreserved) samples upon arrival at the laboratory. Enumeration was conducted on lugols-fixed samples, either by the use of Nageotte Bright Line hemacytometers (depth 0.5 mm) or the Utermöhl settling chamber technique (Lund *et al.,* 1958).

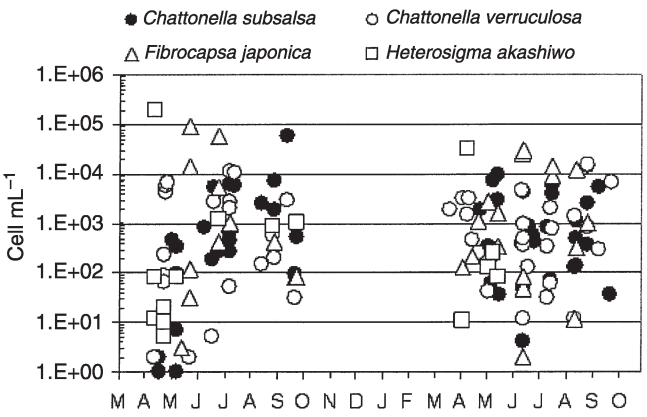


**Figure 3** Cell abundances of four raphidophyte species in pond samples collected from April 2001 to October 2002.

## Results and Discussion

From April 2001 to November 2002, raphidophytes were confirmed by microscopic observations in 47% of the 538 samples collected (Fig. 1A, B). When salinity exceeded 9‰, raphidophytes were observed in 55% of the samples (Fig. 1A), and in only 13% of samples of water with salinities <9‰ (Fig. 1B). A seasonal trend for raphidophyte occurrence was apparent. Raphidophytes were observed in 203 (66%) of the 307 samples from April through August, and only in 1 of 22 total samples collected from November through February (Fig. 1A). The patterns in raphidophyte prevalence in the samples reflected their prevalence in pond occurrence (*i.e.,* the number of ponds with vs. without raphidophyte observations in any given month; Fig. 1C, D).

Four raphidophyte species identified were *Heterosigma akashiwo, Chattonella subsalsa, C.* cf. *verruculosa* (Bourdelais *et al.,* 2002), and *Fibrocapsa japonica.* Based on their prevalence in collected samples, the seasonal distribution appeared to be similar for 3 of the 4 species, with the exception that the prevalence of *H. akashiwo* decreased in the summer (Fig. 2A). Only a few observations were made with low salinity samples (<9‰), but it may be relevant that *F. japonica* was never observed in these waters (Fig. 2B).

Cell abundances were determined from a subset (89) of total samples (Fig. 3). Samples were selected where raphidophytes were observed through initial microscopic screening. Samples with high raphidophyte abundances were common. Cell abundances were $>10^3$ cell $mL^{-1}$ in 44 samples, encompassing 12 different ponds. Maximum abundance for each species exceeded $10^4$ cell $mL^{-1}$. The nutrient properties for 31 samples from 7 different ponds showed some general patterns. Mean $PO_4$ concentration was 9.7 ± 7.1 μM, probably derived from fertilizer and/or sewage inputs. Mean DIN:DIP was 0.72 ± 0.98, well below the Redfield ratio. Mean DOC and DON concentrations were markedly high (1213 ± 584 and 60 ± 20 μM, respectively), and supplementation of N through heterotrophic pathways should not be ruled out (Smayda 1998). The mean DOC:DON ratio in these samples (19 ± 5) is similar to that

associated with some other HAB species (Anderson *et al.,* 2002), however the relevance of this ratio as an indicator of DON use depends on comparisons with pond conditions under non-bloom circumstances.

Given their global association with fish kills, the prevalence of raphidophyte blooms in SC brackish ponds is of concern. The ponds are numerous along the SC coast (>1500), are often used for recreational activities, and are increasing in number as a standard practice for buffering nonpoint source pollutants or for aesthetic purposes. There is an urgency among SC state officials to gain an understanding of the factors promoting raphidophyte blooms in these ponds, and their potential impacts on natural resources, estuarine ecosystem function, and human health.

## Acknowledgements

We thank Sam Baughman, Megan Cook, Krista DeMattio, Brett Epps, Sarah Habrun, Megan Heidenreich, Sabrina Hymel, Chad Johnson, Jiqing Liu, Ian Moody, Arena Richardson, Laura Schmidt, Andrew Shuler, Aaron Shurtleff, Raph Tymowski, and Patrick Williams for technical support, Robert Ball, Rick DeVoe, Fred Holland, and the rest of the SC Task Group on Harmful Algae for paving the way, Carm Tomas for help with raphidophyte identification, and Norm Shea for his support. This research was funded by the U.S. ECOHAB Program grants NA86OP0493 and NA16OP2796, NOAA grants NA90AA-D-SG672, NA06OA0675, and NA86RG0052, and CDC and EPA grants to SCDHEC. Contribution 1306 of USC's Belle W. Baruch Institute, Contribution 530 of SCDNR's Marine Resources Research Institute, and ECOHAB Contribution 87.

## References

D. Anderson, P. Glibert, and J. Burkholder, Estuaries 25, 704–726 (2002).

A. Bourdelais, C. Tomas, J Naar, J. Kubanek, D. Baden, Environ. Health Perspect. 110, 465–470 (2002).

J. Landsberg, Rev. Fish. Sci. 10, 113–390 (2002).

A. Lewitus and A. Holland, Environ. Monit. Assess. 81, 361–371 (2003).

A. Lewitus, L. Schmidt, L. Mason, J. Kempton, S. Wilde, J. Wolny, B. Williams, K. Hayes, S. Hymel, C. Keppler, and A. Ringwood, Popul. Environ. 24, 387–413 (2003).

J. Lund, C. Kipling, and E. LeCren, Hydrobiologia 11, 143–170 (1958).

T. Smayda, in: Physiological ecology of harmful algal blooms, D. Anderson, A. Cembella and G. Hallegraeff, eds. (Springer-Verlag, Berlin), pp. 113–131 (1998).

C. Tomas, in: Harmful algae, B. Reguera, J. Blanco and M. Fernandez, eds. (Santiago de Compostela, Spain), pp. 101–103 (1998).

# Observation of *Prorocentrum lima* in South Carolina

Katherine A. Schaefer and Steve L. Morton
*National Oceanic and Atmospheric Administration, National Ocean Service, Marine Biotoxins Program, Hollings Marine Laboratory, 331 Fort Johnson Road, Charleston, SC 29412, USA*

## Abstract

*Prorocentrum lima* is a tychoplanktonic toxic dinoflagellate which is known to produce okadaic acid and dinophysis toxins. This species has been reported to occur in Florida and the New England states, but no reports of its occurrence appear in the Carolinas and Mid-Atlantic States. Observations of weekly plankton tows taken in several South Carolina tidal creeks showed low numbers of *P. lima.* This is the first report of this species in South Carolina. Because *P. lima* has been associated with the human illness diarrhetic shellfish poisoning (DSP), monitoring of oyster beds is ongoing to determine the possibility of potential toxic events caused by this dinoflagellate.

## Introduction

*Prorocentrum lima* is distributed worldwide from temperate (Lebour, 1925) to tropical oceans (Tindall and Morton, 1990). This species has been observed in Europe (Lebour, 1925), Asia (Yasumoto *et al.,* 1978), and South America (Gayoso *et al.,* in preparation). In North America, this species was first observed by Bomber *et al.* (Kat, 1985) from the Florida Keys and subsequently observed in Nova Scotia, Canada (Quilliam *et al.,* 1990) and Lamoine, Maine (Morton *et al.,* 1999). However, prior to the present study, *P. lima* had not been observed along the mid-Atlantic and Carolina Coast of the United States.

Because *Prorocentrum lima* produces okadaic acid and related congeners, the distribution of this species is important to determine if shellfish are at potential risk for contamination by this toxin. Human ingestion of okadaic acid-contaminated shellfish leads to diarrhetic shellfish poisoning (DSP). Incidents of DSP have been reported in Japan (Yasumoto *et al.,* 1978), Europe (Kat, 1985), North America (Quilliam *et al.,* 1990), and South America (Ferrari *et al.,* 1993). Some of the symptoms of DSP include severe cramping, nausea, vomiting, and diarrhea.

Coastal waters of South Carolina, USA are monitored by the National Oceanic and Atmospheric Administration's South Carolina Phytoplankton Monitoring Network (SCPMN). Volunteer groups consist of middle and high school science classes, environmental citizen organizations, and park facilities. Volunteers are trained on sampling techniques and identification methodologies. One of the purposes of volunteer monitoring is to build a species list of potentially harmful and non-toxic phytoplankton species in South Carolina. These monitoring efforts led to the first observations of *Prorocentrum lima* from the Carolina coast.

## Materials and Methods

Monitoring efforts began on May 17, 2001, and are ongoing off the Cooper River in Charleston, South Carolina, USA. Sampling locations were Amoco Creek (32°57′56″N, 79°54′28″W), Flagg Creek (32°56′56″N, 79°54′34″W), and Goose Creek, South Carolina (32°58′01″N, 79°56′05″W). In addition, samples were collected from Fort Johnson in Charleston, South Carolina (32°45′15″N, 79°54′34″W). Samples were taken once every two weeks to gain an understanding of the phytoplankton composition of the Cooper River.

Discrete water and 20 μm plankton net tow samples were collected at both low tide and high tide at each sampling site. Water samples were then examined for identification of diatoms and dinoflagellates using a Zeiss Axiovert (model S100) inverted microscope and a JEOL (model 5600LV) Scanning Electron Microscope. Samples (1 mL used) for SEM were fixed with glutaraldehyde, dehydrated using a gradient series of acetone, and air dried. A 5 μm polycarbonate filter was used. Samples were then coated with gold for SEM observation.

## Results and Discussion

A variety of diatoms and dinoflagellates were evident in the samples, dominated by *Chaetoceros* spp., *Coscinodiscus* spp., *Odontella* spp., *Rhizosolenia* spp., and *Protoperidinium* spp. *Prorocentrum lima* was observed at all four of the sampling sites. Identification of *P. lima* (Fig. 1) is unequivocal given that the two main thecal plates (valves) are oblong to obvate (length 33–38 μm, width 22–24.2 μm, n = 10) and the morphological features of the cells coincide

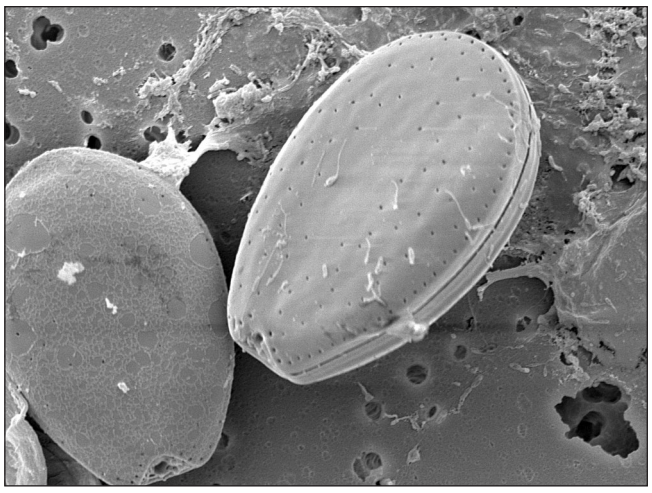

**Figure 1** SEM of *Prorocentrum lima* used for positive identification.

Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

with the descriptions given by Faust (1991) and McLachan *et al.* (1997, as *Exuviaella lima*): a row of conspicuous marginal pores, scattered valve pores, and valve center free of pores.

Further research is ongoing to determine the presence of okadaic acid and related congeners in oyster beds in South Carolina. The monitoring efforts present new information that is valuable to local fisheries with respect to safeguards on human health. Monitoring efforts by SCPMN volunteers are critical in providing preliminary observations of harmful algae in South Carolina.


## Acknowledgements

The authors would like to thank the many volunteer groups which make up the South Carolina Phytoplankton Monitoring Network, whose preliminary observations identified *P. lima.*


## References


J.W. Bomber, D.R. Norris and L.E. Mitchell, in: Toxic Dinoflagellates, D. M. Anderson, A. W. White and D. M. Baden, eds. (Elsevier, Amsterdam), pp. 45–50 (1985).

G. Ferrari, S.M. Méndez and A. Brazeiro, Décimo Simposio Científico Tecnológico de la CTMFM, Montevideo, pp. 63–64 (1993).

A.M. Gayoso, S. Dover, S.L. Morton, M. Busman, P.D.R. Moeller, and L. Maranda. In preparation. J. Shellfish Res.

M. Kat, in: Toxic Dinoflagellates, D. M. Anderson, A. W. White and D. M. Baden, eds. (Elsevier, Amsterdam), pp. 73–77 (1985).

M.V. Lebour, 1925. The Dinoflagellates of Northern Seas. Marine Biological Association United Kingdom, Plymouth, 250 pp.

S.L. Morton, T.A. Leighfield, B.L. Haynes, D.L. Petitpain, M.A. Busman, P.D.R. Moeller, L. Bean, J. McGowan, J.W. Hurst and F.M. VanDolah, J. Shellfish Res. 18, 681–686 (1999).

M.A. Quilliam, M.W. Gilgan, S. Pleasance, A.S.W. deFreitas, D. Douglas, L. Fritz, T. Hu, J.C. Marr, C. Smith and J.L.C. Wright, in: Proceedings of the Second Canadian Workshop on Harmful Marine Algae, D.C. Gordon, ed. Can. Tech. Rep. Fish. Aquat. Sci. 1799, 18–22 (1990).

D.R. Tindall and S.L. Morton, in: Physiological Ecology of Harmful Algal Blooms, D.M. Anderson, A. Cembella and G.M. Hallegraeff, eds. (Springer Verlag, Heidelberg), pp. 293–314 (1998).

T. Yasumoto, Y. Oshima, and M. Yamaguchi, Bull. Jpn. Soc. Sci. Fish. 44, 1249–1255 (1978).

# *Prorocentrum lima* in New England Coastal Waters: Population Dynamics and Toxicity

Lucie Maranda[1], Susannah Corwin[1], Paul E. Hargraves[1], Laurie L. Bean[2], Steven Eaker[3], Tod Leighfield[3], and Steve L. Morton[3]

[1]*Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882-1197, USA;* [2]*Department of Marine Resources, West Boothbay Harbor, ME, USA 04575;* [3]*Marine Biotoxin Program, NOAA/Hollings Marine Laboratory, 331 Ft. Johnson, Charleston, SC 29412, USA*

## Abstract

The epibenthic/epiphytic dinoflagellate *Prorocentrum lima* (Ehrenberg) Dodge is widespread in New England coastal waters. The abundance and seasonality of this toxin producer were followed within the planktonic and epibiotic community, bimonthly at eight sites along the coast of New England, USA. In an effort to evaluate the potential for diarrhetic toxins to contaminate shellfish resources, the digestive glands of wild and cultured shellfish collected at five of the stations were analyzed for okadaic acid (OA) content. After the first of this two-year study, the seasonal distribution of *P. lima* and toxin patterns in mussels and oysters suggest seasonal variations between stations. Although the presence of toxins in shellfish digestive glands indicates uptake, the levels remained below the maximum safety limit.

## Introduction

The dinoflagellate *Prorocentrum lima* is associated with the epibiota in coastal northeast USA, although its spatio-temporal distribution and its involvement in diarrhetic shellfish poisoning (DSP) events are poorly known (Morton *et al.,* 1999; Maranda *et al.,* 2000). The overall goal of our study is to determine and understand the potential for diarrhetic toxins to contaminate shellfish. Two hypotheses are being tested: 1) *P. lima* from New England coastal waters is toxigenic with respect to production of OA and derivative compounds, and 2) Shellfish grown in suspension culture become contaminated with DSP toxins at a faster rate and at a higher level than wild shellfish.

Our specific objectives are to establish the seasonal distribution of *P. lima* associated with wild and cultured shellfish and to determine the toxin load and composition of the epibiotic community and of shellfish, over time. We present data on the first of this two-year investigation.

## Materials and Methods

Collection of epibiota, plankton (10-µm mesh size) and shellfish was performed underwater at eight sites, within one hour of low tide, twice a month between October 2001 and October 2002 (Table 1 and Fig. 1). Plankton and epibiota were processed for identification; the <90-mm fraction of the epibiota was enumerated on a Sedgwick-Rafter chamber (100×) and an aliquot was frozen for later toxin analysis, while the >90-µm fraction was briefly rinsed, dried (2 weeks at 60°C) and weighed. Shellfish digestive

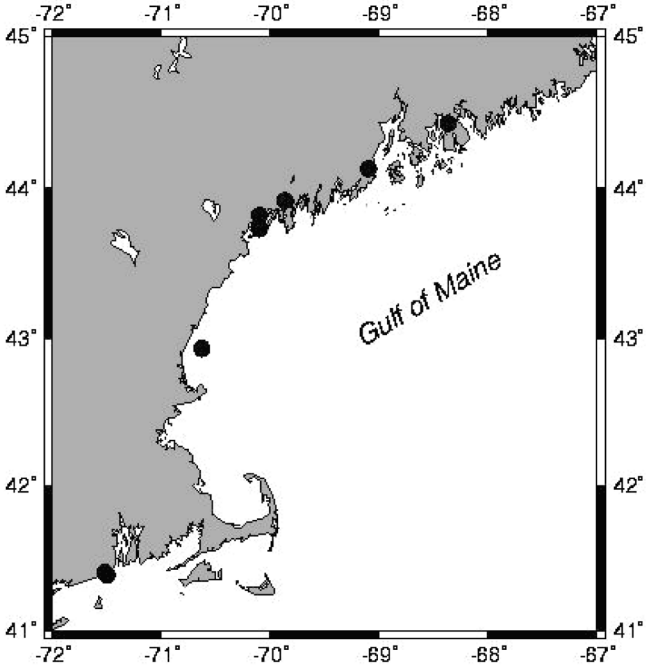


**Figure 1** Location of stations along the U. S. northeastern coast.

**Table 1** Sampling stations within New England coastal waters.

| Sampling Stations | Coordinates | Collections |
|---|---|---|
| Mount Desert Narrows, ME | 44°26′N 68°22′W | Phytoplankton, epibiota, mussels |
| Clam Cove, ME | 44°08′N 69°06′W | Phytoplankton, epibiota, mussels |
| New Meadows River, ME | 43°55′N 69°52′W | Phytoplankton, epibiota |
| Harraseeket River, ME | 43°49′N 70°06′W | Phytoplankton, epibiota, mussels |
| Casco Bay, ME | 43°44′N 70°06′W | Phytoplankton, epibiota, mussels |
| New Hampshire offshore | 42°56′N 70°38′W | Phytoplankton, epibiota |
| Point Judith Pond, RI | 41°24′N 71°31′W | Phytoplankton, epibiota, oysters |
| Bluff Hill Cove, RI | 41°23′N 71°30′W | Phytoplankton, epibiota |

Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

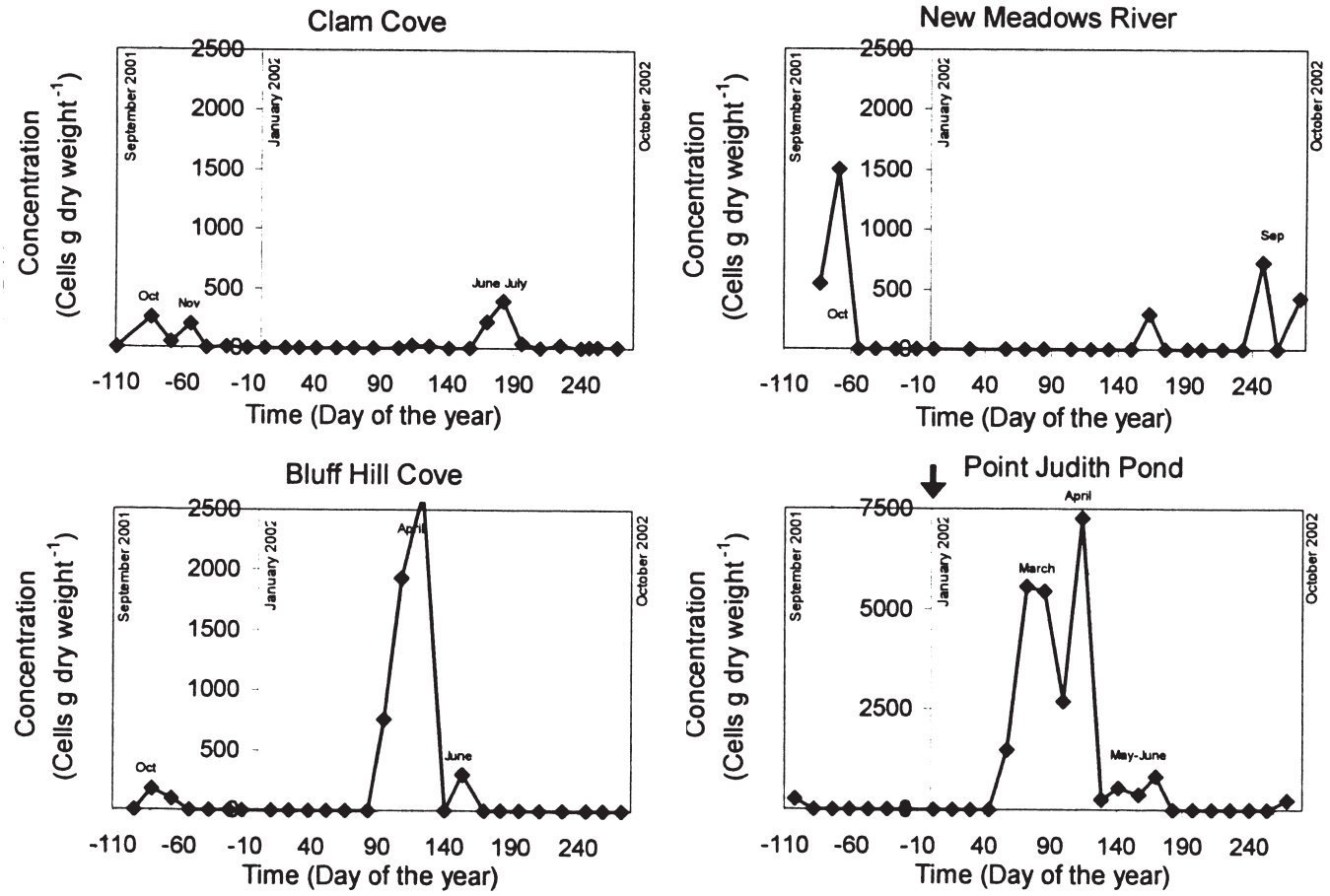


**Figure 2** Cell density of *Prorocentrum lima* per unit dry biomass of intertidal epibiota over time at four stations along the coast of Maine. Note the change of scale in the y axis for the Point Judith Pond station (arrow).

glands were excised, weighed and frozen (–80°C) prior to toxin analysis. Shellfish and epibiota were analyzed for DSP-type toxins by the fluorimetric protein phosphatase inhibition assay (Mountfort *et al.,* 1999); the detection limit is $1 \times 10^{-10}$ M okadaic acid equivalents.

Three cultures of *Prorocentrum lima* in exponential phase were tested for toxin load: two clones (C1-2 and C1-3) isolated from Point Judith Pond and one clone (SLM99-1) isolated from Mount Desert Narrows.

## Results and Discussion

The toxicity of clonal cultures of *Prorocentrum lima* from New England coastal waters is confirmed with 22 and 20 pg OA equivalent cell$^{-1}$ for the Maine and the Rhode Island isolates, respectively.

At four of the eight stations, populations of *P. lima* exceeded 200 cells g dry weight$^{-1}$ more than once during the sampling period (Fig. 2). At the northern stations, *P. lima* was detected during summer and early fall, while at both Rhode Island stations, only 3° of latitude farther south, populations of *P. lima* displayed early spring peaks of 2 to 15 times higher densities than those found at the northern stations. At Mount Desert Narrows and Harraseeket River, populations of *P. lima* never exceeded 100 cells g dry weight$^{-1}$, and none were ever recorded at the New Hampshire offshore station (data not shown). At the Casco Bay station, *P. lima* was detected only once, in May 2002. The dinoflagellate was rarely found in the plankton.

The dominant type of habitats where populations of *P. lima* were found consisted primarily of filamentous algal substrates attached to either the phaeophytes *Ascophyllum nodosum* and *Fucus* spp. or to sea or marsh grass. The most common substrates were *Pilayella littoralis, Hincksia mitchelliae, H. granulosa, Ectocarpus siliculosus,* and three-dimensional structures of the tube-forming diatoms *Berkeleya rutilans* and *Navicula grevillei,* as well as long chains of *Melosira* spp. and extensive tufts of *Licmophora* spp.

At all stations where shellfish are collected, data from October to May showed that toxin content in digestive glands never exceeded 2 ng g digestive glands$^{-1}$ (wet weight) (Fig. 3).

*Prorocentrum lima* from the coastal northeast USA produces DSP-type toxins generally associated with epibiotic filamentous phaeophytes and three-dimensional diatom structures growing in the intertidal zone or on aquaculture rafts. This first year of the study reveals variations in the size and seasonal distribution of *P. lima* populations between stations. Southern stations (Rhode Island) show peaks in abundance in the spring, while northern stations (Maine)

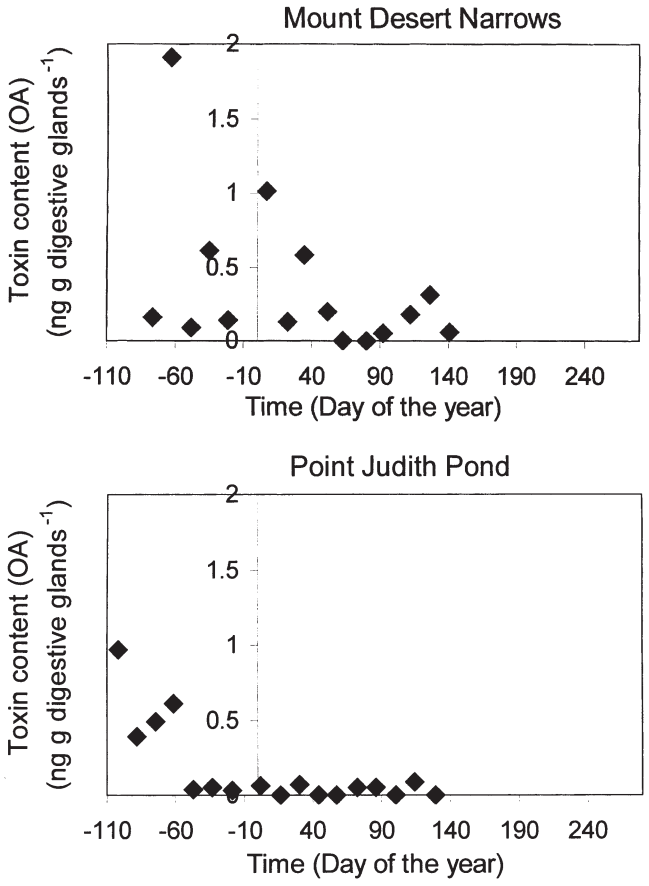


**Figure 3** Toxin content per unit shellfish digestive glands (wet weight) at one station in Maine and one in Rhode Island.

harbor mostly late-summer and fall populations. This pattern, should it be confirmed with data from the second year of the study, may reflect the station locations within two distinct biogeographic entities. Maximum densities reported here are close to those reported for the coast of Nova Scotia and the Gulf of St. Lawrence (Lawrence *et al.,* 2000; Levasseur *et al.,* 2001).

The presence of DSP-type toxins in shellfish digestive glands indicates that uptake occurs; so far, levels remained safely below the accepted tolerance level of 0.2 µg/g whole shellfish (wet weight) (Anon., 2001). Positive samples will be further analyzed by liquid chromatography-tandem mass spectrometry to determine the type of DSP toxins. The limited toxin data do not yet allow evaluation of our second hypothesis.

The difficulty of quantitatively evaluating unicellular populations within epiphytic habitats cannot be ignored. Samples with a high background of detritus and other organisms are particularly tedious to enumerate, especially when *P. lima* is accompanied by other *Prorocentrum* species such as *cassubicum* (Maranda *et al.,* 2000) and cf. *mexicanum.* The advent of molecular probes should significantly ease detection and enumeration of *P. lima.*

## Acknowledgements

This study is funded by ECOHAB-NOAA NA160P1455. Dr. Petra Stegman is acknowledged for map design.

## References

S. L. Morton, T. A. Leighfield, B. L. Haynes, D. L. Petitpain, M. A. Busman, P. D. R. Moeller, L. Bean, J. McGowan, J. John W. Hurst, and F. M. Van Dolah, J. Shellfish Res. 18, 681–686 (1999).

L. Maranda, M. D. Keller, J. W. Hurst, L. L. Bean, J. D. McGowan, and P. E. Hargraves, J. Shellfish Res. 19, 1003–1006 (2000).

D. O. Mountfort, G. Kennedy, I. Garthwaite, M. A. Quilliam, P. Truman, and D. J. Hannah, Toxicon 37, 909–922 (1999).

J. E. Lawrence, J. Grant, M. A. Quilliam, A. G. Bauder, and A. D. Cembella, Mar. Ecol. Prog. Ser. 201, 147–154 (2000).

M. Levasseur, J.-Y. Couture, G. Sauve, and S. Michaud, Rapport technique canadien des sciences halieutiques et aquatiques, 2350, 41 pp. (2001).

Anon., in: Fish and Fishery Products Hazards and Controls Guide, http://www.cfsan.fda.gov/~comm/haccp4.html, U.S. Food and Drug Administration (2001).

# An Unprecedented Bloom of *Dinophysis acuminata* in Chesapeake Bay

Peter Tango[1], Walt Butler[1], Richard Lacouture[2], David Goshorn[1], Robert Magnien[1], Bruce Michael[1], Sherwood Hall[3], Kathy Browhawn[4], Robert Wittman[5], and William Beatty[4]

[1]*Maryland Department of Natural Resources, 580 Taylor Ave. D-2, Annapolis, MD 21401, USA;* [2]*The Academy of Natural Sciences Estuarine Research Center, St. Leonard, MD 20685, USA;* [3]*US Food and Drug Administration FDA HFS-426, 200 C-Street SW, Washington, DC 20204, USA;* [4]*Maryland Department of the Environment, 1800 Washington Blvd. Suite 540, Baltimore, MD 21230, USA;* [5]*Virginia Department of Health, Division of Shellfish Sanitation, 1500 E. Main St. Room 109, Richmond VA 22520, USA*

## Abstract

*Dinophysis acuminata* has been encountered infrequently and typically at low abundance (~$1 \times 10^3$ cells $L^{-1}$) in the Maryland waters of Chesapeake Bay since routine monitoring began in 1985. In February 2002, the Maryland Department of Natural Resources (MD DNR) Chesapeake Bay Monitoring Program initially detected *D. acuminata* with elevated abundance ($5 \times 10^3$ to $3.5 \times 10^4$ cells $L^{-1}$) at several monitoring sites in the lower Potomac estuary. Subsequent sampling by the Maryland Department of the Environment (MDE), the Academy of Natural Sciences (ANSERC), and Virginia Department of Health (VDH) detected concentrations to $4.6 \times 10^4$ cells $L^{-1}$ and revealed a broadly distributed, persistent bloom in the main stem Potomac estuary and its tributaries. Such concentrations found elsewhere in the world have been associated with elevated potential for Diarrhetic Shellfish Poisoning (DSP). MDE and VDH immediately closed shellfish waters of the Potomac River over concern for DSP cases. Trace amounts of okadaic acid were found by the U.S. Food and Drug Administration (FDA) using LC/MS analysis in algae and shellfish meat samples, but they were below food safety levels generally considered a risk for DSP to humans. Shellfish waters were reopened based on this evidence. This event was associated with a severe drought producing salinity levels above the historical range for this region of the Potomac River estuary.

## Introduction

The genus *Dinophysis* is represented in Chesapeake Bay by five species (*D. acuminata, D. acuta, D. fortii, D. caudata* and *D. norvegica*) that are known to produce okadaic acid or other toxins causing DSP (Marshall, 1996). *Dinophysis acuminata* has been encountered infrequently (1–4× per year, total of 7 samples), typically at low abundances ($1 \times 10^3$ cells $L^{-1}$), and only in recent years (1997–2001) in the Maryland waters of Chesapeake Bay since 1985. Worldwide, *Dinophysis* blooms in coastal regions may produce toxic red tides. Shellfish in bloom regions can accumulate the okadaic acid toxins or derivatives of the toxin when feeding on the algae. Diarrhetic Shellfish Poisoning (DSP) has occurred in humans consuming contaminated shellfish, resulting in symptoms that include intestinal discomfort, abdominal pain, nausea, headache, chills and vomiting. Despite thousands of documented cases of DSP worldwide since 1960, there are no reported fatalities associated with the illness (Anderson *et al.,* 2001).

DSP is a common human health concern in countries such as Italy, Norway and Denmark. Management actions in these countries can include intensified monitoring of shellfish harvest waters, toxin testing of the shellfish, and application of restrictions or closures of the fisheries when cell abundance of *D. acuminata* exceed thresholds of 0.5–1.2 $\times 10^3$ cells $L^{-1}$ (Anderson *et al.,* 2001). On the Atlantic coast, DSP from *Dinophysis* species has previously been confirmed in a single shellfish sample from Narragansett Bay, RI, (USA) during a two-year survey (Maranda and Shimizu, 1987). Europe and Japan appear to be the most highly affected areas for cases of DSP, however, outbreaks of DSP in North America have been confirmed in eastern Canada during 1990 and 1992 (Anderson *et al.,* 2001).

A HAB-based shellfish harvest closure was enacted for the first time in Chesapeake Bay based on concern for a potential DSP outbreak in the bloom area of the Potomac River, Maryland. Subsequent sampling from MD DNR, MDE, ANSERC, VDH, Virginia Department of Environmental Quality and the U.S. FDA tracked the bloom. U.S. FDA conducted LC/MS analyses to test for concentrations of okadaic acid present in the algae and shellfish (Eastern Oyster *Crassostrea virginica*).

## Materials and Methods

The Maryland Department of Natural Resources conducts monthly to biweekly water quality and plankton sampling throughout the tidal waters of Maryland's Chesapeake Bay. There are ten stations routinely monitored on the tidal Potomac River. Data histories for the stations date back to 1980–85 through 2002. Physical and chemical water quality measurements are recorded at each site (*i.e.,* Secchi disk depth, temperature, salinity and dissolved oxygen concentrations), complementing lab-derived chemical parameters. Surface water is collected at all stations for phytoplankton counts during water quality sampling. Phytoplankton was identified to the lowest practical taxon. Identification of bloom levels of *Dinophysis* prompted rapid response sampling to assess distribution and abundance of the bloom on the middle Potomac River (Fig. 1). The FDA conducted toxin analysis using LC/MS on algal and shellfish meat (*C. virginica*) samples collected in the bloom region. Shellfish samples were collected in Maryland



Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

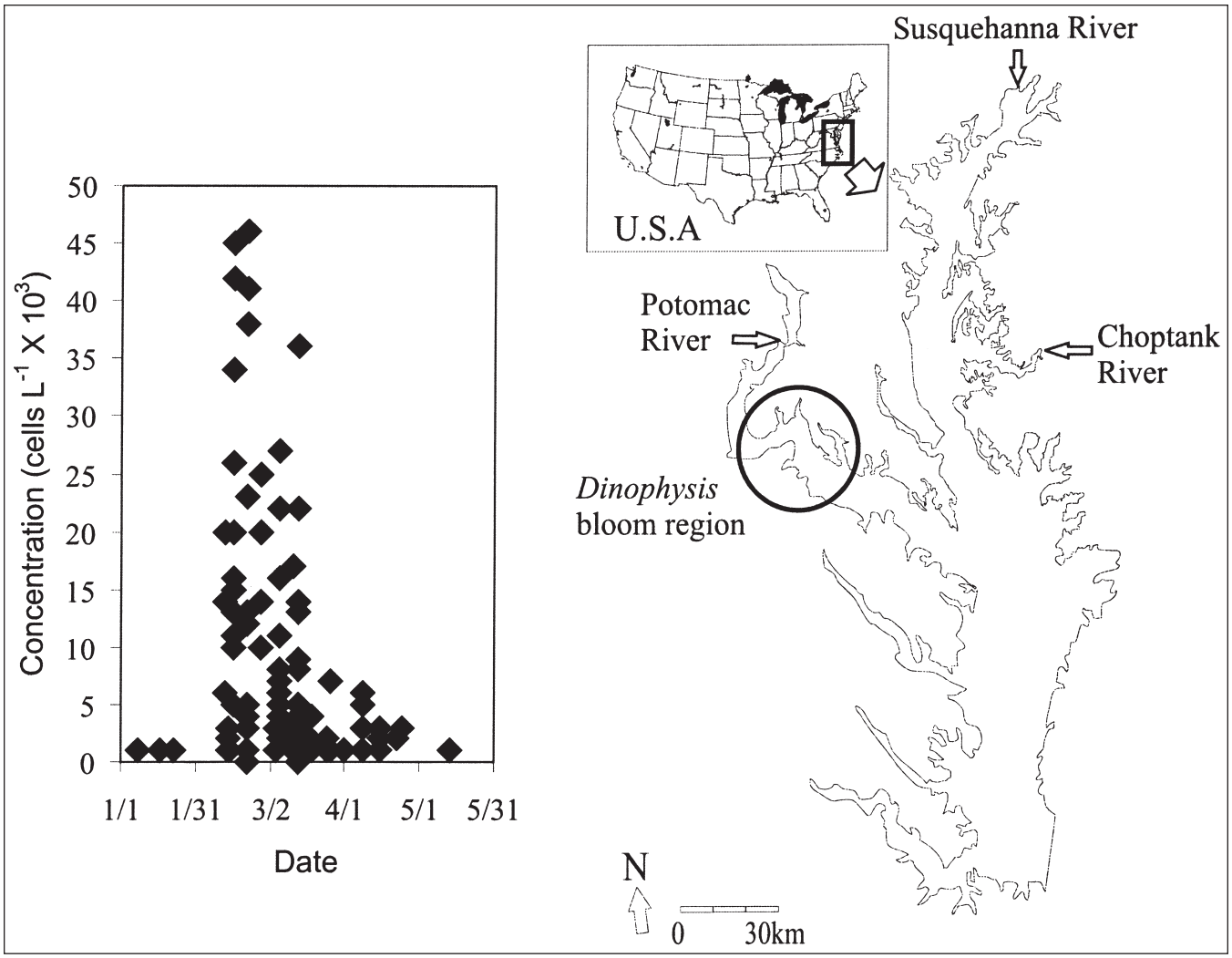


**Figure 1** Time series and location for the *Dinophysis acuminata* bloom during 2002 on the Potomac River, Chesapeake Bay, USA.

and Virginia from the area experiencing the highest algal concentrations in the middle Potomac River (to $4.6 \times 10^4$ cells $L^{-1}$, Fig. 1).

## Results

***2002* Dinophysis *Bloom Detection, Chronology and Response*** *Dinophysis acuminata* was recorded at $1 \times 10^3$ cells $L^{-1}$ from stations on the lower Patuxent and Potomac rivers during January 2002. However, routine samples collected on February 12, 2002, showed *D. acuminata* at elevated concentrations ($6 \times 10^3$ to $2.0 \times 10^4$ cells $L^{-1}$) from the lower Potomac River, with a presence from the main bay upstream to the middle Potomac region where the greatest concentrations were located and enhanced monitoring was put in place (Fig. 1).

Maryland was experiencing a severe drought and salinity levels reached record levels of 18 ppt in the Potomac River segment, 2 ppt above the highest levels recorded since 1985, as bay waters reached farther into the tributaries under the low flow conditions. We believe the plankton bloom was partly a function of the intrusion of the lower bay waters well up into the Potomac estuary delivering the seed population for the bloom.

On February 15, 2002, MDE and ANSERC conducted additional water sampling on the river and confirmed *D. acuminata* at abundances as high as $4.6 \times 10^4$ cells $L^{-1}$, well above normal for our region. Maryland Department of the Environment subsequently initiated a temporary closing of shellfish waters based on the potential human health risks posed by possible toxicity associated with the algal bloom. The presence of the algae also led Virginia officials to close Virginia's Potomac River tributaries south of the Route 301 bridge to shellfish harvesting, including the Little Wicomico River. Weekly water sampling was initiated that defined the lower Potomac River as the significant bloom area. Cell concentrations reached a season high of $4.6 \times 10^4$ cells $L^{-1}$ on February 21, 2002, for Maryland sampling. Additional sampling in the region by Virginia authorities recorded a season high of $2.36 \times 10^5$ cells $L^{-1}$ in the Yeocomico tributary to the Potomac River on February 28, 2002 (R. Wittman, pers. comm.). Routine water sampling programs continued sampling the region, and

the final presence of *D. acuminata* was noted on April 24 on the Potomac River and May 13 on the lower Patuxent River. Salinities had declined to normal historical ranges and water temperatures were increasing in the region.

***Toxin Testing*** The U.S. FDA identified the algal-produced toxin okadaic acid in low concentrations through LC/MS analysis of algae samples collected on February 26, 2002, from the Potomac River. *Dinophysis* concentrations had declined at most sites compared to the previous week but remained well above average levels historically observed for the region. On March 6, 2002, samples of shellfish were collected in Maryland and Virginia waters to be processed for toxin testing. LC/MS analysis of algae and shellfish meats collected from the Potomac River shellfish waters showed only trace amounts of toxin, comfortably below public health standards associated with food safety. In response to these findings, MDE and VDH reopened the waters of the Potomac River to shellfish harvesting for the remainder of the season.

## Discussion

Maryland's Water Quality Monitoring Program with routine sampling provides long-term data for trend analysis but has the added benefit of an early warning of possible HAB events. In response to the *Dinophysis* findings on the Potomac River, rapid response activities were effectively coordinated between states and agencies defining the bloom region. *Dinophysis* abundance is not a definitive indicator of toxicity risk (Anderson *et al.,* 2001), however, countries such as Denmark, Italy and Spain use concentration data to trigger toxin testing in their shellfisheries as we did for this unprecedented event in our monitoring record. Okadaic acid concentrations were detectable below food safety levels, but the findings are significant as the first detection of the algal toxin in Chesapeake Bay.

## Acknowledgements

Appreciation is given to participation in the monitoring and response events by Maryland Department of Natural Resources, Maryland Department of the Environment, the Academy of Natural Sciences Estuarine Research Center, Virginia Department of Health and the U.S. Food and Drug Administration.

## References

D.M. Anderson, P. Anderson, V.M. Bricelj, J.J. Cullen and J.E. Rensel, Monitoring and management Strategies for Harmful Algal Blooms in Coastal Waters, APEC #201-MR-01.1, Asia Pacific Economic Program, Singapore, and IOC Tech Series No. 59, Paris (2001).

H.G. Marshall, Va. J. Sci. 47, 29–38 (1996).

L. Maranda and Y. Shimizu, Estuaries 10, 298–302 (1987).

# Occurrence of *Karlodinium micrum* and Its Association with Fish Kills in Maryland Estuaries

David Goshorn[1], Jonathan Deeds[2], Peter Tango[1], Charles Poukish[3], Allen Place[2], Margaret McGinty[1], Walter Butler[1], Christopher Luckett[3], and Robert Magnien[1]

[1]*Maryland Dept. Natural Resources, Resource Assessment Service, 580 Taylor Ave., D-2, Annapolis, MD 21041, USA;* [2]*Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 701 E. Pratt St., Suite 236, Baltimore, MD 21202, USA;* [3]*Maryland Dept. Environment, 1800 Washington Blvd., Suite 540, Baltimore, MD 21230-1718, USA*

## Abstract

Historic phytoplankton monitoring and fish kill investigation databases were analyzed to draw conclusions regarding spatial and temporal distribution of *Karlodinium micrum* and its association with fish kills in Maryland estuaries. *Karlodinium micrum* is a common dinoflagellate species in Maryland, with highest densities occurring at moderate salinities and warm temperatures. The Patapsco Estuary is a focal point of Maryland blooms during the past two decades. Utilizing analytical chemistry and bioassays in addition to field observations, *K. micrum* was identified as the probable cause of one recent fish kill. The historic databases suggest this species as being involved in nine other kills between 1988 and 2001.

## Introduction

*Karlodinium micrum* is a common estuarine dinoflagellate found along the US east coast and reported from several other locations worldwide (Nielson, 1996; Li *et al.,* 2000; Terlizzi *et al.,* 2000; Kempton *et al.,* 2002; Lewitus and Holland, 2002). Deeds *et al.* (2002) have demonstrated that *K. micrum* produces several lipid-soluble fractions with hemolytic, cytotoxic, and ichthyotoxic properties. This work also suggests that toxin release may be stimulated by agitation of the cells, and that some Maryland isolates may produce sufficient toxin to result in fish mortality in the field at cell densities of 10,000 to 30,000 cells/mL and above. Deeds *et al.* (2002) have presented strong evidence that this species contributed to three fish kills at a Maryland aquaculture facility, and reports by Kempton *et al.* (2002) suggest a similar association between *K. micrum* and a South Carolina fish kill.

We examined data from the Maryland Department of Natural Resources (DNR) long-term phytoplankton monitoring program and the Maryland Department of Environment (MDE) fish kill investigation program in order to better understand the spatial and temporal distribution of this organism and its possible association with historic fish kills in Maryland estuaries.

## Materials and Methods

***Temporal and Spatial Distribution*** Since the early 1980s Maryland has maintained a phytoplankton monitoring program at approximately 20 stations sampled monthly in the tidal portions of the Chesapeake Bay mainstem and tributaries. Numerous other stations have also been sampled throughout Maryland's Chesapeake and Coastal bays and their tributaries for a variety of other reasons with varying spatial and temporal intensity. Maryland DNR has consistently used one staff phycologist to identify algal species collected through these programs. All samples were examined live. Genetic probes developed by Tengs *et al.* (2001) were used to confirm that the species historically identified by Maryland DNR as *Gyrodinium estuariale* was synonymous with *K. micrum* (Daugbjerg *et al.,* 2000).

Temporal and spatial distributions were evaluated using the DNR long-term phytoplankton database. We calculated temporal distribution as the mean monthly density of *K. micrum* cells from all samples in which the dinoflagellate was present at >1 cell/mL. One extremely dense sample (322,968 cells/mL collected September 2001) was omitted from this analysis. To investigate spatial distribution, we calculated mean density per station when *K. micrum* was present. In order to minimize bias resulting from one dense sample collected at a station, or stations sampled primarily during one season, we limited the analysis to those stations sampled April through September and which had a minimum of three samples with *K. micrum* present.

Finally, we also examined associated salinity and temperature as a function of cell density for those samples for which corresponding physical data were available.

***Association with Fish Kills*** We examined the association between *K. micrum* and fish kills in Maryland through a field and laboratory investigation of a 2002 fish kill and by reviewing the 1988–2001 Maryland fish kill database (MDE) for possible *K. micrum*-related kills. Factors considered in determining if *K. micrum* may have been involved in the kill included a) presence and density of associated *K. micrum,* b) laboratory toxin and bioassays of associated water (2002 kill only), and c) other possible causes defined as commercial or recreational fishery discards, dissolved oxygen below 2.5 mg/L, obvious death due to disease or natural stress, chemical spill, entrapment, or stranding. Based on these criteria, the role of *K. micrum* toxin as the primary causative agent in the kill was classified as "probable" (*K. micrum* present at potentially lethal densities, laboratory evidence of toxic activity, and no other cause evident), "possible" (*K. micrum* present at potentially lethal densities, and no other cause evident), or "associated" (*K. micrum* present at sub-lethal densities, and no other cause evident).

## Results and Discussion

***Temporal and Spatial Distribution*** Of approximately 7,000 phytoplankton samples collected, *K. micrum* was

Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

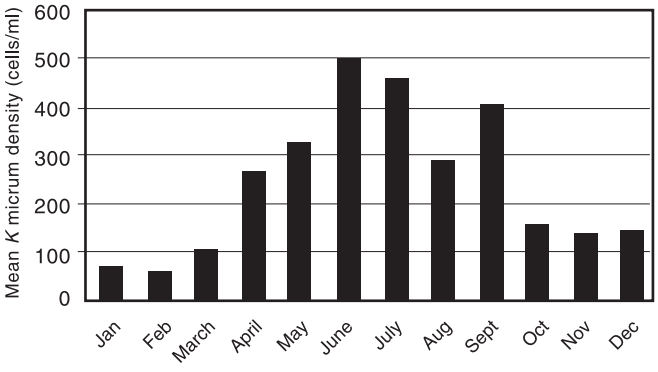


**Figure 1** Mean monthly *K. micrum* density, when present, at all Maryland stations (n = 1,311). One extremely high September sample (322,968 cells/mL) was omitted.

present in 1,312 (18%). Densities when present ranged from 1 cell/mL to 322,968 cells/mL. Mean density when present was 589 cells/mL. Mean density omitting the 322,968 cells/mL sample was 343 cells/mL. *Karlodinium micrum* was present in all months, but highest mean monthly densities (>200 cells/mL) occurred April through September (Fig. 1). Highest mean densities at routinely sampled sites were observed in the Patapsco, lower Patuxent, and mid-Potomac estuaries in the Chesapeake Bay, and the upper St. Martin estuary in the Coastal Bays. Twelve one-time bloom events of greater then 10,000 cells/mL were also plotted (Fig. 2). The greatest density recorded at a bloom event occurred on Mill Creek (a small tributary of the Severn River) on September 6, 2001, with a *K. micrum* density of 322,968 cells/mL. The Patapsco Estuary and its tributaries contained both the routine monitoring station with the highest mean *K. micrum* density (mean = 1,117 cells/mL when cells present, n = 126), and was the site of six of the 12 bloom events exceeding 10,000 cells/mL. The concentration of bloom events in the Patapsco Estuary may partially be an artifact of the greater likelihood that bloom events and fish kills would be reported in this densely populated area, leading to the collection of additional non-routine samples. However, the fact that the routine monitoring station at this site had a mean cell density almost twice that of the next highest routine station, and that eight of the nine instances of cell counts exceeding 5,000 cells/mL found at routine stations were in this estuary, is strong evidence that conditions in the Patapsco regularly promote growth of this organism to bloom densities.

Of the 1,312 samples containing *K. micrum,* 882 had accompanying salinity and temperature data. *Karlodinium micrum* was found over a wide range of these variables, occurring at salinities from 0.6 ppt to 24.8 ppt and temperatures from 3.3°C to 31.6°C. The highest densities of *K. micrum* were found at salinities in the middle of the range and at temperatures in the higher end of the range. Population densities above the 99th percentile (>4,824 cells/mL) occurred at salinities ranging from 8.6 ppt–10.6 ppt and temperatures ranging from 21.5°C to 27.5°C.

***Association with Fish Kills*** Analysis of the historic fish kill database revealed 21 kills at which *K. micrum* was present, ten of which were identified for potential *K. micrum* involvement (Fig. 3).

On June 24, 2002, the MDE investigated a fish kill at Fishing Creek of approximately 1,700 Atlantic menhaden (*Brevoortia tyrannus*) and a variety of other species including Atlantic silversides, mummichog, hogchoker, Atlantic croaker, skilletfish, and bay anchovy. Mid-day dissolved oxygen concentrations ranged from 3.9–9.2 mg/L. The water was observed to have a distinct red / brown coloration. Live water samples analyzed by DNR within five hours of collection revealed a *K. micrum* bloom of 40,810 cells/mL. Splits of water samples were sent to the University of Maryland Center of Marine Biotechnology (COMB) for analysis of toxin presence and hemolytic activity as per Deeds *et al.* (2002). Results indicated that significant quantities of *K. micrum* hemolytic fractions were present in the water, and that near 100% hemolysis was obtained

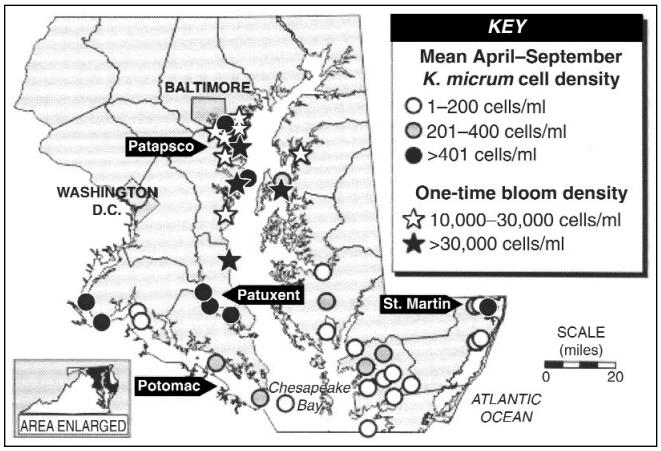


**Figure 2** Mean April– September *K. micrum* densities (cells/mL) at 30 stations in Maryland's Chesapeake and Coastal bays 1981–2001. Also presented are sites of one-time *K. micrum* bloom events greater than 10,000 cells/mL.

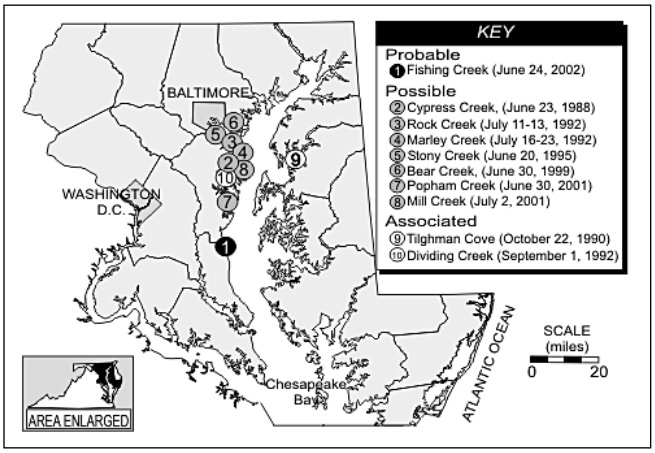


**Figure 3** Maryland fish kills, 1988–2002, with potential *K. micrum* involvement.

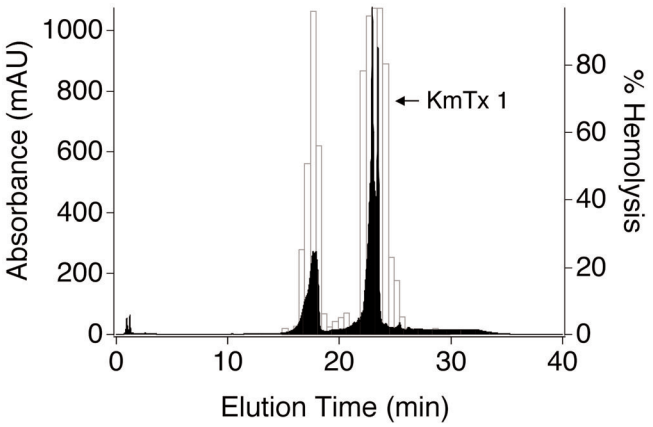


**Figure 4** Reversed-phase HPLC elution profile of the filtrate from the June 24, 2002, Fishing Creek fish kill. The overlaid histogram is the hemolytic activity associated with the same sample. KmTx 1 is further described in Deeds *et al.* (this Proceedings).

with sample aliquots corresponding to these fractions (Fig. 4). Based on these results, and the lack of any other apparent cause of this fish kill, we conclude that *K. micrum* toxin was the "probable" cause of this kill.

*Karlodinium micrum* was identified as the "possible" cause of seven other fish kills ranging in size from 28 to 30,000 fish (Fig. 3). All involved multiple species with daytime dissolved oxygen concentrations above 5.0 mg/L. *Karlodinium micrum* densities at the time of the investigations ranged from 10,270–39,750 cells/mL. Four days after one of these kills (Mill Creek, July 2, 2001), follow-up investigations revealed the highest *K. micrum* densities yet recorded in Maryland at 322,968 cells/mL.

It is important to note that MDE monitoring has identified four other *K. micrum* blooms of greater than 10,000 cells/mL with no associated fish kill. Analysis by the COMB laboratory of water from one of these blooms (Back Creek, August 21, 2002), determined that little or no toxic fractions were present.

Finally, *K. micrum* was identified as "associated" with two other kills (Fig. 3). These two kills involved 50 and 2,500 fish, had daytime dissolved oxygen concentrations above 5.0 mg/L, but *K. micrum* densities at the time of collection below the lethal limit of 10,000 cells/mL suggested by Deeds *et al.* (2002) (1,375 and 8,037 cells/mL).

## Acknowledgements

The authors thank the numerous field and laboratory staff at Maryland Departments of Natural Resources and Environment and at the University of Maryland Biotechnology Institute who assisted in these investigations. Thanks are also given to Tom Parham (Md DNR) for assistance in graphics. This research was funded by grants from NOAA ECOHAB (NA860PO492) and NIEHS (PO1-ES9563). This is contribution #04-609 from the Center of Marine Biotechnology. This is contribution #91 from the ECOHAB program.

## References

N. Daugbjerg, G. Hansen, J. Larsen, and Ø. Moestrup, Phycologia 39, 302–317 (2000).

J. Deeds, D. Terlizzi, J. Adolf, D. Stoecker, and A. Place, Harmful Algae 1, 169–189 (2002).

J. Kempton, A. Lewitus, J. Deeds, J. Law, and A. Place, Harmful Algae 1, 233–241 (2002).

A. Lewitus and A. Holland, in: Proceedings of the EMAP Symposium, 2001 (special issue), 366–375 (2002).

A. Li, D. Stoecker, and D. Coats, J. Plankton Res. 22, 2105–2124 (2000).

M. Nielson, Mar. Ecol. Prog. Ser. 136, 205–211 (1996).

T. Tengs, H. Bowers, A. Ziman, D. Stoecker, and D. Oldach, Mol. Ecol. 10, 551–523 (2001).

D. Terlizzi, D. Stoecker, and P. Glibert, in: Abstracts of Contributions Presented at the International Conference AQUA 2000, Responsible Aquaculture in the New Millenium, R. Flos and L. Cressel, eds. p. 700 (2000).

# Extended Bloom Concentration of the Toxic Dinoflagellate *Dinophysis acuminata* in Virginia Estuaries During Late Winter Through Early Spring, 2002

Harold G. Marshall[1], Todd Egerton[1], Todd Stem[1], Jeremy Hicks[1], and Mikolaj Kokocinski[2]
[1]*Department of Biological Sciences, Old Dominion University, Norfolk, VA 23529-0266;*
[2]*Department of Hydrobiology, Adam Mickiewicz University, Poznan, Poland*

## Abstract

*Dinophysis acuminata* blooms and discolored water occurred in February 2002 in the Potomac River and several of its small embayments. Further blooms followed at 17 sites along the Virginia inlets and creeks and southward along the Chesapeake Bay western shoreline. Blooms of *D. acuminata* continued at several sites until April and then declined in May. Blooms were more common within mesohaline conditions and temperatures <18°C. The Food and Drug Administration found trace amounts of okadaic acid in oysters, below the regulatory limit. This is the first report of *D. acuminata* blooms in Virginia waters of the Chesapeake Bay estuarine system.

## Introduction

*Dinophysis acuminata* Claparède and Lachmann (Dodge, 1982) is a common bloom-producing dinoflagellate that has a broad global distribution (Aune and Yndestad, 1993) including the Atlantic coastal regions of North America (Freudenthal and Jujina, 1988; Cembella and Todd, 1993). Blooms have frequently occurred during upwelling events from late spring to autumn, often patchy in development in subsurface waters and at the pycnocline (Chang, 1996, Dahl *et al.,* 1996, Palma *et al.,* 1998; Marcaillou *et al.,* 2001). *D. acuminata* may produce the lipophilic toxin okadaic acid (OA) that is responsible for diarrhetic shellfish poisoning (DSP) (Yasumoto *et al.,* 1980; Kat, 1983; Aune and Yndestad, 1993). Reports of cell concentrations of *D. acuminata* producing DSP include a range from ca. 200 cells/L, and >$10^3$ cells/L, to 20,000 cells/L (Yasumoto *et al.,* 1980; Lassus *et al.,* 1985; Kat, 1983). This range has been attributed to the variability in the toxin content of these cells (Anderson *et al.,* 1996; Marcaillou *et al.,* 2001). Thus, high cell concentrations will not necessarily indicate greater incidence of DSP (Sato *et al.,* 1996), and deficient levels in phosphorus and nitrogen have been associated with increased OA production (Granéli *et al.,* 1998). Bloom concentrations of this species in Chesapeake Bay have not previously been reported, nor has any major development of this species occurred in the regional rivers entering Chesapeake Bay. However, between February and May 2002, bloom concentrations of *D. acuminata* occurred in the Potomac River and the small bays and creeks along the Virginia shoreline of this river.

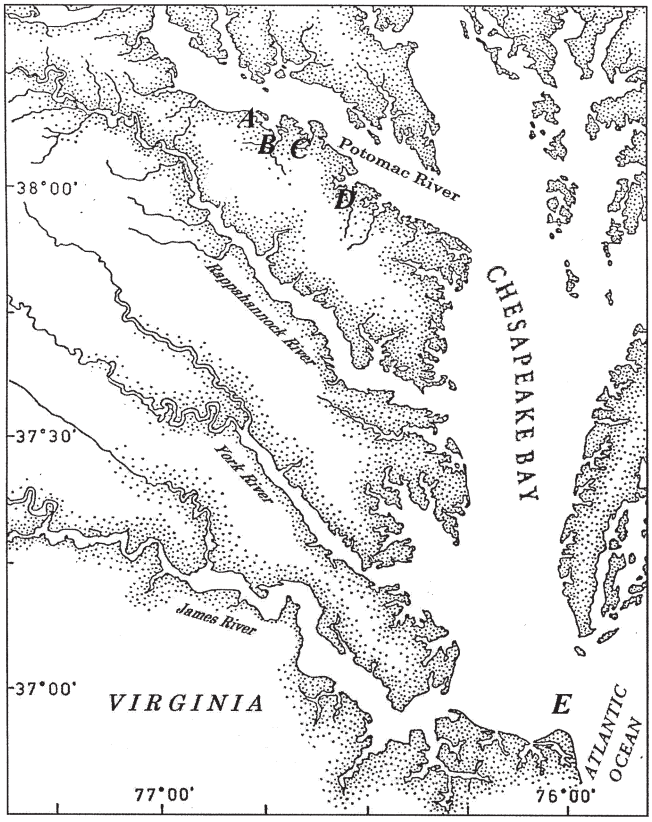


**Figure 1** Station locations for Currioman Bay (A), Lodge Creek (B), Lower Machodoc Creek (C), Yeocomico River (D), and Chesapeake Bay (E).

## Materials and Methods

Personnel from the Virginia Department Health Division of Shellfish Sanitation provided water samples (n = 312) from 17 stations located in several Virginia rivers, creeks, and inlets (Fig. 1) from February through May 2002. Subsurface one-liter samples (1 m depth) were collected and fixed immediately with Lugol's solution (4 mL). Aliquots from these samples were examined using a Palmer-Maloney counting cell at 500× magnification using a Nikon TS100 microscope. The entire field of the Palmer-Maloney cell was scanned for determining cell abundance. Replicate counts were made for quality control. Epifluorescent microscopy was used to determine presence of chloroplasts. Representative samples were processed for examination using a Leo Model 435VP SEM. Additional water samples (n = 84) were obtained from the Chesapeake Bay from above and below the pycnocline, which were examined microscopically for cell counts. On station, Secchi depths and water tem-



Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

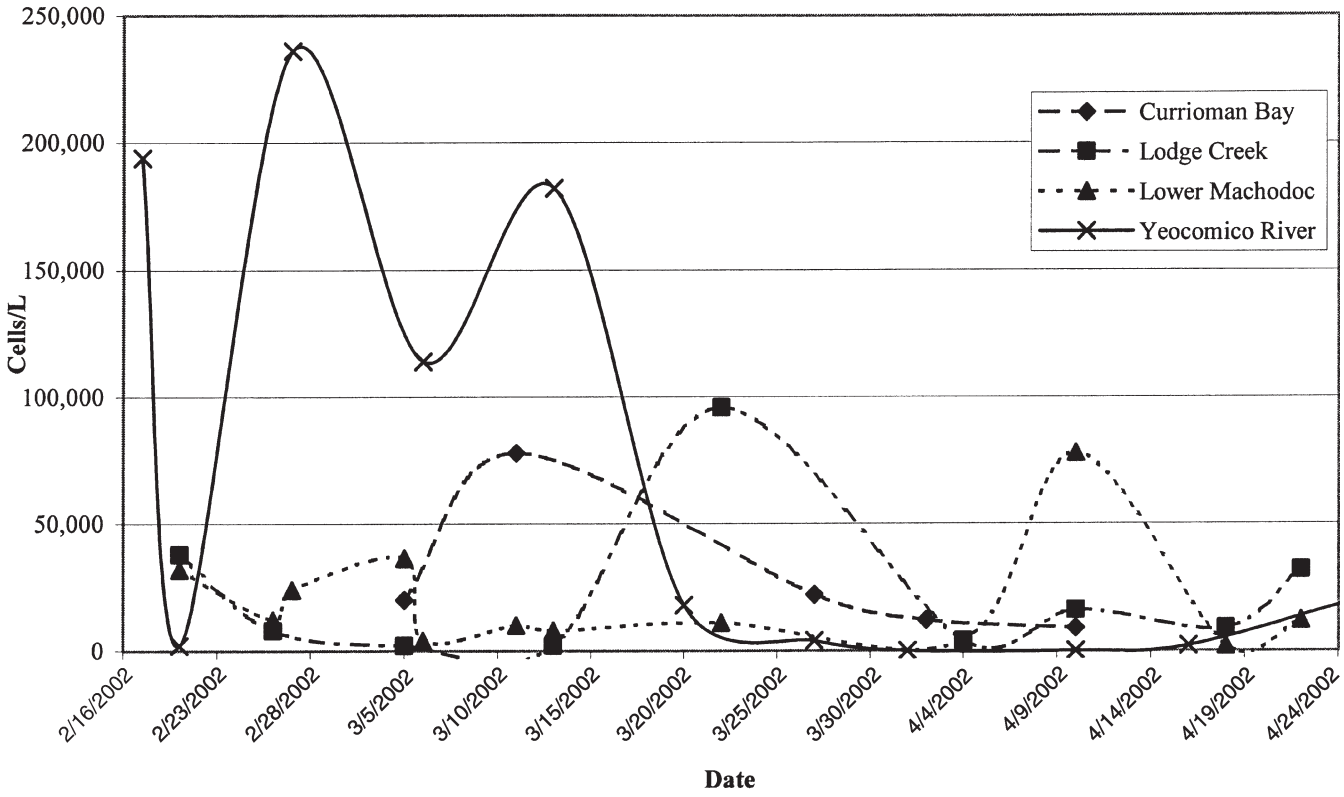


**Figure 2** Monthly concentrations of *D. acuminata* at major bloom stations, February–April 2002.

perature were recorded, with water samples taken for salinity, pH, and oxygen determination.

## Results

Bloom conditions and discolored water occurred February 17, 2002, in the Potomac River and several of its small embayments with *D. acuminata* at ca. 14,000 cells/L. Additional blooms followed at 17 sites along the Virginia inlets and creeks, and southward along the Chesapeake Bay western shoreline between the Potomac and Rappahannock Rivers. Bloom concentrations of *D. acuminata* continued at several sites until April, before declining in May. The greatest abundance of *D. acuminata* (>20,000 cells/L) occurred at 4 of these sites. These were Currioman Bay, Lodge Creek, Lower Machodoc Creek, and the Yeocomico River (Fig. 2). At 6 other locations, the mean cell concentrations ranged between 5,000 and 20,000 cells/L. The other locations (7) had mean values less than 5,000 cells/L. Peak concentrations for February, March, April, and May were 236,000, 150,000, 94,000, and 32,000 cells/L, respectively. The U.S. Food and Drug Administration tested shellfish meat (oysters) for OA and found trace concentrations of OA, and closure of shellfish harvesting beds followed. During these blooms, there was considerable variability in the morphology of the hypotheca and its lists of the *Dinophysis* cells, which contained chloroplasts. At these sites no other *Dinophysis* taxon was observed. However, during these blooms there were other dinoflagellates in high concentrations (*e.g., Prorocentrum minimum, P. micans, Heterocapsa triquetra*), and in lesser numbers were also common diatoms (*e.g., Skeletonema potamos, S. costatum*). In analysis, there were no close statistical correlations between *D. acuminata* blooms to temperature, salinity, dissolved oxygen, pH, or Secchi depth. High concentrations of *D. acuminata* occurred within the following ranges: temperature (4.4 to 20.7°C), salinity (10.6 to 21.3 ppt), DO (3.8 to 13.1 mg/L), pH (6.9 to 8.4), and Secchi (0.4 to 3.5 m). The blooms were more common within mesohaline conditions and temperatures <18°C. The Chesapeake Bay samples in November–December 2001 had *D. acuminata* concentrations <500 cells/L, but these counts were increasing in January 2002 below the pycnocline to ca. 3,000 cells/L. Prior to these blooms, the above pycnocline waters in the Bay contained <500 cells/L of *D. acuminata.* However, these less saline surface waters moving out of the bay had increased cell concentrations of this species beginning in March 2002 (ca.1000 cells/L).

## Discussion

The bloom of *D. acuminata* was a unique event. This is the first report of a major bloom by this taxon in the Chesapeake Bay estuarine system, and more specifically in the Potomac River. It is suggested that the species originated in coastal waters and was introduced to these locations via passage in sub-pycnocline waters moving northward in Chesapeake Bay. This sub-pycnocline advancement may

have been enhanced due to reduced regional rainfall preceding this event, with subsequently less freshwater moving into these estuaries. Associated with the *Dinophysis* development were abundant subdominant dinoflagellates and diatoms. This is indicative of adequate nutrient levels that were present that may also been counterproductive to *Dinophysis* toxin production (Granéli *et al.,* 1998). The absence of a major shellfish contamination by *D. acuminata* toxins during these periods of high abundance may also be attributed to the variability of toxin production that has been associated with this species (Anderson *et al.,* 1996; Marcaillou *et al.,* 2001; Sato *et al.,* 1996).

## References


P. Anderson, B. Hald, and H. Emsholm, In: Harmful and Toxic Algal Blooms, T. Yasumoto and Y. Oshima, eds., Intergov. Oceanogr. Comm. of UNESCO, pp. 281–284 (1996).

T. Aune and M. Yndestad, In: Algal Toxins in Seafood and Drinking Water, I. Falconer, ed., Academic Press. Harcourt Brace and Co. Publ., London, pp. 87–104 (1993).

A.D. Cembella and E. Todd, In: Algal Toxins in Seafood and Drinking Water, I. Falconer, ed., Academic Press. Harcourt Brace and Co. Publ., London, pp. 129–144 (1993).

F.H. Chang, In: Harmful and Toxic Algal Blooms, T. Yasumoto and Y. Oshima, eds., Inter. Oceanogr. Comm. of UNESCO, pp. 93–96 (1996).

E. Dahl, T. Aune, and B. Aase, In: Harmful and Toxic Algal Blooms, T. Yasumoto and Y. Oshima, eds., Inter. Oceanogr. Comm. of UNESCO, pp. 93–96 (1996).

J.D. Dodge, Marine Dinoflagellates of the British Isles. Her Majesty's Stationery Office, London, 1–304 pp. (1982).

A.R. Freudenthal and J.L. Jijina, J. Shellfish Res. 7:695–695 (1988).

E. Granéli, N. Johansson, and R. Panosso, In: Harmful Algae, B. Reguera, J. Blanco, M. Fernandez, and T. Wyatt eds., Xunta de Galicia and Intergov. Oceanogr. Comm. of UNESCO, pp. 321–324 (1998).

M. Kat, Sarsia 68:81–84 (1983).

P. Lassus, N. Bardouil, I. Truquet, P. truquet, C. LeBaut, and M. Pierre, In: Toxic Dinoflagellates, D. Anderson, A. White, D. Baden, eds., Elsevier Publ., N.Y. pp. 159–164 (1985).

C. Marcaillou, P. Gentien, M. Lunven, J. LeGrand, F. Mondeguer, M. Danilou, M. Crassous, and A. Youenou, In: Harmful Algal Blooms 2000, G. Hallegraeff, S. Blackburn, C. Bolch, and R. Lewis eds. Intergov. Oceanogr. Comm. of UNESCO, pp. 356–359 (2001).

A.S. Palma, M.G. Vilarinlho and M.T. Moita, In: Harmful Algae. B. Reguera, J. Blanco, M. Fernandez, and T. Wyatt, eds., Xunta de Galicia and Intergov. Oceanogr. Comm. of UNESCO, pp. 124–127 (1998).

S. Sato, K. Koike, and M. Kodama, In: Harmful and Toxic Algal Blooms, T. Yasumoto and Y. Oshima, eds, Inter. Oceanogr. Comm. of UNESCO, pp. 285–288 (1996).

T. Yasumoto, Y. Oshima, W. Sugawara, Y. Fukoyo, H. Oguri, T. Igarashi, and N. Fujita, Bull. Jpn. Soc. Sci. Fish. 46:1405–1411. (1980).

# Volunteer Phytoplankton Monitoring in the Inland Bays of Delaware, USA

Edward B. Whereat[1], Joseph G. Farrell[1], and Edythe M. Humphries[2]
[1]*Delaware Inland Bays Citizen Monitoring Program, University of Delaware, Sea Grant Marine Advisory Service, 700 Pilottown Road, Lewes, DE 19958, USA;* [2]*Delaware Department of Natural Resources and Environmental Control, Division of Water Resources, 89 Kings Hwy, Dover, DE 19901, USA*

## Abstract

The Delaware Inland Bays Citizen Monitoring Program was established to enlist area volunteers in the collection of water quality data, and was expanded in 2001 to include a harmful algal bloom (HAB) monitoring component. Volunteers attended training sessions that included an introduction to phytoplankton taxonomy and the use of laboratory and field microscopes. Episodic blooms of *Chattonella subsalsa, C.* cf. *verruculosa, Prorocentrum minimum* and *Gyrodinium instriatum* in the Inland Bays (IB) were identified by volunteers and confirmed by the volunteer coordinator or by phytoplankton taxonomists at various state and university laboratories. The ability of volunteers to sample frequently and at sites not sampled by the State provided valuable spatial and temporal coverage and can be an effective early warning system for HABs.

## Introduction

In 1991, the Delaware Inland Bays Citizen Monitoring Program was established to enlist area volunteers in the collection of water quality data used to augment monitoring efforts by the state in compliance with the Clean Water Act. The program is managed by the University of Delaware, Sea Grant Marine Advisory Service (UD SGMAS) through the Division of Water Resources (DWR) of the Delaware Department of Natural Resources and Environmental Control (DNREC). Currently, thirty dedicated volunteers collect water quality data at 25 sites around the IB weekly. The IB and its tributaries are relatively small, covering 34 square miles along the mid-Atlantic coast.

In 2001, in recognition that many species of potentially harmful algae could exist in the IB, a pilot phytoplankton monitoring component was added to the program. Inspiration came from other states that utilize volunteer phytoplankton monitors as a cost-effective early warning system for shellfish toxicity (Ely, 1998 and 2000). Dr. Sherwood Hall, US Food and Drug Administration encouraged states to initiate these programs. Ten of our volunteers were interested in phytoplankton identification and participated in the program.

The purpose of the phytoplankton monitoring program is to

- increase the spatial and temporal coverage of monitoring for HABs in the IB,
- improve the predictive capability of HAB occurrence for water quality management and public health alerts,
- improve public understanding of phytoplankton blooms by engaging citizen volunteers as IB stewards, and
- provide significant samples to the DNREC HAB Monitoring Program, and to researchers at the University of Delaware, College of Marine Studies (UD CMS) and other institutions.

## Materials and Methods

Each of the volunteers attended three, two-hour training sessions that included an introduction to the taxonomy of phytoplankton, reference materials, and the use of laboratory and field microscopes. During the training, volunteers examined UD CMS cultures of phytoplankton, as well as live and preserved water samples. The number of species identified increased with the volunteers' experience in phytoplankton identification. Learning phytoplankton identification is an ongoing process, and is proportional to time spent under the scope and perusing reference material (Tomas 1997, and a variety of web sites). The volunteer coordinator, with support from CMS staff and laboratory personnel under contract to DNREC, confirmed volunteer identifications and cell density estimates.

Volunteers utilize their own conventional laboratory microscopes or are provided a Swift FM-31 Field Microscope, noting the magnification and the diameter of the field of view of each objective lens. We also encourage volunteers to screen samples in our laboratory utilizing CMS "teaching" microscopes (American Optical Corp., model 60), because these offer a mechanical stage and precise light control.

Volunteers are cautioned that unpreserved phytoplankton samples must be screened within a few hours after collection because some aids to identification involve shapes, behavior or color that are lost as the cells die. Volunteers are also familiarized with preserved samples since circumstances may prevent timely screening, or fast-moving species might have to be killed for examination.

A surface sample (0–30 cm depth) is collected by bucket or sample collection pole, usually from a dock or bulkhead. After gentle mixing, a wet mount is prepared using a conventional glass slide/cover slip or a glass capillary tube (VitroCom Inc., 0.3 mm × 3 mm × 10 cm). Volunteers use a semi-quantitative scale to gauge cell concentration based on the average number of cells seen per field of view at 100× across many fields of view, or at low concentrations, based on the number seen in a drop of water. Cell density estimates are reported as "present" (<0.1 million cells $L^{-1}$), "common" (0.1–1.0 million cells $L^{-1}$), "many" (1.0–10.0 million cells $L^{-1}$) or "abundant" (>10.0 million cells $L^{-1}$). Although this

Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

process might miss cells found at low densities, volunteers can easily spot blooms in the "many" or "abundant" ranges. The process of collecting, screening and preserving a sample takes from 0.5 to 1 hour, and only a few drops of water are actually examined.

Temperature and salinity, and to a lesser extent, water quality measurements, were made with every sample. Volunteer sampling efforts were expanded during blooms to define the spatial and temporal extent of the event. Samples were used to confirm identifications, acquire cultures to be utilized for physiological experiments or the development of genetic probes, analyze for toxins, or to compliment species identification training.

Observations were reported bi-weekly to DNREC resource managers, UD researchers, The Center for the Inland Bays, and our volunteers during the summer months. When HAB events were encountered, we immediately notified DNREC staff by email or phone.

## Results and Discussion

*Chattonella* cf. v*erruculosa,* a Raphidophyte with the potential to produce brevetoxin (Bourdelais *et al.,* 2002), was the species found in the IB that poses the greatest threat to human health and fisheries. It has been implicated as the cause of local fish kills, and brevetoxin can accumulate in shellfish, posing a human health hazard. *C.* cf. *verruculosa* was reported as present or common (<1 million cells $L^{-1}$) at most sites. Blooms were restricted to upper IB tributaries (>1 million cells $L^{-1}$) and a few residential dead-end canal systems (>10 million cells $L^{-1}$). Blooms were found during August and September, although the species was detected in lower abundance from June through October. Blooms in the residential dead-end canals, where cells may have been concentrated by prevailing winds, persisted for several days. Brevetoxin was detected in the water on one occasion. No fish kills were observed in conjunction with the blooms, which were not near any shellfish harvesting areas.

Other Raphidophytes detected in bloom concentrations include *C. subsalsa, Heterosigma* sp., and *Fibrocapsa japonica.* In general, the spatial and temporal distribution of these blooms was similar to that of *C.* cf. *verruculosa.* Except for the occasional mixed bloom of both species of *Chattonella,* Raphidophyte blooms did not coincide. Toxic dinoflagellates detected in bloom concentrations included *Prorocentrum minimum* and *Gyrodinium instriatum.*

Phytoplankton blooms are often patchy and ephemeral. Since volunteers live on the water, they are apt to notice blooms and have easy sampling access. Although some species cannot be reliably identified using light microscopyand may require expert assistance, adequately trained volunteers can accurately identify HAB species. Volunteer programs are a useful tool to expand the spatial and temporal monitoring efforts of state HAB programs.

## Acknowledgements

The Pilot Program was funded by a grant from The Center for the Inland Bays (CIB). CIB and DNREC provided field microscopes and reference books. CIB and DNREC grants and funds from the United States Environmental Protection Agency and from DE Department of Health and Social Services have continued the program. We are grateful for the support of the University of Delaware, Sea Grant Marine Advisory Service and the UD College of Marine Studies. Most importantly, we thank our volunteers for their efforts and enthusiasm.

## References

A.J. Bourdelais, C. R. Tomas, J. Naar, J. Kubanek, and D. Baden, Environ. Health Perspect. 110, 465–470 (2002).

E. Ely, ed., The Volunteer Monitor, The National Newsletter of Volunteer Water Quality Monitoring 10(2), 4–7 (1998), and 12(1), 20–21 (2000).

C. R. Tomas, ed., Identifying Marine Phytoplankton (Academic Press, San Diego CA), 1–858 (1997).

# Blooms of the Ichthyotoxic Flagellate *Prymnesium parvum* in U.S. Waters: An Emerging or a Perennial Problem?

Carmelo Tomas[1], Joan Glass[2], Jack Ralph[2], and Alan Lewitus[3]

[1]*University of North Carolina at Wilmington, Center for Marine Science, 5600 Marvin Moss Lane, Wilmington, NC 28409, USA;* [2]*Texas Parks and Wildlife, 1601 East Crest Drive, Waco, TX 76705, USA;* [3]*South Carolina Department of Natural Resources, Hollings Marine Laboratory, 331 Ft. Johnson Road, Charleston, SC 29442 USA*

## Abstract

Since 1981, persistent blooms of *Prymnesium parvum* caused recurrent fish-kills in rivers, lakes and reservoirs in Texas. Fish mortality exceeding $12.4 \times 10^6$ fish and a loss of $4.4 million dollars was estimated for a 20-year period. In 2002 alone, $2–5 \times 10^6$ fish were killed in the Brazos, Pecos, Colorado, and Red rivers, with blooms that persist to the present day. In 2001, a bloom of $6.1 \times 10^8$ cells $\cdot$ $L^{-1}$ of *P. parvum* occurred in a golf course pond at Kiawah Island, SC, with no fish mortality reported. During the period from March 2001 through October 2002, one aquaculture facility in northeastern North Carolina had *P. parvum* blooms in 18 of 22 ponds during the warmer months, resulting in a complete loss of stock totaling $318,000 harvest value. The bloom persisted until the low salinity (~4) of the ponds restored to freshwater. In addition, a 3-year study of the New River, NC, also demonstrated the presence of *P. parvum* each year as part of the indigenous flora. While no fish-killing events could be attributed to *P. parvum,* this species is now recognized as a perennial damaging species in Texas, the New River, and northeastern regions of North Carolina. With these observations, *P. parvum* is now established as a resident species and a potentially toxic one for U.S. brackish waters.

## Introduction

Within a decade from its first description (Carter, 1937), *Prymnesium parvum* was identified as causing fish kills in Lake Kinneret, Israel, and in aquaculture facilities established for tilapia culture (Reich, 1947). Since then, this ichthyotoxic flagellate caused massive fish kills in brackish waters in Scandinavia, Europe, Asia, Africa, Australia and China (Edvardsen *et al.,* 2000). Blooms of this species are episodic, often resulting in fish-killing hemolytic activity and, once established, tend to be permanent. Toxicity of *P. parvum* appears to be associated with N or P nutrient stress (Granéli and Johansson, 2003), requires a dark phase for toxin production and is stimulated by aeration. It also can survive extended periods in the dark when supplemented with organic nutrient sources (Antia *et al.,* 1969) and is known for its mixotrophy, both as an auxotroph (osmotrophy, Paster *et al.,* 1966) and as a phagotroph (Nygaard and Tobiesen, 1993, Legrand 2001). To document the emergence of this organism in the U.S., we present observations from three regions of the U.S. having *P. parvum* blooms.

## Materials and Methods

Samples for identification, enumeration and bioassay testing were obtained in Texas from the Texas Parks and Wildlife, in South Carolina from SC DNR (Kiawah Island) and from two sources in North Carolina: Artesian Aquafarms, Elizabeth City, NC (G. Sawyer, manager) and New River Estuary, NC, C. Tomas and the NC Department of Environmental and Natural Resources. This last study consisted of samples from 12 stations taken monthly over a two-year period. All samples were examined for live toxic flagellates. Samples were enumerated for abundance and tested for toxicity using the *Gambusia affinis* fish bioassy (after Ulitzer and Shilo, 1964). Samples containing *P. parvum* were fixed with osmium vapors, rinsed and dried according to the method described by Moestrup and Thompson (1980), and scales observed after staining with uranyl acetate with a Phillips Transmission Electron Microscope. Scales were compared to those of known *Prymnesium parvum* cells.

## Results and Discussion

The area having the longest record of *P. parvum* blooms is Texas (Fig. 1). Rivers located in the central and western region differ from those in the eastern portion of the state in that they are heavily influenced by alkaline soils, giving water a hardness capable of supporting *P. parvum* populations, which persist into summer in the Pecos, Colorado, and Brazos rivers. A *P. parvum* bloom was observed in a golf course pond on Kiawah Island, South Carolina, that persisted for a short period of time. It was associated with high temperatures (>30°C) and brackish salinity of 4, with a population of $6.8 \times 10^8$ cells $\cdot$ $L^{-1}$. No dead fish were noticed,

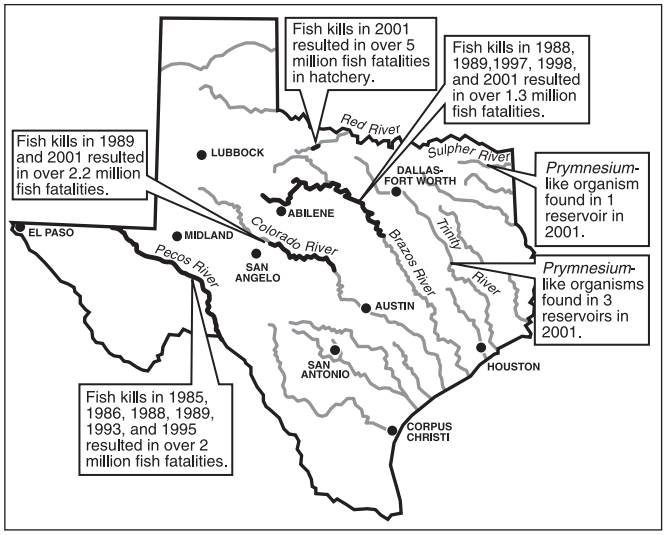


**Figure 1** Location of *P. parvum* blooms in Texas from 1988–2001.

Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

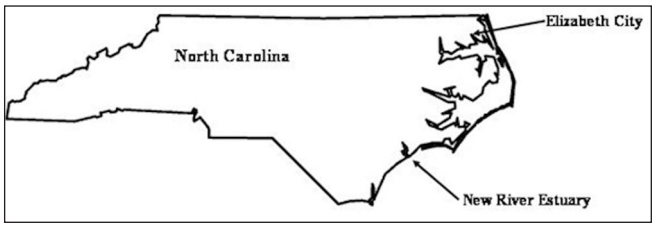


**Figure 2** North Carolina and sites of *P. parvum* blooms.

although this pond has limited exchange with marine waters and did not have a large resident fish population. Conditions of elevated phosphorus levels (>30 µM/L) prevailed while nitrogen, primarily as ammonia, was very low. This species was isolated into clonal culture and was found to be highly toxic using the *Gambusia affinis* fish bioassay, causing mortality within 30 minutes of exposure.

Blooms of *P. parvum* persisted from March 2001 through October 2002 in an aquaculture facility in Elizabeth City, North Carolina (Fig. 2). Newly dug ponds filled with artesian well water were used to raise hybrid striped bass, largemouth bass, koi and catfish. Poor fish growth prompted the facility manager to seek slightly brackish water from a deeper artesian well to replace the water in the holding ponds. The salinity was raised to 4, and within 3 months, fish mortalities were experienced in 18 of the 22 ponds. Cell concentrations exceeding $10^8$ cells $L^{-1}$ were found in the 18 ponds where fish mortalities occurred. For the one-year period, fish stocks in excess of $318,000 were lost from the ponds. While *P. parvum* persisted throughout the full year period, fish mortalities were continuously experienced in those ponds. Salinity at 4, high temperatures (>25°C) and high nitrogen, as ammonia, accompanied these blooms. All ponds having confirmed *P. parvum* cells were positive using the *Gambusia affinis* assay. A temporary decline in toxicity followed pond treatments with potassium permanganate, but the bloom did not terminate until the salinity of the ponds was restored to 0. *Prymnesium parvum* cells were also observed during the 2-year study in the New River Estuary (Fig. 2). Here the cells were found in the upper regions of the estuary where salinities were normally below 5 and nutrient levels were in excess of 10 µM $L^{-1}$ nitrogen as ammonia, nitrate and urea. While no large blooms were observed, fish kills in this region were common but could not be directly associated to *P. parvum* blooms. Further studies are needed and data should be discussed in greater detail elsewhere.

To confirm the identity of *Prymnesium,* cell morphology (Fig. 3 A,B) as well as scales from all samples (Fig. 3C) were observed. All cells were confirmed as *P. parvum.* The presence of this species in Texas and South and North Carolina clearly establishes its presence as a resident harmful alga. Further studies are required to define the extent and impacts of this species in fish-killing events.

## Acknowledgements

This research was supported by Texas Parks and Wildlife (J. Glass and J. Ralph), University of South Carolina and SC DNR (A. Lewitus), North Carolina Water Resources Research Institute and NC Sea Grant projects #50298 and #50970, respectively (C. Tomas).

## References

N. J. Antia, J. Y. Cheng and F.J.R. Taylor, Proc. Int. Seaweed Symp. 6, 17–19 (1969).
N. Carter, Arch. Protistemk. 90, 1–68. (1937).
B. Edvardsen, W. Eikrem, J. C. Green, R. A. Anderson and S.Y. Moon-vander Staay and L. K. Medlin, Phycologia 39(1), 19–35 (2000).
E. Granéli and N. Johansson Harmful Algae 2, 135–145 (2003).
C. Legrand, Limnol. Oceanogr. 45, 1208–1214 (2001).
K. Nygaard and A. Tobiesen, Limnol. Oceanogr. 38, 273–279 (1993).
Ø. Moestrup and H. Thompsen, Handbook of Phycological Methods, pp. 265–85 (1980).
Z. Paster, K. Reich, F. Bergmann and M. Rahat, Experientia 22, 970–971 (1966).
K. Reich, Palestine J. Bot. 14, 14–23 (1947).
S. Ulitzer and M. Shilo, Gen. Microbiol. 36, 161–169 (1964).

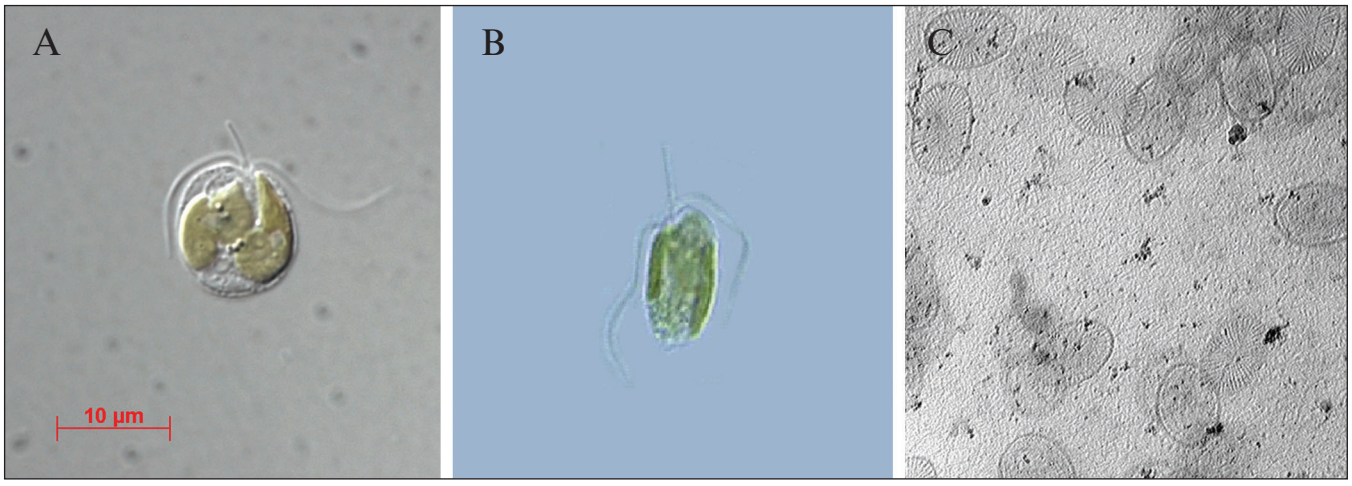


**Figure 3** *P. parvum* cells from Artesian Aquafarm **A**, Texas **B**, and scales **C** from bloom samples. Size bar for **A** and **B** the same = 10 µm.

# *Pfiesteria* Distribution Along the Texas (USA) Coast

Tracy Villareal[1], James D. Simons[2], and Parke Rublee[3]
[1]*Marine Science Institute, The University of Texas at Austin, 750 Channel View Dr., Port Aransas, TX 78373, USA;*
[2]*Texas Parks and Wildlife Dept., 6300 Ocean Dr., NRC, Suite 2501, Corpus Christi, TX 78412, USA;*
[3]*Biology Dept., University of North Carolina at Greensboro, Greensboro, NC 27402, USA*

## Abstract

A two-year monitoring program examined the Texas coast for the presence/absence of *Pfiesteria piscicida* and *P. shumwayae*. The sampling included a variety of bays and estuaries with differing degrees of nutrient input, sewage treatment outfalls, and heavily urbanized channels. Nine stations were monitored monthly and eight stations were monitored on a bi-monthly basis from April through September 2000. In 2001, a selected set of stations was monitored at two week intervals. Water samples were collected for nutrient and chlorophyll analyses. Two polymerase chain reaction assays were used in the study. A conventional PCR assay for detection of a large amplified fragment was less sensitive than a second assay using a fluorogenic probe and quantitative PCR. Despite evidence of DNA degradation after prolonged storage at –20°C, the follow-up Q-PCR technique yielded substantially more positives. Using this method, we found both *Pfiesteria* species occurred at some time during 2001 at every station sampled. Water collected after a fish kill in Dickinson Bayou, Texas, tested positive for *Pfiesteria,* but there is no supporting data to indicate that this was the cause of the fish kill. The two species occurred at a wide range of chlorophyll and nutrient concentrations. They appear to be a common and widely distributed members of the Texas coastal dinoflagellate community.

## Introduction

The heterotrophic dinoflagellate *Pfiesteria* has been implicated in both fish kills and human health issues along the eastern U.S. coast (Burkholder *et al.,* 2001). In response, significant resources have been devoted to understanding the nature of its toxicity, response to environmental variables and importance in estuarine environments. The *Pfiesteria*-like complex appears to be broadly distributed (Rublee *et al.,* 2001), but difficulty in identifying the two described *Pfiesteria* species along with the extensive discussions surrounding this group has slowed advance in this area. The Texas coast appears to be a likely habitat for *Pfiesteria* but there have been no attempts to determine if the species is present on this coast. The shallow, estuarine systems typical of the Texas coast are similar to its habitat along the eastern U.S. coast and the records of this species from the eastern Gulf of Mexico suggest it is likely to be present. Along the eastern U.S. seaboard, *Pfiesteria* is considered to be a major cause of fish mortality (Glasgow *et al.,* 2001).

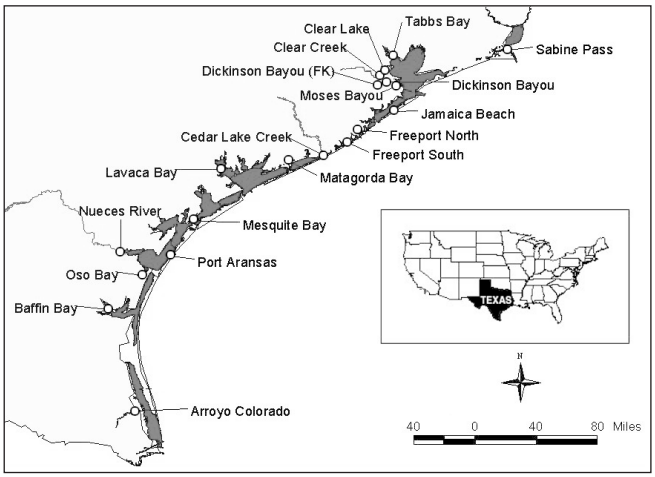


**Figure 1** Sampling sites in this study.

Fish kills are common along the Texas coast, and while there is no evidence to suggest *Pfiesteria* has been a causative agent, information on its distribution and occurrence in these waters is an important part of the state's overall resource management program. We report here the results of two years of sampling using two different gene assays for both *Pfiesteria piscicida* and *P. shumwayae*.

## Methods

Presence/absence of *P. piscicida* and *P. shumwayae* was determined using gene assays. 100 mL of water were filtered and returned to UNC, Greensboro, for processing. Initially, samples were analyzed by a conventional PCR approach (M1) using probes for *P. piscicida* from Rublee *et al.* (1999) and for *P. shumwayae* by Oldach *et al.* (2000). Samples were later re-assayed by real-time PCR (M2) using primers and Taqman probes (Bowers *et al.,* 2000) on a Cepheid Smart Cycler. Although the real-time PCR assay is more sensitive, storage of samples at –20°C and additional freezing and thawing of samples prior to this second assay

**Table 1** Total number of occurrences using the two gene probes. Joint records by M1 and M2 are recorded as a single occurrence; hence the sum of the columns may not equal the total.

| | | *P. piscicida* | | |
|---|---|---|---|---|
| Year | n = | M1 | M2 | Total |
| 2000 | 90 | 5 | 9 | 12 |
| 2001 | 68 | 5 | 26 | 22 |
| | | *P. shumwayae* | | |
| Year | n = | M1 | M2 | Total |
| 2000 | 90 | 5 | 2 | 7 |
| 2001 | 68 | 7 | 28 | 33 |

Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

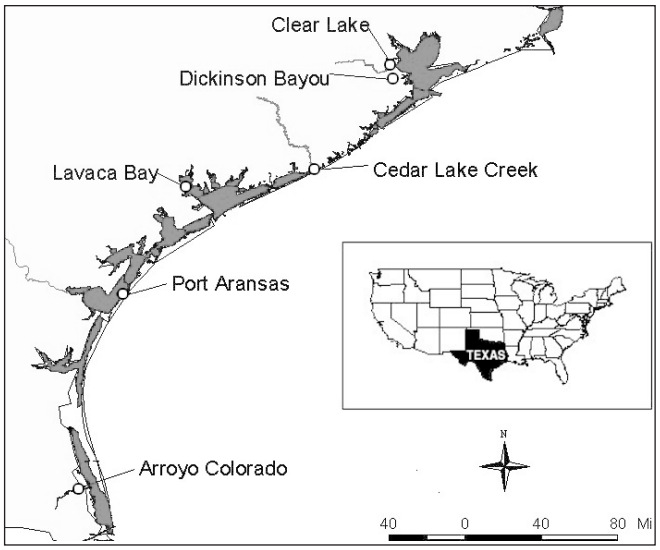


**Figure 2** *Pfiesteria* presence in 2000.

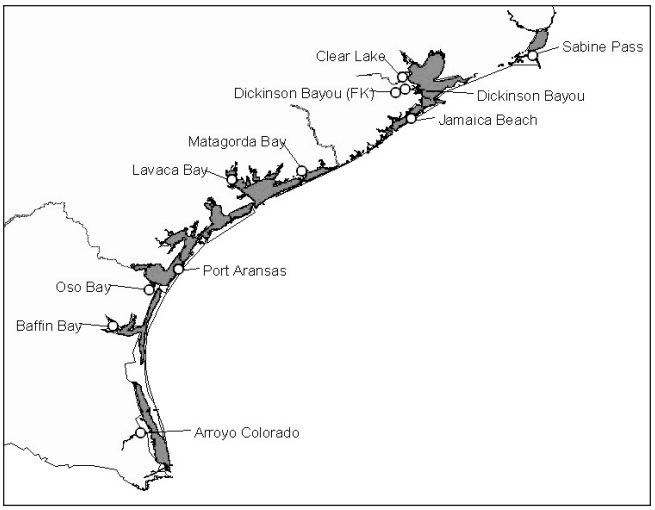


**Figure 3** *Pfiesteria* presence in 2001.

may have resulted in some degradation of DNA between assays. At each station, additional samples were collected for chlorophyll (Welschmeyer 1994) and automated nutrient analysis (Lachat Quikchem 8000) for nitrate+nitrite, silicate, phosphate, ammonium and dissolved organic nitrogen. The M2 assay was not available until 6 months after all samples were run using M1. Station selection in Year 1 was based on the need to do a broad coast-wide survey that would sample both impacted and pristine areas. In Year 2, more intensive sampling (two-week intervals) focused on a subset of sites that represented extremes found in Year 1. Statistical analysis used Statview 5.01. Sample sites for both years are noted in Fig. 1.

## Results

Both *Pfiesteria* spp. were present along the coast in both years (Table 1, Fig. 2, 3). *Pfiesteria* was present across the range of environmental conditions found (Fig. 4). Substantially more positive records were found using the M2 assay. There were more records in 2001 than 2000, probably due to sample degradation upon re-analysis. In 2001, nutrients, chlorophyll and hydrographic parameters showed no consistent difference in relation to presence or absence except for Si. Silcate concentrations were significantly higher at stations that showed positive for *P. piscicida* (57.3 vs. 36.3 μM, $P = <0.0001$). The data from 2000 was not analyzed in this way due to the likelihood of sample degradation over time.

## Discussion

*Pfiesteria* spp. appear to be a broadly distributed member of the coastal dinoflagellate community along the Texas coast. Both species of *Pfiesteria* were present along the entire Texas coast. The temperature-salinity conditions where the species could be found spanned essentially the entire range found along the coast during the May–Sept. sampling period. We found no evidence of preferred conditions although we recognize that presence/absence is not a useful indicator for biological response. It is relevant to note that the distribution of either species was not statistically linked to any inorganic nutrient, DON or chlorophyll concentration except for silicate. The curious link to silicate concentration for *P. piscicida* cannot be explained at this time by a direct relation to *Pfiesteria* physiology. It may be a proxy for other environmental or biological factors such as residence time, benthic processes, or preferred prey items; however, the data cannot resolve this issue.

The genetic test used cannot distinguish toxic from non-toxic forms of the species. Although one sample from a fish kill tested positive for *Pfiesteria,* there is no ancillary evidence to suggest *Pfiesteria* was responsible. Thus at this time, we cannot state whether we have Tox-1, Tox-2, non-inducible strains or any combination of the above.

## Acknowldegements.

This study would not have been possible without the collections of numerous Texas Parks and Wildlife Dept. field teams. Contribution number 1294 from The University of Texas at Austin, Marine Science Institute.

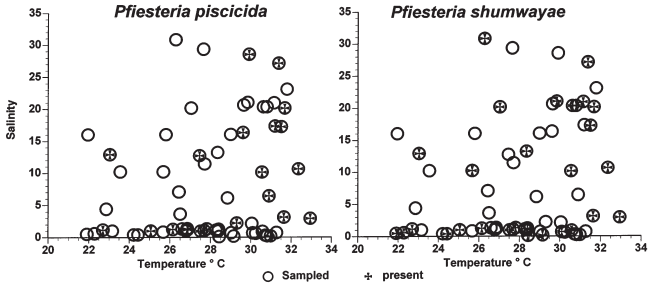


**Figure 4** Temperature and salinity conditions at *Pfiesteria* sites. Open circles indicate conditions at all sites sampled, crosses indicate sites where one or both species were found.

## References


H. A. Bowers, T. Tengs, Glasgow Howard B, Jr., J. M. Burkholder, P. A. Rublee and D. W. Oldach, Applied and Environmental Microbiology 66, 4641–4648 (2000).

J. M. Burkholder, H. B. Glasgow and N. Deamer-Melia, Phycol. 40, 186–214 (2001).

H. B. Glasgow, J. M. Burkholder, M. A. Mallin, N. J. Deamer-Melia and R. E. Reed, Environ. Health Perspect. 109, 715–730 (2001).

D. W. Oldach, C. F. Delwiche, K. S. Jakobsen, T. Tengs, E. G. Brown, J. W. Kempton, E. F. Schaefer, H. A. Bowers, H. B. Glasgow, J. M. Burkholder, K. A. Steidinger and P. A. Rublee, Proc. Natl. Acad. Sci. U. S. A., 97, 4303–4308 (2000).

P. A. Rublee, J. Kempton, E. Schaefer, J. M. Burkholder, H. B. Glasgow, Jr. and D. Oldach, Va. J. Sci., 50, 325–336 (1999).

N. A. Welschmeyer, Limnol. Oceanogr., 39, 1985–1992 (1994).

# Regional Distribution of the Texas Brown Tide (*Aureoumbra lagunensis*) in the Gulf of Mexico

Tracy A. Villareal[1], Teresa Chirichella[2], and Edward J. Buskey[1]
[1]*Marine Science Institute, The University of Texas at Austin, 750 Channel View Dr., Port Aransas, TX 78373, USA;* [2]*Environmental Sciences Program, University of Massachusetts, Boston, 100 Morrisey Blvd., Boston, MA 02125, USA*

## Abstract

Samples collected from Florida, Louisiana, Texas and Mexico in 1996–1997 were examined for the presence of the Texas Brown Tide species *Aureoumbra lagunensis.* Low abundance (<200 cells $mL^{-1}$) was noted at one or more stations in Florida Bay from November 1996 to June 1997. The species was present along the Texas coast from Matagorda Bay to the Laguna Madre during July/October 1997, but was not present at stations near Galveston, Texas (northeast Texas), or in the northern Gulf of Mexico near the Mississippi River. A wide distribution in the Laguna Madre de Tamaulipas, Mexico, was noted in June 1997, but the species was not present in the lagoon south of La Pesca, Mexico. Low salinity alone did not seem to limit its distribution—it was noted at $10^2$ cells $mL^{-1}$ at salinities from 0.5 to 25 in October 1997 in central Texas. The singular HAB event in the Laguna Madre, Texas, appeared to result from a confluence of events unique to this hypersaline embayment that allowed a cryptic, but widespread, member of the coastal flora to bloom and dominate.

## Introduction

The Texas brown tide was the longest documented HAB event in U.S. history. It comprised over 95% of the phytoplankton community ($10^6$ cells $mL^{-1}$) in the upper Laguna Madre, Texas, for the period 1990 to 1998, and disrupted zooplankton, benthic and seagrass communities (Whitledge *et al.,* 1999). The pelagophyte that caused this brown tide (*Aureoumbra lagunensis*) is a small (4–6 μm) cell that is morphologically difficult to distinguish from other cells using a light microscope (DeYoe *et al.,* 1997). Part of the mystery of the bloom was the sudden dominance of this previously undescribed species in a nearly pristine ecosystem with chlorophyll levels that were often 5–10 times pre-bloom levels. An atypical severe freeze resulting in a massive fish kill and subsequent ammonium pulse was initially suggested to be responsible for the bloom outbreak (DeYoe and Suttle, 1994); however, it is now known that hypersalinity suppression of grazers (Buskey *et al.,* 1998), an effective grazing inhibition mechanism (Buskey *et al.,* 1997), and unusual P requirements (Liu *et al.,* 2001) probably played a dominant role in the initiation and maintenance of the bloom. In addition, archived samples examined with a polyclonal antibody showed a clear increase in *A. lagunensis* populations prior to the fish kill and subsequent ammonium increase (Buskey *et al.,* 1999). The species also was present in the Laguna Madre, Texas, at least six months prior to the bloom at ≤$10^2$ cells $mL^{-1}$. Much less is known about its distribution outside this area.

## Materials and Methods

The development, specificity and application of the polyclonal antibody have been described in Lopez-Barrerio et al (1998). Our study used a series of samples collected in the Gulf of Mexico to study the broad regional distribution of the species. Sample sites are illustrated in Fig. 1. In all cases, formalin-preserved (1–3%) samples were examined within 2 months of collection. Salinity is expressed using the practical salinity scale.

## Results and Discussion

*Aureoumbra lagunensis* was widely distributed in the Gulf of Mexico. Concentrations of $10^1$–$10^2$ cells $mL^{-1}$ were present in Florida Bay, several Texas bays, and the more northern Mexican bays (Fig. 1, 2; Table 1). The species was not present in samples from Belize in May 1997. Bloom concentrations were only noted in the Laguna Madre of Texas (Table 1). The concentration found in Laguna Madre, La Pesca was much higher than background concentrations found in other areas ($10^5$ cells $mL^{-1}$). The species was commonly present in Laguna Madre de Tamaulipas at $10^2$ cells $mL^{-1}$ during this time. Cell counts versus salinity plots (Fig. 3) revealed that below a salinity of approximately 38, cell counts were ≤103 cells $mL^{-1}$. However, $10^2$ cells $mL^{-1}$ were noted at salinities of 0.5 to 25 in Matagorda Bay, Texas, in 1997. Bloom concentrations (>$10^4$ cells $mL^{-1}$) were found

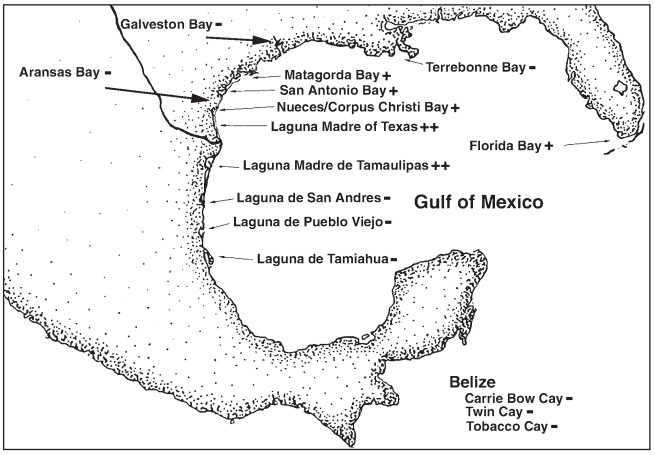


**Figure 1** Sampling sites and summarized results of the survey. A "–" indicates no cells were found, a "+" indicates $10^2$–$10^3$ cells $mL^{-1}$, and "++" indicates $10^4$–$10^6$ cells $mL^{-1}$. The Terrebonne Bay sampling was a 5–9 station transect that ran from Dec. 1996 to Oct. 1997. Florida bay was a set of 3–5 stations collected on dates shown in Table 1. The Belize samples were collected in May 1997.



Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

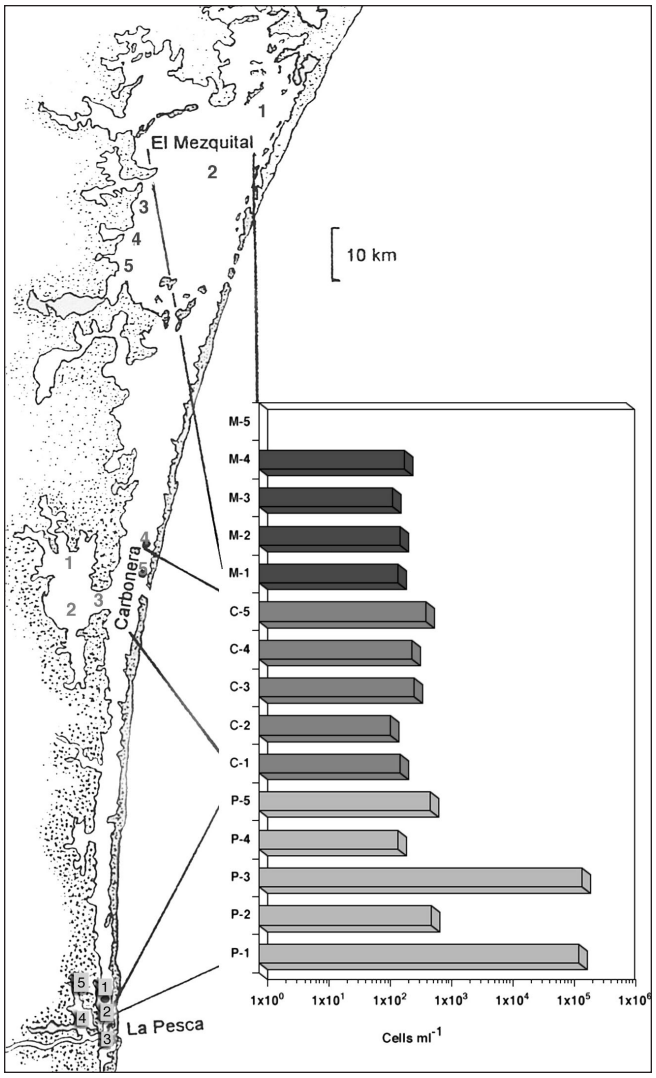


**Figure 2** Quantitative counts from along the east coast of Mexico in June 1997.

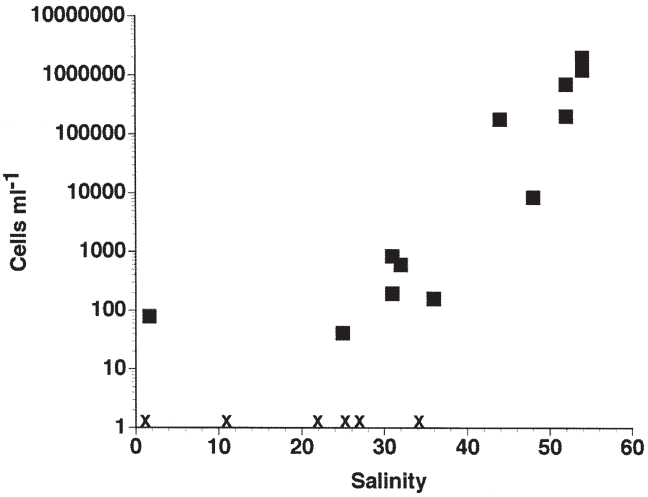


**Figure 3** Cell abundance versus salinity. Sites include south Texas, Mexico and Florida Bay. X indicates a zero count.

only at salinities >40 (Fig. 3). Unfortunately, no salinity data was available from the sites in Mexico, so this relationship is only a function of observations in the Laguna Madre, Texas.

This study extends the known range of *Aureoumbra lagunensis* to bays on both sides of the Gulf of Mexico. Much like the New England brown tide *Aureococcus anophagefferens,* the range greatly exceeds that of known bloom sites (Anderson *et al.,* 1993). The observational data confirms the strong influence that hypersalinity has on this species' dynamics (Buskey *et al.,* 1998). Although present at many coastal sites at salinities ≤35, blooms only occurred when salinity was elevated. Of particular interest is the observation that Terrebonne Bay did not host the species. This region is strongly influenced by the Mississippi River; however, we cannot determine whether the absence

**Table 1** Maximum abundance of *A. lagunensis* observed at the study sites.

| Location | Sample Dates | Maximum Cells mL$^{-1}$ | Location | Sample Dates | Maximum Cells mL$^{-1}$ |
|---|---|---|---|---|---|
| **USA** | | | | | |
| **Texas** | | | **Louisiana** | | |
| Baffin Bay/Laguna Madre | 4/97, 7/97 | 1,998,270 | Terrebonne Bay | 12/96 – 10/97 | 0 |
| Nueces/Corpus Christi Bay | 1/97, 10/97 | 837 | | | |
| San Antonio Bay | 1/97, 10/97 | 240 | **MEXICO** | | |
| Matagorda Bay | 1/97, 10/97 | 478 | Laguna Madre/Mezquital | 6/97 | 234 |
| Aransas Bay | 1/97, 10/97 | 0 | Laguna Madre/Carbonera | 6/97 | 538 |
| Galveston Bay | 7/97 | 0 | Laguna Madre/La Pesca | 6/97 | 191,400 |
| | | | Laguna de San Andres | 6/97 | 0 |
| **Florida** | | | Rio Panuco | 12/96, 6/97 | 0 |
| Rabbit Key Basin | 11/96, 1/97, 3/97, 6/97 | 95 | Laguna Pueblo Viejo | 12/96, 6/97 | 0 |
| Sandy Key Basin | 11/96, 1/97, 3/97, 6/97 | 99 | Laguna de Tamiahua | 12/96, 6/97 | 0 |
| Johnson Key Basin | 11/96, 1/97, 3/97, 6/97 | 85 | Laguna de Tampamachoco | 12/96, 6/97 | 0 |
| Rankin Lake | 11/96, 1/97, 3/97, 6/97 | 53 | | | |

of the species was a low salinity effect or water quality effect. The dissipation of the *A. lagunensis* bloom from the Laguna Madre, Texas, during sustained rains and a concurrent salinity drop (Buskey *et al.,* 2001) suggests that freshwater inflows do not favor this species. However, it does appear to be able to tolerate low salinity as evidenced by its presence in October 1997 at several low salinity bays in Texas. *A. lagunensis* appears to be part of the cryptic flora of the coastal zone in the Gulf of Mexico. Despite the elevated concentrations observed at Laguna Madre, La Pesca, the sustained bloom in the Laguna Madre, Texas, still appears to be a singular event.


## Acknowledgements

This study would not have been possible without the generous collections of Leanne Flewelling, Dr. Quay Dortch, Rick Kalke Maria Llosa, Teresa Barreiro and Luis Lopez. Contribution No. 1293 from The University of Texas at Austin, Marine Science Institute.


## References


D. M. Anderson, B. A. Keafer, D. M. Kulis, R. M. Waters and R. Nuzzi, J. Plankton Res. 15, 563–580 (1993).

E. J. Buskey, H. Liu, C. Collum and J. G. F. Bersano, Estuar. 24, 337–346 (2001).

E. J. Buskey, P. A. Montagna, A. F. Amos and T. E. Whitledge, Limnol. Oceanogr. 42, 1215–1222 (1997).

E. J. Buskey, T. A. Villareal and T. López-Barreiro, Plankton Biol. Ecol. 46, 159–161 (1999).

E. J. Buskey, B. Wysor and C. Hyatt, J. Plankton Res. 20, 1553–1565 (1998).

H. DeYoe and C. A. Suttle, J. Phycol. 30, 800–806 (1994).

H. R. DeYoe, D. A. Stockwell, R. R. Bidigare, M. Latasa, P. W. Johnson, P. E. Hargraves and C. A. Suttle, J. Phycol. 33, 1042–1048 (1997).

H. Liu, E. A. Laws, T. A. Villareal and E. J. Buskey, J. Phycol. 37, 500–508 (2001).

T. López-Barrerio, T. A. Villareal and S. L. Morton, In: Harmful Algae, B. Reguera, J. Blanco, M.L. Fernandez, T. Wyatt, eds. (Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, Santiago de Compostela), pp. 263–265 (1998).

T. Whitledge, D. A. Stockwell, E. J. Buskey, K. C. Dunton, G. J. Holt, S. A. Holt and P. A. Montagna, In: The Gulf of Mexico Large Marine Ecosystem, H. Kumpf, K. Steidinger, K. Sherman, eds. (Blackwell Science, Inc., Malden, MA), pp. 338–359 (1999).

# Florida's Black Water Event

Merrie Beth Neely[1,3], Erich Bartels[2], Jennifer Cannizzaro[3], Kendall L. Carder[3], Paula Coble[3], David English[3], Cynthia Heil[1,3], Chuanmin Hu[3], John Hunt[1], Jim Ivey[3], Gil McRae[1], Erich Mueller[2], Ernst Peebles[3], and Karen Steidinger[1]
[1]*Florida Marine Research Institute, Florida Fish and Wildlife Conservation Commission, St. Petersburg, FL, USA;*
[2]*Mote Marine Laboratory, Center for Tropical Research, Summerland Key, FL, USA;*
[3]*University of South Florida, College of Marine Science, St. Petersburg, FL, USA*

## Abstract

In January 2002, fishermen first noticed dark, discolored water in the southeastern Gulf of Mexico near Florida's Marquesas Islands, which they called "black water." The accumulated evidence suggests the dark water was caused by a series of algal blooms, from red tide to diatoms, which were supported by both marine and estuarine sources of nutrients. The passage of fewer fronts during the winter of 2001–2002, combined with local circulation patterns and heavy rainfall, contributed to the formation of this expansive bloom that persisted for many months.

## Introduction

The health of Florida's coastal environment came under worldwide scrutiny in March 2002, following media reports of an expansive area of discolored water on the Southwest Florida Shelf, dubbed "black water." Although first noticed by fisherman and small aircraft pilots in January 2002, it was not reported to resource officials until March 1, 2002. Some observers quoted in the media stated that fish were avoiding the water mass and that it was a dead zone, devoid of life. Based upon satellite images (Hu *et al.,* 2002), this dark water event occurred between late November 2001 and mid-April 2002. Coordinated sampling efforts by the Florida Marine Research Institute (FMRI), the University of South Florida (USF) and Mote Marine Laboratory (MML) occurred in March and August 2002.

## Materials and Methods

On March 19, 2002, water samples were collected from a small boat (R/V *Acropora,* MML) with buckets, and hydrographic parameters were measured with a Hydrolab. Subsequently, water samples were collected from shipboard operations with Niskin bottles on a rosette sampler equipped with a CTD (late March on the R/V *Subchaser* and August 2002 on the R/V *Suncoaster,* both from Florida Institute of Oceanography). Nutrient samples were analyzed using an Astoria Pacific autoanalyzer. Particulate carbon and nitrogen samples were analyzed using a Carlo Erba Model 1106 elemental analyzer. Total dissolved phosphate and particulate phosphate were analyzed following Solorzano and Sharp (1980). Chlorophyll *a* and phaeopigment were determined following Holm-Hansen and Reimann (1978) using a Turner Designs 10-AU fluorometer. Phytoplankton cell count and identification were made on both live and unacidified Lugol's-preserved whole water samples using a Nikon inverted light microscope. Water-leaving radiance profiles were collected using a submersible hyperspectral radiometer. Colored dissolved organic matter (CDOM) measurements are reported as quinine sulfate equivalents in the 300/420 wavelength region for humic measurements.

## Results and Discussion

Contrary to the media characterization of this as a dead zone, March samples indicated the dark water was a widespread Rhizosoleniaceae bloom coincident with high populations of ctenophores (*Mnemiopsis mccradyi*) and low to moderate counts of the red tide dinoflagellate *Karenia brevis* (Table 1). Chlorophyll *a* values were moderate to high (2.45–10.24 mg $L^{-1}$) and were higher at the bottom than at the surface. Chlorophyll in the area was the highest recorded in recent years. However, higher chlorophyll values have been recorded for algae blooms on the West Florida Shelf (WFS) and in adjacent estuaries like Florida Bay. The phaeopigment content of the samples was low to average, indicating a growth or maintenance bloom rather than a senescent one (Barlow *et al.,* 1993). In general, nutrient levels (Table 1) were comparable to those typically found on the WFS (Vargo *et al.,* 2001). Dissolved oxygen levels were saturated or supersaturated throughout the water column. All March water samples had near-oceanic salinity of 36.2, and concentrations of CDOM were only slightly elevated above expected levels for a mixture of water from south Florida rivers with coastal seawater. CDOM fluorescence fingerprints indicated some contribution from new marine productivity but were not specific to any one group of phytoplankton.

During the fall and winter of 2001–2002, a red tide was co-occurring along the WFS from Tarpon Springs to Marco Island (FWC FMRI, unpublished data). *Karenia brevis* was either absent or found in low-to-moderate numbers in samples taken near the dark water (Table 1). However, sampling coverage was limited temporally and spatially in this remote region, especially during the winter months. High densities of the ctenophore *M. mccradyi* were coincident with the darkest water sampled in late March 2002.

The flushing of dark water from terrestrial areas of Florida is commonly associated with heavy rainfall (Clark *et al.,* 2002). Dark and discolored water, often referred to as black water by observers, has also been reported during and after red tide blooms along the WFS (FMRI data) and in Texas waters (David Buzan, personal communication). Central and southern Florida received considerable rainfall during the months of September and October 2001 due to the passage of two tropical storms and other fronts. Following decadal peak salinities in excess of 26 in Febru-

Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

**Table 1** This table presents values for various parameters measured from three dark water sampling events. All measurements are from surface samples unless otherwise indicated.

| Parameters | 03/19/2002 | 03/28/2002 | 08/06/2002 |
|---|---|---|---|
| [a]$NH_4$ (μg $L^{-1}$), inorganic | 2.29 | 0.2–1.65 | ND |
| [a]$NO_3$+$NO_2$ (μg $L^{-1}$), inorganic | BLD | 0.02–0.22 | 0.02S/0.38B |
| [a]$PO_4$ (μg $L^{-1}$), inorganic | 0.05 | BLD–0.02 | 0.48S/0.35B |
| [a]$SiO_4$ (μg $L^{-1}$), inorganic | 1.45 | 0.25–1.37S/2.34–2.85B | 18.6S/>20.0B |
| [a]Total dissolved $PO_4$ (μg $L^{-1}$) | 0.16 | 0.07–0.25 | BLD–0.08S/BLD–0.09B |
| [a]Particulate $PO_4$ (μg $L^{-1}$) | 0.40 | 0.24–0.35 | 0.28–0.51S/ 0.3–0.46B |
| [a]C:N (molar) | 4.5 | 0.15–0.21 | NA |
| [a]N:P (molar) | 106.2 | 2660–303S/3008–3133B | NA |
| [b]CDOM, 300/420 λ (ppb QSE) | 8.9 | 3.8–4.4S/2.7–3.7B | 28.4 |
| [a]Chlorophyll *a* (μg $L^{-1}$) | 10.24 | 0.68S/2.64B | 5.36S/1.68B |
| [a]Phaeopigment (μg $L^{-1}$) | 2.19–2.43 | 0.3S/1.05B | 2.14S/0.85B |
| [e, d, a]Salinity | 36.2 | 36.2 | 32.4 |
| [c]Brevetoxin (μg $L^{-1}$) | ND | BLD–4.55 | ND |
| [c]*Karenia brevis* counts (cells $L^{-1}$) | 0–333 | 0–118 × $10^3$ | 0 |
| [c]Rhizosoleniaceae counts (cells $L^{-1}$) | 67–1170 × $10^3$ | 25–140 × $10^3$ | ND |

[a]Cynthia A. Heil, USF; [b]Paula G. Coble, USF; [c]FWC FMRI; [d]Ken Carder, USF; [e]Erich Bartels, MML.
BLD = below limits of detection. ND = no data. NA = not analyzed. S = surface. B = bottom.

ary 2001, decadal record flows in excess of 16000 cfs from the Caloosahatchee River at Ft. Myers Yacht Club were noted in late September and early October 2001 (http://www.sfwmd.gov/org/wrp/wrp_ce/2_wrp_ce_estuary/ftmyers_histor_salin.gif). In addition, several seasonally heavy rainfall events occurred in November 2001 and January–March 2002 (Southeast River Forecast Center/NOAA, South Florida Water Management District).

Diatom blooms, particularly Rhizosoleniaceae blooms, are seasonal occurrences in this region of the WFS in the winter months resulting from riverine flows containing silicate (Jurado and Hitchcock, 2001; Steidinger, unpublished data). The drought, which was particularly severe in South Florida in early 2001, followed by peak rainfall events, likely led to a diatom bloom of increased magnitude and duration during late 2001 and early 2002.

The water mass containing the diatom bloom must have remained stable for at least one month for zooplankton and one generation of ctenophores to have reached high densities (Peebles, unpublished data; Purcell *et al.,* 2001). The National Weather Service station at Key West International Airport recorded fewer and less severe meteorological fronts passing the Florida Bay and lower Keys area in the winter of 2001–2002, which may have reduced mixing and led to increased water column stability on the West Florida Shelf. Trajectories of drifters released west of Florida Bay and off the Shark River (U Miami/RSMAS and NOAA data, drifters 24792 and 29526, http://mpo.rsmas.miami.edu/flabay/latest.html) indicated that the water circulation in this area was dominated by a persistent gyre during the period of the dark water. This gyre would have entrained the Rhizosoleniaceae bloom water from the Ten Thousand Islands area, and water containing a decaying red tide off Naples, FL. With a notable lack of fronts passing through South Florida, this type of circulation pattern would have maintained stratified coastal water masses, contributing to the magnitude and duration of the dark water event and allowing high populations of ctenophores to exploit the zooplankton population feeding on the algae blooms.

Ctenophores are also known to release dissolved organic matter (DOM) (Kremer, 1994) and remove particles from the water. CDOM absorbs light in the blue and green wavelengths, and fewer particles in the water column would reduce light scattering. These combined effects reduce water-leaving radiance, making the water appear dark. High densities of gelatinous zooplankton probably accounted for reports by lay observers of gelatinous blobs on the surface of the dark water. Reported avoidance of the dark water by macrofauna could have been due to reduced visibility, mechanical obstruction of gills by large diatoms, or chemoreception of residual brevetoxin in the water.

A second event, also dubbed "black water" by the media, was reported in August 2002 near Sanibel Island, FL. Data collected during this event indicated two separate algal blooms were occurring: a cyanobacteria bloom at the mouth of the Caloosahatchee River and a diatom-dominated bloom in the Ten Thousand Islands area. All analyses performed to date on these samples identify river water as the nutrient source for these blooms (Table 1).

In summary, phytoplankton species identification and cell counts indicate the dark water was dominated by large centric diatoms (Fam. Rhizosoleniaceae) and that the Florida red tide organism *K. brevis* and its toxin was either

absent or found in low-to-moderate amounts. Climatic extremes were present in the region throughout 2001, with severe drought conditions followed by the passage of fewer and less severe weather fronts in the winter/spring of 2002. Drifter trajectories indicate the water between Naples and the Marquesas circulated in a gyre during the event. We propose that under these conditions the normal winter diatom bloom from western Florida Bay shifted spatially and became superimposed over a waning red tide from the West Florida Shelf, reflected in decreasing *K. brevis* counts. Both blooms coincided with optically dark water exhibiting CDOM that was primarily riverine in origin. Water column stability of at least one month's duration would have allowed high populations of ctenophores to develop, and the presence of these zooplankton predators may have contributed to the maintenance of the phytoplankton blooms by reducing grazing pressure. The oceanographically unique features of this event were the magnitude and duration of the cohesive water mass, the high densities of gelatinous zooplankton observed, and the climatic extremes in 2001 followed by a mild winter in South Florida.

## Acknowledgements

The authors gratefully acknowledge data provided by Dr. Tom Lee and Elizabeth Williams, University of Miami, Rosenstiel School of Marine and Atmospheric Sciences, Elizabeth Johns and Ryan Smith, NOAA/AOML and Marsha Kirchhoff, South Florida Water Management District. We also thank Earnest Truby and Leanne Flewelling, FMRI and Julie Havens, USF. Shiptime and salary support for some investigators was provided by NOAA and EPA ECOHAB.

## References

R. G. Barlow, R. F. C. Mantoura, M. A. Gough, and T. W. Fileman, Deep-Sea Res. I. 40(11/12):2229–2242 (1993).

C. D. Clark, J. Jimenez-Morais, G. Jones II, E. Zanardi-Lamardo, C. A. Moore, and R. G. Zika, Mar. Chem. 78(2–3), 121–135 (2002).

O. Holm-Hansen and B. Reimann, Oikos 30, 438–441 (1978).

J. Jurado and G. Hitchcock, Florida Seagrant Report FLSGP–G-010006 (2001).

P. Kremer, ICES J. Mar. Sci., 51, 347–354 (1994).

L. Solorzano and J. H. Sharp, Limnol. Oceanogr. 25, 754–758 (1980).

C. Hu, F. E. Muller-Karger, Z. Lee, K. L. Carder, B. Roberts, J. J. Walsh, C. Heil, P. G. Coble, K. Steidinger, G. McRae, R. H. Weisberg, R. He, E. Johns, T. Lee, B. Keller, N. Kuring, J. Cannizzaro, J. Ivey, G. A. Vargo, R. G. Zepp, J. Boyer, R. Jones, G. Kirkpatrick, R. P. Stumpf, E. Mueller, R. Pierce, J. Culter, J. Hunt, EOS 83(26), 281, 284 (2002).

J. E. Purcell, T. A. Shiganova, M. B. Decker, and E. D. Houde, Hydrobiologia 45, 145–176 (2001).

G. A. Vargo, C. A. Heil, D. Spence, M. B. Neely, R. Merkt, K. Lester, R. H. Weisberg, J. J. Walsh and K. Fanning, in: Harmful Algal Blooms 2000, G. M. Hallegraeff, S. I. Blackburn, C. J. Bolch, and R. J. Lewis, eds. (UNESCO, Paris), pp. 157–160 (2001).

# Toxic and Harmful Dinoflagellates in the Southern Gulf of Mexico

Sergio Licea[1], María E. Zamudio[1], Ruth Luna[1], Yuri B. Okolodkov[2], and Samuel Gómez-Aguirre[3]
*[1]Instituto de Ciencias del Mar y Limnología, Apartado Postal 70-305, México 04510 D.F.; [2]Departamento de Hidrobiología, Universidad Autónoma Metropolitana-Iztapalapa, Col. Vicentina, México 09340, D.F.; [3]Instituto de Biología, Apartado Postal 70-153, México 04510 D.F.*

## Abstract

Previous analyses of phytoplankton have shown that several toxic and harmful species are a permanent component in the southern Gulf of Mexico. Because of their potential toxicity, this study was initiated with the aim of tracing the occurrence of some of these species in Mexican waters. The study material consisted of water bottle and net samples collected in four coastal lagoons and on the continental shelf at 183 sites from June 1979 to July 1994. Results revealed the presence of 17 species, of which 13 are toxic (DSP, PSP, and NSP) and four are non-toxic red tide producers (*Ceratium furca, Pyrodinium bahamense* var. *bahamense, Scrippsiella trochoidea* and *Gonyaulax polygramma*). Data on frequency and relative abundance show that in July–August, toxic and harmful dinoflagellates had the widest distribution in the study region. Two blooms of the species *Karenia brevis* and *Scrippsiella trochoidea* were observed in summer. *Ceratium furca, Dinophysis caudata, Gonyaulax polygramma, Prorocentrum rhathymum* and *P. micans* are widely distributed. Micrographs of most identified species are illustrated.

## Introduction

Toxic and harmful species of dinoflagellates have received worldwide recognition in recent years due to the increase in poisoning events reported in coastal marine ecosystems (Hallegraeff, 1993, 2002). In the northwestern Gulf of Mexico, several species have been observed in great numbers, as is the case for *Karenia brevis* (Steidinger *et al.,* 1988). In the southern region, Gómez-Aguirre and Licea (1998) and Licea *et al.* (2002) previously reported on most of the species discussed in this paper. The widespread occurrence of potentially toxic species poses a significant danger for human health, and it is essential to identify the species correctly in order to survey their field distribution. Although harmful algal blooms (HAB) are relatively well-known events in Mexican coastal waters, there has been no consistent assessment and inventory of the species involved. This study discusses the species composition and distribution of potentially harmful dinoflagellates found throughout the shelf in the southern Gulf of Mexico from June 1979 to July 1994.

Hydrographic conditions in the southern Gulf of Mexico are highly influenced by the Loop Current and its eddies as well as by the occurrence of winter storms between October and February (Hulburt and Thompson, 1980). The intensification of the Yucatan Current entering the Gulf of Mexico through the Yucatan Canal occurs mainly in summer and autumn. The Campeche Bank seems to depend on interannual fluctuations of the intensity of the Yucatan Current to a greater extent (Bessonow *et al.,* 1971). The main zones of upwelling and downwelling are stable in both time (within a year) and space, resulting in small variability in the quantitative characteristics of the plankton in the Campeche Bank area (Bogdanow *et al.,* 1968; de la Cruz, 1971; Furnas and Smayda, 1987). In addition, this region is impacted mainly by two rivers, the Coatzacoalcos and the Grijalva-Uumacinta system, that account for approximately one-third of all fluvial discharge into the coastal waters (Tamayo, 1990). The Yucatan shelf is highly influenced by the upwelling along Cape Catoche and other areas of the northern Yucatan coast (Cochrane, 1969; Belousov *et al.,* 1996; Merino, 1997).

## Materials and Methods

Water-bottle samples were obtained during eight cruises from June 1979 to July 1994. Samples were taken by a CTD Neil Brown with a rosette of Niskin bottles and were fixed in Lugol's solution. In addition, samples were collected by plankton net, mesh size 54 μm. Observations and counts were made of concentrated water-bottle samples of 250 mL in an inverted Zeiss ICM-405 light microscope (Hasle, 1978). Water mounts with Trypan blue (= diamine blue 3B) were examined to obtain information on dinoflagellate plate pattern (Lebour, 1925). In addition, a scanning electron microscope (SEM) was used to observe specimens.

## Results and Discussion

Three hundred and twenty samples were collected from 183 sites. Seventeen species of toxic and harmful dinoflagellates were identified (Fig. 1). Qualitative data show that *Ceratium furca, Gonyaulax polygramma, Dinophysis caudata, Prorocentrum rhathymum* and *P. micans* are widely distributed in the study area (Fig. 2). In contrast, *Dinophysis fortii, D. rotundata, D. tripos, Prorocentrum minimum, Protoperidinium crassipes* and *Pyrodinium bahamense* var. *bahamense* had a more limited distribution (Fig. 3). In addition, the major frequency of species was found in summer, where maximum blooms occurred (Fig. 4). Relative abundance of toxic species fluctuated from a few cells to millions per liter. The maximum cell concentration observed was for *Karenia brevis* ($13.9 \times 10^6$ cells/L) in October 1997 on the Veracruz coast. This species had two blooms along the southwestern shelf during the summers of 1980 and 1987 coincident with red tide events associated with fish kills with densities of $<2 \times 10^5$ cells/L. In contrast, *Prorocentrum micans* had the min-



Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

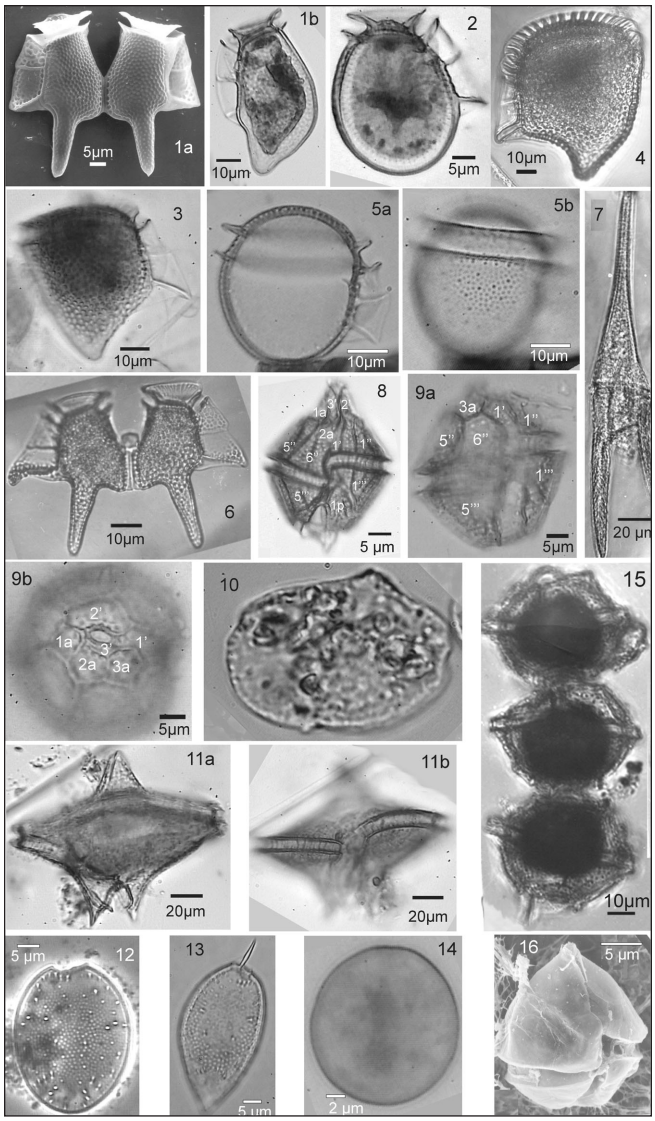


**Figure 1** Toxic and harmful dinoflagellates in the southern Gulf of Mexico. **1a** *Dinophysis caudata* Saville-Kent, 1881, SEM. **1b** *D. caudata* f. *acutiformis* Kofoid et Swezy. **2** *D. fortii*. Pavillard, 1923 **3** *D. mitra* (Schütt) Abé vel Balech, 1967. **4** *D. rapa* (Stein) Balech, 1967. **5a**, **b** *D. rotundata* Claparède et Lachmann. **6** *D. tripos* Gourret, 1883. **7** *Ceratium furca* (Ehrenberg) Claparède, 1858. **8** *Gonyaulax polygramma* Stein, 1883. **9a** *Lingulodinium polyedrum* (Stein) Dodge, 1989, ventral view. **9b** Ibid., apical view. **10** *Karenia brevis* (Davis) G. Hansen et Moestrup, 2000. **11a** *Protoperidinium crassipes* (Kofoid) Balech, 1974, lateral-ventral view. **11b** Ibid., ventral view. **12** *Prorocentrum rhathymum* Löeblich, Sherley et Schmidt, 1974, a valve. **13** *P. micans* Ehrenberg, 1883. **14** *P. minimum* (Pavillard) Schiller, 1931. **15** *Pyrodinium bahamense* Plate, 1906 var. *bahamense,* three cells in chain. **16** *Scrippsiella trochoidea* (Stein) Loeblich III 1976, SEM.

imum density, fluctuating between $>10^3$ and $<10^4$ cells/L at some stations in February. *Protoperidinium crassipes* had a brief peak in the southeastern region ($2 \times 10^4$ cells/L), and *Dinophysis caudata* was abundant during this time ($<10^4$ cells/L). *Gonyaulax polygramma* was recorded mostly in autumn. *Prorocentrum micans* showed the maximum

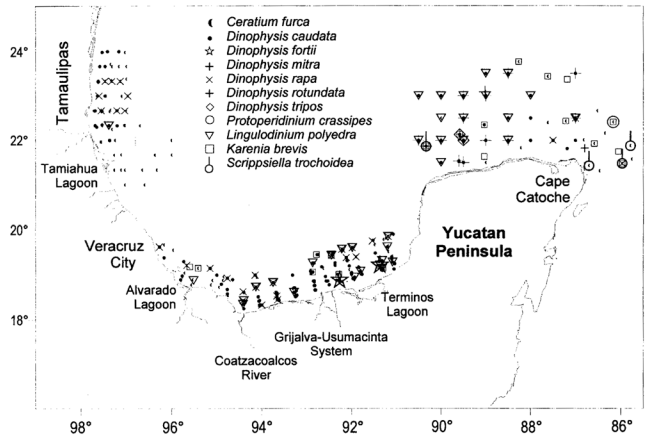


**Figure 2** Sampling area showing the distribution of some toxic and potentially harmful dinoflagellates in the southern Gulf of Mexico (June 1979–July 1994).

abundance in summer and autumn along the southern coast and in Sian K'aan lagoon (a biosphere reserve).

*Pyrodinium bahamense* occurred in chains mostly in coastal lagoons. Another bloom of *Scrippsiella trochoidea* was found with a maximum concentration of 300,000 cells/L at stations located near Cape Catoche in the summer of 1994, where a regular upwelling occurs. In both cases the species caused water discoloration. The latter was a typical brown-greenish color in July 1994, in which 99.6% of the total phytoplankton population was maintained by this species, associated with 6.08 mg of chlorophyll $a \cdot m^{-3}$ and 1.21 mg of carotenoids $m^{-3}$. In contrast, *Ceratium furca* and *Gonyaulax polygramma* had densities of $<10^3$ cells/L. *Pyrodinium bahamense* var. *bahamense* had values of $<10^5$ cells/L in four coastal lagoons, usually in the summer near some coastal lagoons.

A complex of factors favorable for the development of toxic and harmful algae exists in the southern Gulf of Mexico. In our opinion, the major factors are high levels of mineral and dissolved organic substances as well as the vertical stability of water layers during summer, associated with the river discharge. Mapping harmful algae will help to predict their invasion and to prevent misidentifications. Although algal blooms have been considered characteristic of temperate waters, the red tide dinoflagellates are neither boreal nor cosmopolitan. The global distributional patterns of harmful dinoflagellates show that more than 50% of red tide species are tropical-boreal. However, it is still questionable whether the red tide tropical-boreal dinoflagellates prefer tropical or temperate waters in terms of causing bloom events.

## Acknowledgments

This work was partly supported by CONABIO (grant No. FB683/S088). We thank Casimiro Ramirez for providing net samples and information on *Karenia brevis.* We also thank the crew of the R/V *Justo Sierra.* Jorge Sepulveda gave us expert sample preparation. Jesus Soto is thanked for technical support.


**Figure 3** General occurrence of dinoflagellate species obtained from qualitative data. Species code equals the figure number in Figure 1.

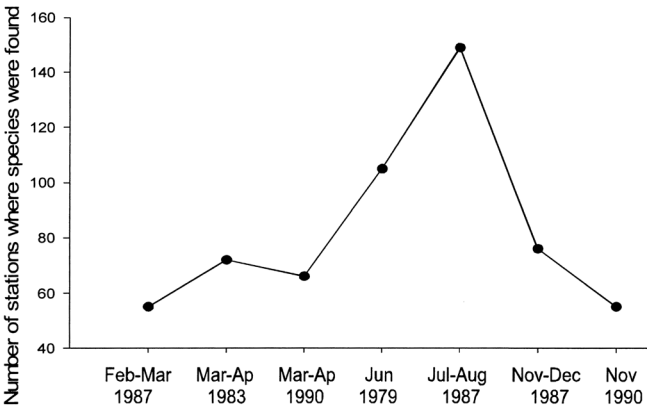


**Figure 4** Total frequency of potentially toxic species of dinoflagellates recorded during seven sampling dates.

## References


I. M. Belousov, Y. A. Ivanov, S.A. Pasternak, T. S. Russ and V. V. Rossov, Oceanology, USSR Acad. Sci. 6, 312–320 (1996).

N. Bessonow, O. Gonzalez, and A. Elizarov, in: Coloquio Sobre Investigaciones y Recursos del Mar Caribe y Regiones Adyacentes (UNESCO, Paris), pp. 317–323 (1971).

D. V. Bogdanov, V.A. Sokolov and N. S. Khromov, Oceanology, USSR. Acad. Sci. 8, 371–381 (1968).

J. D. Cochrane, Bull. Jpn. Soc. Fish. Oceanogr. Spec. No. (Prof. Uda's Commemorative Papers), pp. 123–128 (1969).

A. de la Cruz, in: Coloquio Sobre Investigaciones y Recursos del Mar Caribe y Regiones Adyacentes (UNESCO, Paris), pp. 375–383 (1971).

M. J. Furnas and T. J. Smayda, Cont. Shelf Res. 7, 161–175 (1987).

S. Gómez-Aguirre and S. Licea, in: Harmful Algae, B. Reguera, J. Blanco, M. L. Fernández and T. Wyatt, eds. (UNESCO, Paris), pp. 61–62 (1998).

G. M. Hallegraeff, Phycologia 32, 79–99 (1993).

G. M. Hallegraeff, Aquaculturists' Guide to Harmful Australian Microalgae (University of Tasmania, Australia), 1–136 (2002).

G. R. Hasle, in: Phytoplankton Manual, A. Sournia, ed., (UNESCO, Paris), pp.136–142 (1978).

H. E. Hulburt and J. D. Thompson, J. Phys. Oceanogr. 10, 1611–1651 (1980).

M. V. Lebour, The Dinoflagellates of the Northern Seas (Marine Biological Association, Plymouth), 1–250 (1925).

S. Licea, R. Luna, Y. B. Okolodkov and R. Cortés-Altamirano (in preparation, 2002).

M. Merino, J. Mar. Syst. 13, 101–121 (1997).

K. A. Steidinger, G. A. Vargo, P. A. Tester and C. R. Tomas, in: Physiological Ecology of Harmful Algal Blooms, D. M. Anderson, A. D. Cembella and G. M. Hallegraeff, eds. (Springer, Berlin), pp. 135–153 (1998).

J. L. Tamayo, Geografía Moderna de México. (Trillas, México), 1–390 (1990).