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Amnesic Shellfish Poisoning: Domoic Acid Production by *Pseudo-nitzschia* Diatoms

Background

Most phytoplankton (microscopic one-celled algae) are beneficial, forming the base of the food web and providing oxygen to the atmosphere. However, a small but increasing number of phytoplankton species – about 90, or 2% of the total – also produce potent toxins, called phycotoxins (or biotoxins). The occasional rapid growth of these algae to high concentrations results in Harmful Algal Blooms (HABs; popularly known as “red tides”). These toxic algal cells are filtered out of the water by molluscan shellfish (mussels, oysters, quahogs, etc.) during their normal feeding activity. Without the existing monitoring efforts, these animals could then cause serious illness or death, if consumed by humans.

In late November to December, 1987, three deaths and over 100 illnesses were traced to the consumption of cultivated mussels (*Mytilus edulis*) harvested from Cardigan Bay, PEI (Figure 1).

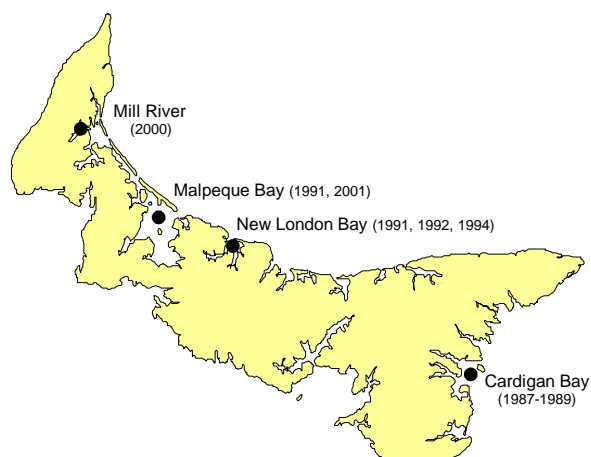


Figure 1. Locations and dates of closures due to toxic *Pseudo-nitzschia multiseries* blooms in PEI.

Symptoms of the new poisoning included nausea, vomiting, diarrhea and abdominal cramps. In more severe cases neurological symptoms occurred, including headaches, confusion, loss of short-term memory, difficulty breathing, seizures, and in extreme cases, death. Because of the memory loss that could persist indefinitely, the syndrome was named Amnesic Shellfish Poisoning (ASP).

After an unprecedented 104 hours of investigative work by National Research Council Canada, Fisheries and Oceans Canada (DFO), University of Prince Edward Island and other federal agencies, domoic acid (DA) was identified as the contaminating agent in the shellfish. The identification of DA as a toxin was at first treated with skepticism. This small amino acid was known as a folk medicine to treat intestinal worm infestations in young children in Japan. However, a 10 times greater dose was consumed in the eastern Canadian toxic episode, and those most affected were elderly or had previous underlying medical conditions. This neurotoxin has a chemical structure similar to that of glutamic acid, a brain neurotransmitter. Because of this structure, DA binds to the same receptors on the neurons as glutamic acid – but it remains bound. The neurons therefore begin to fill with calcium, swell and eventually burst. These nerve cells are located in the part of the brain associated with memory retention (the hippocampus), hence the memory loss characteristic of ASP.

Closure Level for Domoic Acid

The action limit for the amount of DA allowed in molluscan shellfish was established at 20 µg DA/g of wet weight tissue. The Canadian Food



Inspection Agency (CFIA) is responsible for analyzing shellfish for biotoxins, to help ensure the safety of the product for human consumption. When DA levels start to approach the action limit, the CFIA recommends to DFO that the area be closed to shellfish harvesting. DFO then issues a Prohibition Order to legally prevent harvesting; the closure is enforced by Fishery Officers from DFO's Conservation and Protection Branch. An area is reopened only when three samples over a 14 day period are found to contain less than 20 µg DA/g and the DA levels are decreasing.

Sources of Domoic Acid

Pseudo-nitzschia multiseries

The source of DA in the 1987 episode on PEI was rapidly traced to a phytoplankton species called *Pseudo-nitzschia multiseries* (at the time, it was known as *Nitzschia pungens* forma *multiseries*). As a pennate diatom, it is characterized by having a long, narrow cell with a delicate wall – called a frustule – made of silica. The frustule is formed in two halves that fit together like the lid and base of a pillbox. *Pseudo-nitzschia* cells are stuck to each other by their overlapping tips, and can form chains made up of hundreds of cells (Figure 2).

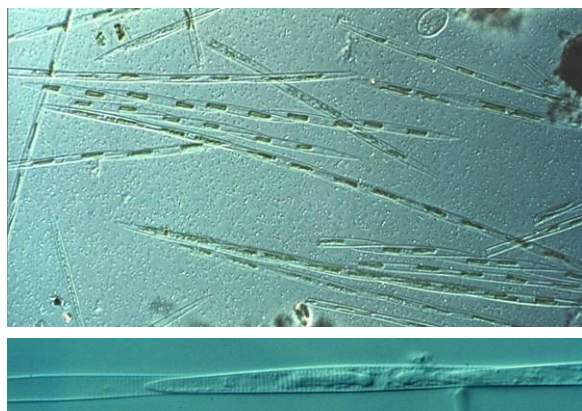


Figure 2. Chains of *Pseudo-nitzschia multiseries* cells, as seen using a light microscope, in seawater collected from Cardigan Bay in 1987 (upper photo) and in culture (lower photo).

Neither the length of the chains nor the presence of DA harms the shellfish that continue to feed upon them. The DA is nevertheless toxic to the humans (and some marine mammals) who consume contaminated shellfish. This was the first time that a diatom had been shown to produce a phycotoxin!

Toxic blooms of *P. multiseries* have always appeared during the autumn, in eastern and northern embayments of PEI. The highest observed levels of DA (790 µg/g) and of *P. multiseries* cell numbers (15 million cells per litre) occurred during the original 1987 bloom. Toxic blooms reappeared the following two years, but with much lower DA levels and *P. multiseries* cell numbers. There have been no closures due to this species on eastern PEI since then. However, closures have since occurred at several embayments in northern PEI (Figure 1).

The circumstances that promoted the original, very intense *P. multiseries* blooms in eastern PEI are thought to be related to the unusual meteorological conditions: 1987 was characterized by a prolonged dry period in summer, followed by an unusually rainy autumn. This may have provided nutrients, via river runoff, to feed the blooms. The following three years were less severe, and these exact conditions have not reappeared since.

A complicating factor is that there is another species of *Pseudo-nitzschia* that tends to bloom just prior to, and during, blooms of *P. multiseries*. The difficulty is that the accompanying species, called *P. pungens*, looks identical to *P. multiseries* in terms of length and width, when viewed with the classical light microscope. It is important to distinguish these two species, because *P. multiseries* is toxic, whereas *P. pungens* is not. During some autumns, there have been intense blooms of *P. pungens*, which have not resulted in any DA in cultivated shellfish.

How then does one distinguish the two species? We are able to take advantage of the diatoms' silica frustule that forms a solid "glass shell". The inside of the frustule has an elaborate pattern of pores and other structures that are unique to each species. In order to view the frustules, the cells must first be cleaned with an acid solution to remove the outer organic layer, leaving the inorganic silica frustule intact. A scanning electron microscope (SEM) is then used to view the patterns on the frustules. The SEM at the Digital Microscopy Facility (DMF) of Mount Allison University (Sackville, NB) produced the images of four species of *Pseudo-nitzschia* found in PEI waters (Figure 3).

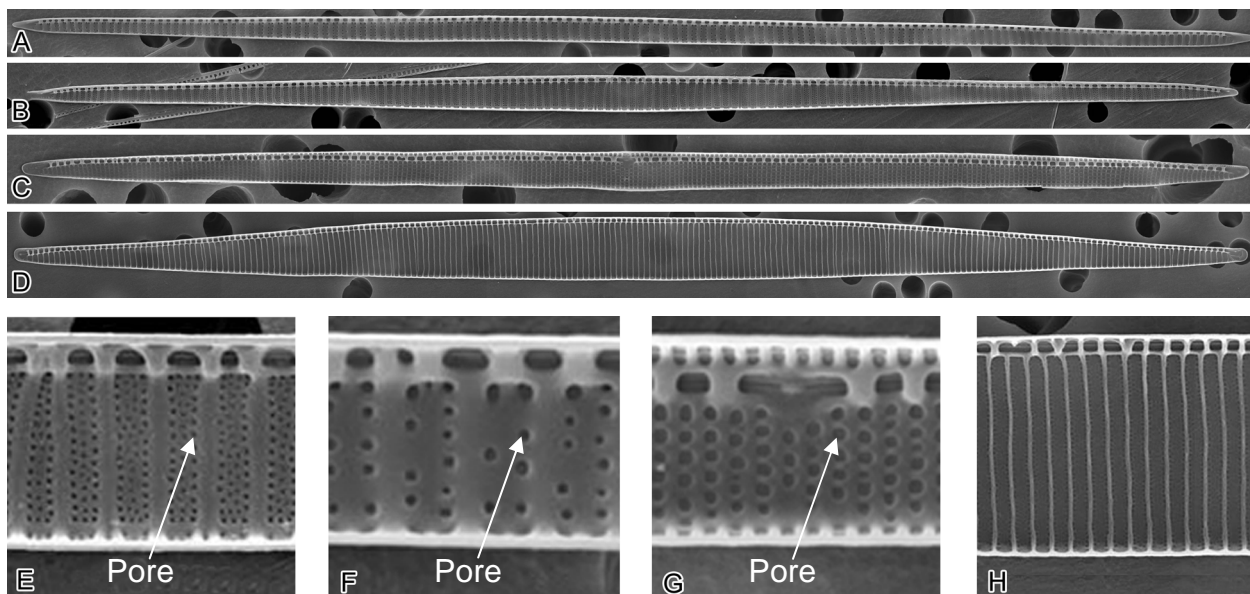


Figure 3. Scanning electron micrographs (SEMs) of acid-cleaned *Pseudo-nitzschia* species at low magnification (A-D) and at high magnification of the central part of the cells (E-H). (A, E) *P. multiseriata*; (B, F) *P. pungens*; (C, G) *P. calliantha*; and (D, H) *P. seriata*. Notice differences in the size and shape of pores (but too small to be seen in *P. seriata* image H). Images not to scale. Source: http://www.mta.ca/dmf/psn_compare.htm.

Up to the autumn of 2000, the above two species of *Pseudo-nitzschia* were the most common in PEI waters. Then, another species began to show up in abundance. That species, *P. calliantha* (but originally called *P. pseudodelicatissima*), is much thinner than the above two, so it can easily be distinguished using light microscopy. It also has some obvious differences when viewed with the SEM (Figure 3G). During the autumn of 2001, and again in 2002, this species reached very high numbers in Cardigan Bay (1.3 to 1.6 million cells per litre), and was a cause of great concern. However, cells isolated into culture at DFO Moncton showed that they did not produce DA. No toxins were found in the shellfish.

Pseudo-nitzschia seriata

Up to 2001, the CFIA had never detected DA at any other time of year except the autumn. Then, on April 5, 2002, there was an unexpected closure, due to DA for the first time ever in the spring, of shellfish harvesting in bays of northern PEI. The closure was expanded to the Baie des Chaleurs, the entire coast of eastern NB, and to western and eastern Cape Breton Island (NS), when the DA was discovered to be more widespread. These

closures prevented any contaminated shellfish from reaching the market.

Such a broad extent of closures, encompassing most of the southern Gulf of St. Lawrence (sGSL), suggested that a large and wide-spread bloom of toxic diatom cells was involved. The bloom was likely distributed throughout the sGSL by the prevailing southeast water currents. Water samples collected at each affected site showed that *Pseudo-nitzschia seriata* was the only toxic diatom present. It can easily be distinguished from the other *Pseudo-nitzschia* species because of the wider cells (Figures 3D, 4).

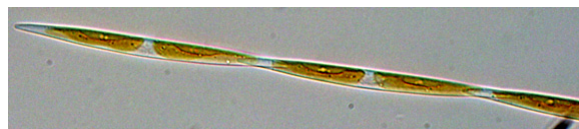


Figure 4. Chains of *Pseudo-nitzschia seriata* cells, as seen using a light microscope, in culture.

On northern PEI, the highest observed cell concentration was 403,000 cells per litre on April 10; the highest toxicity in mussels was 71 µg DA/g, on April 16. Known as a “cold-water” diatom, *P. seriata* is found only in the north

Atlantic. It is normally present at low concentrations during most of the year in the Gulf of St. Lawrence, and was associated with high levels of DA in the digestive glands of Magdalen Island sea scallops since 1998; the adductor muscle is free of DA. Tests at DFO Moncton confirmed that *P. seriata* produced DA in culture.

One cannot say with certainty what caused this first spring toxic bloom. However, we know that *P. seriata* can grow on the underside of sea ice in the Arctic Ocean, as well as freely in the water column. We also know that the winter and spring of 2001-2002 had anomalous weather conditions. Above normal winter and spring temperatures delayed the ice freeze-up by 2-3 weeks. Total ice coverage was the sixth lowest on record, and the ice departed two weeks early – by mid-March. One can therefore hypothesize that the early ice break up released cells into the water, where they continued to bloom. Prevailing southeast currents then moved the blooms towards the coast, where tides drove the toxic cells into the bays, thus contaminating the cultivated mussels. This event demonstrates that we must remain continuously vigilant for new species of toxic phytoplankton to appear at unexpected times of the year.

PEI has the above two species of toxic *Pseudo-nitzschia*, but there are now 8 other species capable of producing DA (although some strains are not toxic). These species are found in many coastal waters around the world; the problem is therefore not unique to PEI!

Laboratory Studies

Using a microscope and an extremely thin glass tube, single diatom cells can be isolated and placed into a seawater culture medium. This has allowed studies to determine which conditions promote growth and DA production. *Pseudo-nitzschia* species grow well under a wide range of salinities and temperatures, except for *P. seriata*, which requires temperatures below about 15°C. Sufficient light is needed for growth, but also to provide enough energy for toxin biosynthesis. The cells start producing high amounts of DA as soon as their growth slows or stops, when they run out of the nutrients silicate or phosphorus.

Monitoring Programs

The CFIA monitors for the presence of biotoxins in molluscan shellfish. However, monitoring for the diatoms that produce the DA can give an early warning of impending biotoxin accumulation in the shellfish. The PEI Department of Agriculture, Fisheries, Aquaculture and Forestry, in collaboration with the CFIA, collects water samples weekly from 15 embayments for counts of toxic phytoplankton, as part of the PEI Mussel Monitoring Program. Water sampling is from early September to early December. *Pseudo-nitzschia* cell counts are sent to the industry, the CFIA and DFO Science. Under contract to DFO, the *Pseudo-nitzschia* species are identified using the SEM at the DMF of Mount Allison University. This information can then be used to follow the progress of the bloom and to assess the potential for the bloom to render the shellfish toxic.

Conclusions

Blooms of toxic *Pseudo-nitzschia* cells do not occur every year. However, they are a reality that the shellfish industry must deal with from time to time. Biotoxin monitoring by the CFIA provides reasonable assurance of the safety of the product for human consumption. Scientific research aims at understanding the conditions under which toxic blooms occur, with the ultimate goal of being able to predict them. In the meantime, phytoplankton monitoring can help the industry manage the harvesting of their product by knowing when and where toxic cells begin to bloom.

This information sheet was made possible by the following partners:

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 - PEI Department of Agriculture, Fisheries, Aquaculture and Forestry
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