# EVIDENCE FOR DIARRHETIC SHELLFISH POISONING IN MAINE COASTAL WATERS

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Diarrhetic Shellfish Poisoning (DSP) is one of several classes of seafood poisoning caused by naturally occurring marine microalgae. The state of Maine has annual blooms of the paralytic shellfish poisoning (PSP) producing dinoflagellates, *Alexandrium tamarense* and *Alexandrium fundyense*, and has a comprehensive phytoplankton monitoring and shellfish testing program for PSP which successfully protects the public health (Shumway et al., J. Shellfish Res. 7:643-652,1988). Nonetheless, the occasional incidence of unexplained, shellfish-associated gastroenteritis, as well as the rejection of a single lot of shellfish tested for DSP for export to the Netherlands, has raised the question of whether DSP is also an issue of public health significance for Maine.

DSP causes severe cramping, nausea, vomiting and diarrhea, distinguishable from microbial food poisoning in its rapid onset (as early as 30 min), and generally lasts 2-3 days. The toxins responsible for DSP are a suite of polyethers containing transfused or cyclic ether rings, including okadaic acid and the dinophysis toxins (DTX1-4), which are potent inhibitors of Ser/Thr protein phosphatases. The first incidence of shellfish poisoning identified as DSP occurred in Japan in the late 1970's. The dinoflagellate, *Dinophysis fortii,* was identified as the causative organism, and its toxin identified as the causative agent was termed dinophysis toxin (DTX-1) (Yasumoto et al., Bull Japan Soc. Sci. Fish. 44:1249-1255,1978). Retrospective analysis of similar disease outbreaks in the Netherlands and Scandinavia confirmed that these were also associated with *Dinophysis*. DSP is now a frequently encountered problem in Europe and Japan, where it significantly impacts extensive aquaculture industries. The first confirmed outbreak of DSP in North America occurred in 1990 in Nova Scotia, Canada (Quilliam et al., Can Tech. Rep. Fish. Aquat. Sci. No. 1799:18-22, 1990). However, the causative organism responsible for the DSP outbreak in Canada was not *Dinophysis* but an unrelated, benthic dinoflagellate, *Prorocentrum lima.*

Two species of *Dinophysis* present in Maine coastal waters, *D. acuminata* and *D. norvegica*, are frequently found in high numbers from June and September. An anecdotal report from the PSP testing station in La Moine indicated that *Dinophysis* was present in Salisbury Cove, ME in July 1998 at sufficient concentrations to discolor the water, prompting a "red tide" report from a local citizen (J. McGowan, pers. comm.). *Prorocentrum lima,* in contrast, has not been identified in Maine coastal waters. In this study, we therefore surveyed 31 sites along the Maine coast for DSP producing dinoflagellates. Both phytoplankton and mussels (*Mytilus edulis*) collected from each site were then tested for DSP toxin activity using a rapid protein phosphatase inhibition assay (Tubaro et al., Toxicon 34:743-752, 1996). Following this rapid screen, toxin identification was confirmed by HPLC/MS/MS.

Of the 31 sites tested, mussels from 4 sites in Eastern Bay and Frenchman Bay consistently displayed protein phosphatase inhibition activity in the assay for DSP toxins (Figure

1): Lamoine Beach (6-8 ng okadaic acid equiv./g), Bar Harbor Airport (8-14 ng okadaic acid equiv./g), Salisbury Cove (6-10 ng okadaic acid equiv./g), and Bar Harbor Bar (7-20 ng okadaic acid equiv./g). Each site was sampled on four separate dates between June 14-July 16, 1999. The activities found were below the detection limit of the HPLC/MS/MS method used and were at least 100-fold below the European and Canadian regulatory limits of 2 µg/g digestive gland. This indicates that although DSP-like toxins were present in the Frenchman Bay-Eastern Bay region, they were not present at levels that represent a significant public health issue. Two additional sites, Castine and Blue Hill had one out of four positive assays but were not significantly different from negative sites. All other sites from Maine were consistently negative.

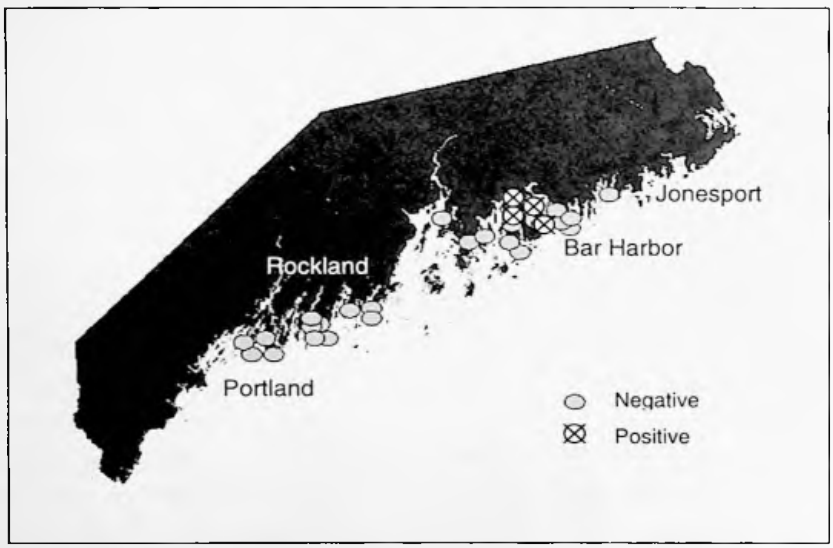


Figure 1. Sampling sites tested for DSP- producing dinoflagellates and toxins. Mussels from four sites in Frenchman Bay-Eastern Bay were found to have okadaic acid-like activity.

*Dinophysis* species, primarily dominated by *D. norvegica,* were observed throughout the sampling period from Rockland to Bar Harbor. Two additional species, *D. acuminata* and *D. rotunda,* were also observed in low numbers. The highest concentrations of *D. norvegica* were routinely observed at Salisbury Cove. All plankton tows that were dominated by *Dinophysis*, were negative for phosphatase inhibition activity and displayed no OA or DTX-1 when analyzed by the LC-MS/MS.

Sites where *Mytilis* tested positive for okadaic acid-like activity were therefore further studied in order to identify a causative organism. At all sites where *Mytilus* tested positive, the dominant macrophyte found was the brown alga, *Ectocarpus* sp. At these locations *Ectocarpus* had an epiphytic community that included the toxic dinoflagellate, *Prorocentrum lima* (Figure 2). This dinoflagellate was not found at any other locations. A large sample (535 g wet weight) of the epiphytic microalgae associated with *Ectocarpus* was collected from the Lamoine Airport site, concentrated, and extracted in methanol. Density of *P. lima* from this sample was approximately 200 cells/g wet weight. This extract displayed protein phosphatase inhibitory activity and subsequent analysis by HPLC-MS/MS showed the presence of DTX-1 in the wild population of *P. lima* (Figure 3). Furthermore, microscopic analysis of the digestive gland from

*Mytilus* collected from the Lamoine Airport and Bar Harbor sites showed empty thecae, consistent with the morphology of *P. lima*, suggesting that *P. lima* is consumed by the mussel.

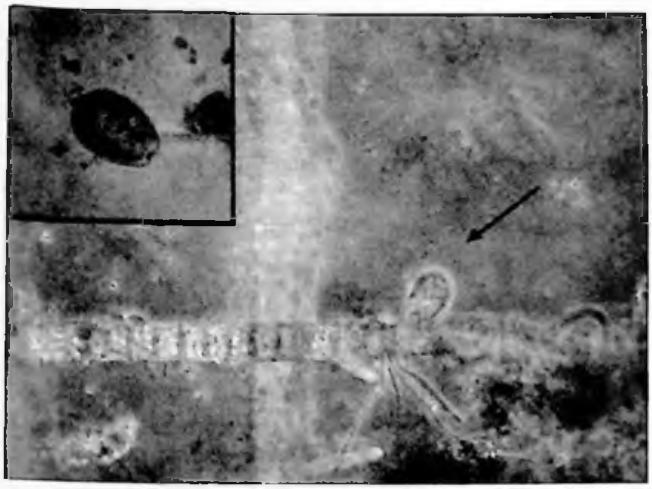

Figure 2. *Prorocentrum lima*. Inset and attached to *Ectocarpus* sp. (arrow).

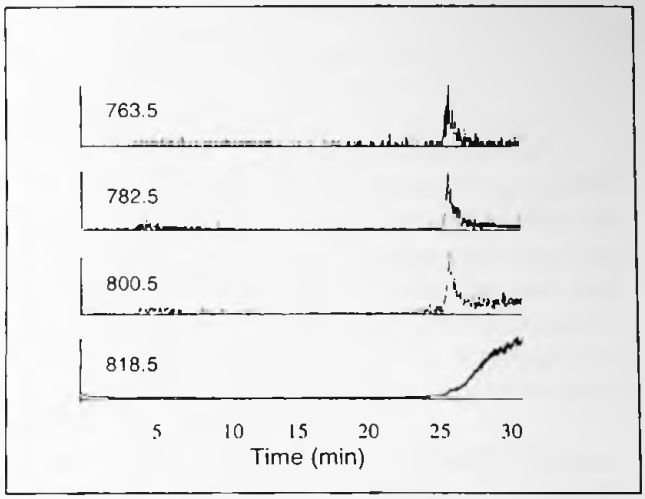


Figure 3. HPLC-MS/MS chromatograph of DTX-1 from P. lima. Traces reflect selected ion monitoring of distinctive fragment ions at M/Z 763.5,782.5, and 800.5, as well as parent ion of M/Z 818.5.

The results of this study identify DSP toxins in Maine coastal waters. *P. lima* and DSP toxins were found only in the Frenchman Bay-Eastern Bay region. In the current study, OA-like activity found in mussels were not at levels that present a public health issue. However, the presence of DSP as a potential problem in Maine coastal waters must be considered in future management decisions regarding shellfish aquaculture. DSP became a problem in Canadian maritime waters when the practice of raft culture was introduced, creating suitable conditions for the growth of *Pilayella littoralis*, a filamentous brown macrophyte that is provides an excellent substrate for the dinoflagellate, *P. lima* (Lawrence et al., *In* Reguera et al.(eds). Harmful Algae. UNESCO. Pp. 78-79.1998). The sampling sites exhibiting both *P. lima* and OA-like activity in *Mytilus* were protected embayments with muddy to gravel substrates that supported the growth of *Ectocarpus*. These sites differ substantially in character and location from the primary monitoring sites used by the State of Maine's PSP monitoring program. This, in addition to the benthic behavior of the causative organism, *P. lima*, indicates that a different sampling strategy than that currently employed by the state for the long established PSP monitoring program maybe required to monitor for occurrences of DSP.

This study was supported by funds from NOAA National Ocean Service and by a New Investigator Award from the MDIBL NIEHS Marine and Freshwater Biomedical Sciences Center to FMVD.