Harmful Algal Blooms on the North American West Coast

Edited by Raymond RaLonde

Proceedings of Harmful Algal Blooms (HABs): The Encroaching Menace
A conference to organize a West Coast effort for
monitoring and research on harmful algal blooms,
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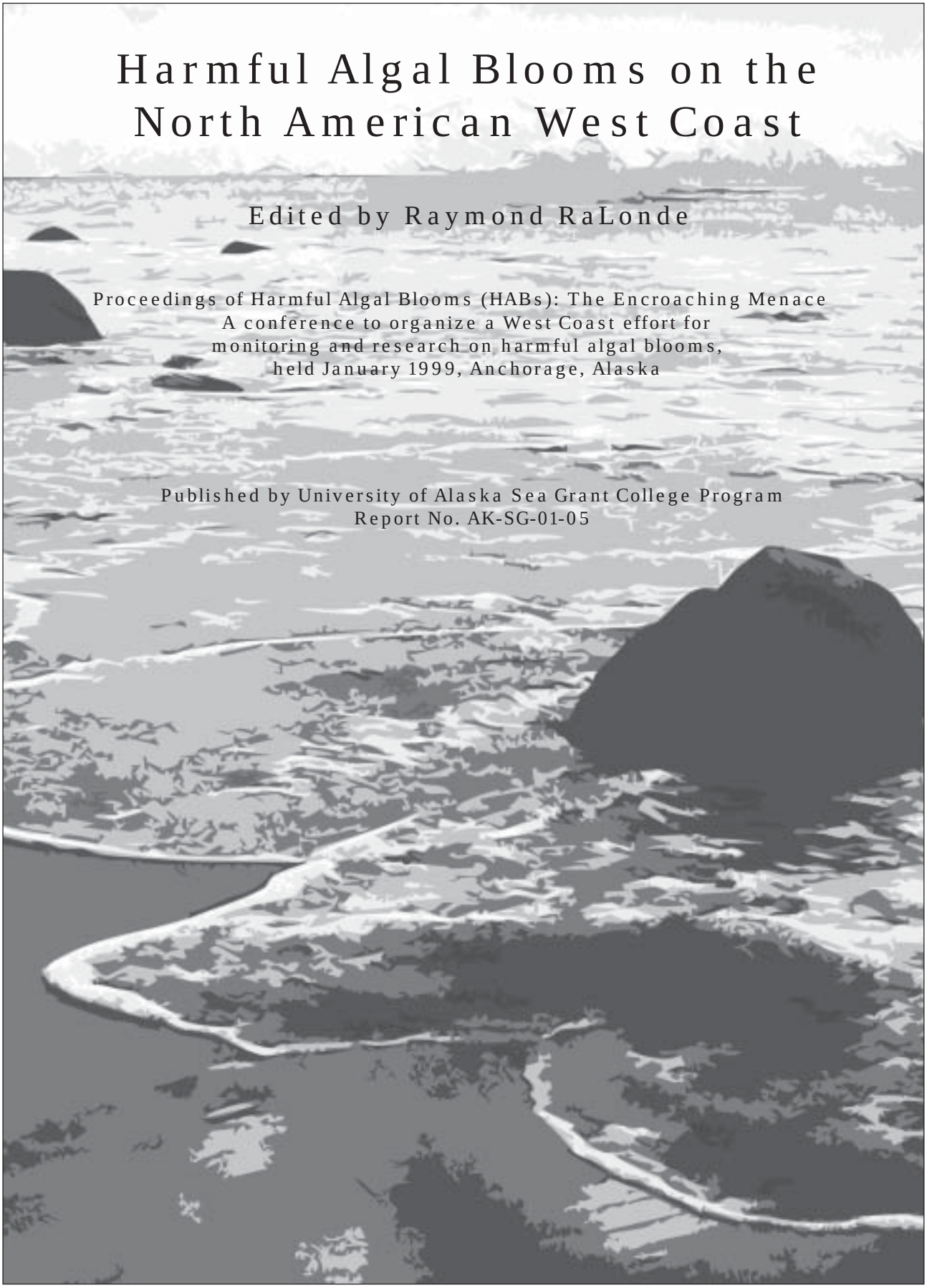

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University of Alaska Sea Grant
P.O. Box 755040
205 O'Neill Bldg.
Fairbanks, Alaska 99775-5040
Toll free (888) 789-0090
(907) 474-6707 • Fax (907) 474-6285
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Harmful Algal Blooms (HABs): The Encroaching Menace
A 1999 conference to organize a West Coast effort for monitoring and research on harmful algal blooms

Raymond RaLonde
University of Alaska Marine Advisory Program, Anchorage, Alaska

Background

Harmful algal blooms (HABs) are a worldwide problem and increasing in frequency and intensity, causing severe economic hardship, episodes of illness, and death. In Alaska, the most damaging HABs are *Alexandrium* dinoflagellate blooms which cause paralytic shellfish poisoning (PSP). A persistent problem for Alaska, PSP fatalities date back to 1799 when the crew of Alexander Baranof, of the Russian American Trading Company, ate tainted blue mussels at the now notorious Poison Cove in southeast Alaska. Since 1990, one fatality, a number of illnesses, and economic losses to shellfish fisheries have occurred, caused by PSP problems.

PSP directly affects three sectors of Alaska's marine enterprises—the commercial fishery, aquaculture, and recreational/subsistence harvest. The geoduck clam dive fishery and the crab fisheries feel the effects when PSP is in the viscera, and/or the cumbersome nature of the testing process impedes live shipment and reduces the value of the harvest. Shellfish aquaculture operations, as production diversifies and expands, must comply by testing an increasing number of product samples, and must certify it safe for human consumption. Recreational and subsistence harvesters do not have a testing program that certifies the safety of personally harvested shellfish.

1995 HABs Conference

To address these issues, a conference and workshop was held May 8-9, 1995. A primary goal of the conference was to assemble PSP experts from across the nation to discuss means of dealing with the Alaska PSP problem. The conference was titled "Living with Paralytic Shellfish Poisoning: A conference to Develop PSP Research and Management Strategies for Safe Utilization of Shellfish in Alaska." Participants discussed Alaska's PSP problem and developed the following set of recommendations.

Recreational/Subsistence

1. Determine which state agency should have lead responsibility for monitoring noncommercial harvests of shellfish to ensure public health is protected.
2. Form an interagency group to develop an effective public education program for safe noncommercial use of shellfish in Alaska.
3. Develop and implement immediately a pilot PSP monitoring for noncommercial shellfish beaches.

Commercial bivalve shellfish (oysters, clams)

1. Examine PSP test results, seasonal data, and species variability to determine appropriate sample size for oysters, clams, and mussels.
2. Study the rates of toxification and detoxification for each commercial bivalve species.
3. Expand Alaska's current phytoplankton monitoring program to include more active shellfish farmers and clam producers and compare the data with results of PSP tests.

Commercial production of whole-cooked or live crab

1. Raise the action level from 70 to 80 µg per 100 grams.
2. Conduct preseason PSP testing of critical crab stocks.
3. Change crab PSP monitoring districts to reflect harvesting and oceanographic patterns.

General issues

1. Form an intergovernmental/industry working group to develop a comprehensive state program for marine biotoxins.

2. Appoint a committee to follow up on conference action plans.

Accomplishments since the 1995 HABs Conference

Since 1995, several of the recommendations received attention, and other issues were added. The actions taken on the recommendations are as follows.

Recreation/Subsistence

By February 1999 no state agency had been assigned the responsibility of monitoring noncommercial harvests of shellfish. Two letters were sent to Governor Tony Knowles, neither receiving a reply.

To address better public education about PSP, the University of Alaska Marine Advisory Program has published an *Alaska Marine Resources* newsletter titled "Paralytic Shellfish Poisoning: The Alaska Problem." Over 2,000 of the newsletters have been distributed and it is posted on the Alaska Sea Grant Program Web page (www.uaf.edu/seagrant/bookstore/M-02.html). A lecture series titled "Paralytic Shellfish Poisoning: What You Don't Know Might Kill You" was presented at three locations in Alaska. Several radio shows and newspaper articles have been published. Ray RaLonde, the MAP aquaculture specialist, included a session on HABs and marine toxins in the training course of water quality monitoring for the Native American Fish and Wildlife Society. Since the 1995 conference, RaLonde has contacted officials in Kodiak, Sitka, and Craig to possibly begin a pilot toxin monitoring program.

Commercial bivalve shellfish

Some progress has been made in reducing the sampling requirements for commercial and cultured shellfish. Kachemak Bay, previously subjected to the lot sampling program where each harvested lot was tested for marine toxins, now has a regional sampling program. Examination of historic data, and the minor problems Kachemak Bay had with marine toxins, has allowed ADEC the regulatory opportunity to reduce the Kachemak Bay sampling program to a once-each-week sample.

The ADEC Uniform Sampling Program has undergone significant modification that reduces sampling requirements with historical data documenting PSP-free lot samples.

A toxification/detoxification study for Dungeness crab was proposed for the summer of 1997, but toxic shellfish were unavailable for feeding the crab. Subsequently, the study has been postponed in favor of other priority research.

A detoxification study funded by the Alaska Science and Technology Foundation (ASTF) was conducted to search for a solution to the visceral PSP problem in geoduck clams. Specifically the study tested the prospect that geoduck clams could be detoxified when held at a location known to be free of PSP. The result of the experiment was not encouraging. Although visceral PSP levels dropped 22.5% to 55.7% over a four-week holding period, more than 50% of the clams still retained PSP levels above the regulatory limit. Also, the mortality from holding clams was unacceptably high at 25.5% (R. Painter, 1998. Purging geoducks of PSP toxins. Final report to the Alaska Science and Technology Foundation, Grant 97-1-008).

The phytoplankton monitoring program is very much reduced from 1996 with only a single sampler continuing in southeastern Alaska. Both MAP and ADEC have little time to dedicate to a sampling program.

Commercial production of whole-cooked or live crab

Since 1996, southeastern Alaska has not had a crab visceral PSP problem. The Kodiak Island area, however, continues to process and section crab because of chronic PSP. In fact, Kodiak has not had a shipment of live or whole cooked crab since 1996. To monitor the PSP in crab, ADEC requires 12 samples per week per processing plant be tested for PSP.

General issues

An intergovernmental/industry/university HAB work group was organized in 1998. The 1999 HABs conference was the first conference organized by and for the work group. A seven-person committee was appointed in 1999 to follow up on the action plans laid out in the 1995 conference.

Other activities since 1995

A new PSP diagnostic testing procedure, developed by a Canadian biotechnology company, was field-tested in Alaska (see contribution by J. Jellett in these proceedings). The new technology uses mouse

cells rather than live mice in detecting and quantifying the presence of toxin in a sample. Trials used both the new maritime in vitro shellfish test (MIST™) kits and the ADEC live mouse bioassay. Trials include PSP-screening tests using a derivation of the quantitative MIST™ tests at three different aquaculture sites. The desired outcome is the acceptance of the new MIST™ technology by ADEC for use in Alaska, and the development of a satisfactory strategy for transferring the technology to Alaska.

1999 HABs Conference

BRAINSTORM SESSION

During the 1999 HABs Conference, contributors presented the following issues that need to be addressed:

Research

- Intense systems approach in key areas with a multi-project collaboration (logistical considerations).
- Local volunteer assistance (aquatic farms).
- Historical records (global perspective and how it relates).
- Draw upon state and regional experience.
- Utilize new and emerging technologies to get dense data sets.
- Cooperation with institutions outside the area (cost share).
- Look at existing expertise and experience.
- Managing a regional research program (based on primary participants, consent agreements among the research team).
- Publishing research results.

Outreach activities

- Newsletters (continued funding).
- Cultural diversity.
- Every other year meetings, with funding (outreach product as a result of the meetings).
- State high school programs.
- National visibility, international participation in toxic algae conferences.
- Media presentations.

Funding

- Need a coordinated regional approach.
- Window of opportunity with Alaska's Washington, D.C., delegation.
- Proposals must pass peer review.
- Good concept to communicate to our delegates.
- Who's going to manage this funding effort?
- What funding sources, federal or state?
- Communicate the economic, human, and ecological impact of harmful algal blooms.
- Develop a short fact sheet of impacts.

Monitoring

- Sampling methodologies (how samples are collected, pooling vs. individual, are they valid?)
- New improved methodologies for testing to drive costs down.
- Funding for monitoring (commercial, subsistence, recreational).
- Development of monitoring programs in key locations.
- Correlation between algae and the toxicity of the shellfish (focusing on a single key location).
- Indicator species, by location and species, is a problem in Alaska (there is a specific species for a specific fisheries).
- Frequency of algae sampling (2 to 3 times a week).
- Differences in monitoring between regulatory and research objectives.
- Nondestructive technique for sample collection (small sample extraction).

Alexandrium and *Pseudo-nitzschia*: Two of the Genera Responsible for Toxic Algal Blooms on the U.S. West Coast

Rita A. Horner
School of Oceanography, University of Washington, Seattle, Washington

A number of toxin-producing algae occur on the U.S. West Coast, but public health and economic problems are related primarily to species of only two genera: the dinoflagellate genus *Alexandrium* that causes paralytic shellfish poisoning (PSP) and the diatom genus *Pseudo-nitzschia* that causes amnesic shellfish poisoning (ASP). The two genera are very different from each other in many respects including morphology, motility, life cycles, and toxins. Unfortunately, the general biology of many of the toxigenic species is not well-known and their identification is often difficult.

Alexandrium

There are about 30 species of *Alexandrium* occurring primarily in temperate and tropical waters in both hemispheres with six species known to occur on the U.S. West Coast. The cells are generally 20-50 µm in size, are usually spherical or oval in shape, have no spines or horns, and have a theca (cell wall) composed of cellulose plates that are arranged in a very specific pattern (Fig. 1). A girdle, or groove, encircles the middle of the cells with the ends being displaced by about 1-1.5 times the girdle width. The transverse flagellum lies within the girdle and enables the cell to swim forward, while at the same time causing the cell to rotate. The sulcus is a depression on the ventral side running from the girdle to the posterior end of the cell. The trailing flagellum lies in the sulcus and acts primarily as a rudder. All species contain chloroplasts and are photosynthetic. However, some species are also known to ingest other organisms (Jacobson and Anderson 1996). In some species, the cells are held together to form chains.

Species are differentiated based on cell shape and cell dimensions; the shape and position of a pore plate (Po plate) on the top of the cell (the apical pore complex or APC; Fig. 1); the presence and size of the ventral pore (Vp; Fig. 1) on the 1' plate; the displacement of the 1' plate with regard to the APC, i.e., how the two are or are not connected; the shape and size of some of the sulcal plates (difficult to see without special dissection and staining methods); and chain formation.

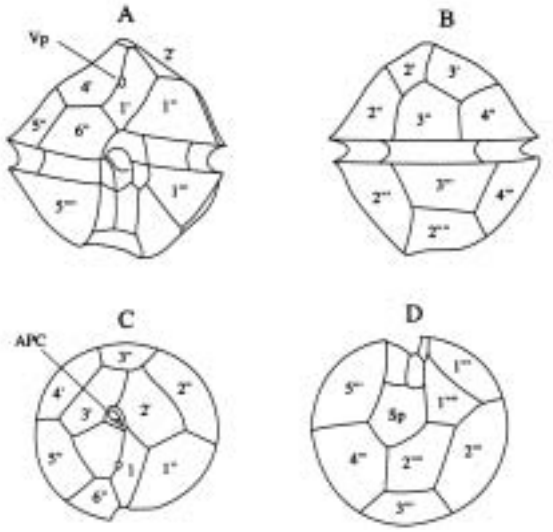

Figure 1. Line drawing of *Alexandrium* showing thecal plates and the apical pore complex. (A) Ventral, (B) Dorsal, (C) Epitheca, (D) Hypotheca. Vp = ventral pore, APC = apical pore complex, Po = extreme anterior plate (the same as the APC), ' = 4 apical plates, " = 6 precingular plates, c = 6 cingular plates, s = 9-10 sulcal plates; "' = 5 postcingular plates; "" = 2 antapical plates (redrawn from Balech 1995).

The most abundant species in Pacific Northwest waters is *Alexandrium catenella* (Whedon & Kofoid) Balech. It is also the best known because it causes PSP and is the organism most people associate with the term "red tide," although it rarely causes discolored water. The cells are somewhat compressed from top to bottom, the pore plate (Po) touches the 1' plate, there is no ventral pore, and the cells usually form chains. On the West Coast, it is known from southern California at least to Bristol Bay, Alaska. Three additional species do not form chains and are difficult to distinguish from each other. *A. acatenella* (Whedon & Kofoid) Balech has cells longer than

wide and has a ventral pore. It occurs from northern British Columbia into Alaska. *A. tamarense* (Lebour) Balech is relatively small, with a broadly rounded epitheca and a ventral pore. In North America, it is known primarily from the eastern United States and Canada, but has been found in the Gulf of Alaska. *A. fundyense* Balech is similar to *A. tamarense*, but lacks the ventral pore. It is known primarily from eastern Canada (Bay of Fundy), but has been identified from Porpoise Island, Alaska, using molecular techniques (Scholin et al. 1994). *A. ostenfeldii* (Paulsen) Balech & Tangen and *A. hiranoi* Kita & Fukuyo have also been identified from the Pacific Northwest (Taylor and Horner 1994).

The life cycle of *Alexandrium* is somewhat complex (Fig. 2). In general, motile vegetative cells divide and produce more motile cells, a process called asexual reproduction. However, when environmental conditions are not quite right, the vegetative cells round up and become thin-walled cysts called pellicle, or temporary, cysts. These cysts do not last very long and produce vegetative cells again when the stress is relieved. Sexual reproduction frequently occurs when nitrogen is limiting. In this case, the vegetative cells produce gametes that fuse forming a planozygote that is similar to the vegetative cells except that it has two trailing flagella. The planozygote may last for several weeks, but eventually rounds up, loses its flagella and becomes a thick-walled hypnocyst, sometimes called a resting cyst. The hypnocyst has a required dormancy period, usually several months, and is an overwintering cyst. After the dormancy period and often in response to increasing temperature, the hypnocyst germinates, producing an oval cell with two trailing flagella. The young germling will eventually undergo meiosis and generate motile vegetative cells thus completing the cycle.

The hypnocysts are important for a number of reasons. They can be involved in bloom termination if conditions are such that vegetative cells no longer divide, but form cysts instead. The motile cells then disappear from the water column and the cysts eventually settle to the sea bed where, because they are resistant to environmental extremes, they survive and act as seed populations for new generations of cells. Cysts are also a dispersal mechanism because they are first formed in the water column and can be transported long distances by water currents. Another important attribute of cysts is that they are the product of sexual reproduction so provide for genetic variability in the organism. Finally, cysts are toxic, although there is some question whether shellfish can become toxic from cysts.

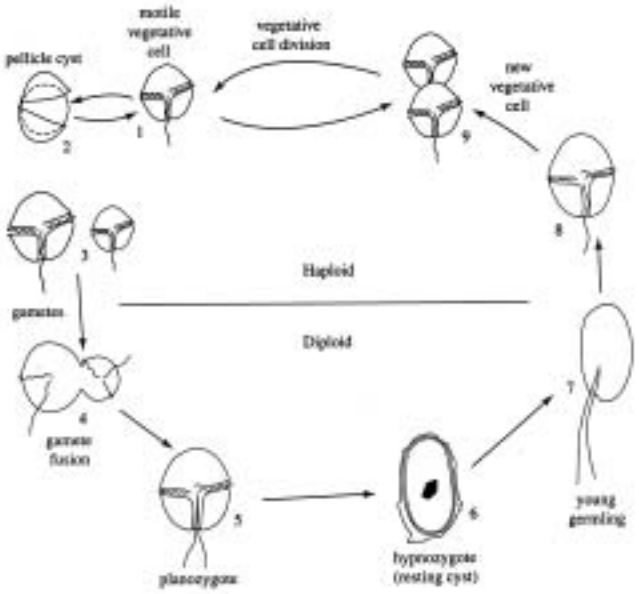

Figure 2. Generalized life cycle of *Alexandrium* (redrawn from Anderson 1998).

Pseudo-nitzschia

Currently there are 21 taxa in the genus and of these, seven, possibly eight, are known to produce domoic acid. Species of *Pseudo-nitzschia* occur in coastal and oceanic waters throughout the world, including polar regions. At least eight species have been identified to date from Pacific Northwest waters, including five of the known domoic acid producers. Cells range in size from ca. 20 to 144 µm long (apical axis) and from 1 to 10 µm wide (transapical axis) and are strongly elongate, rectangular, or fusiform in girdle view (Fig. 3). The cells are usually united in stepped chains by overlapping of the cell ends, but are sometimes solitary. Flagella are not present, but chains are motile, moving with the slow, smooth to jerky gliding motion typical of many pennate diatoms. As with all diatoms, the cell wall is composed of silica and the fine structure of the cell wall is one character used to determine species. The cells are photosynthetic having two chloroplasts lying along the girdle on either side of the transapical plane.

Characters used to differentiate species include: the valve outline; width of the valve; linear density of

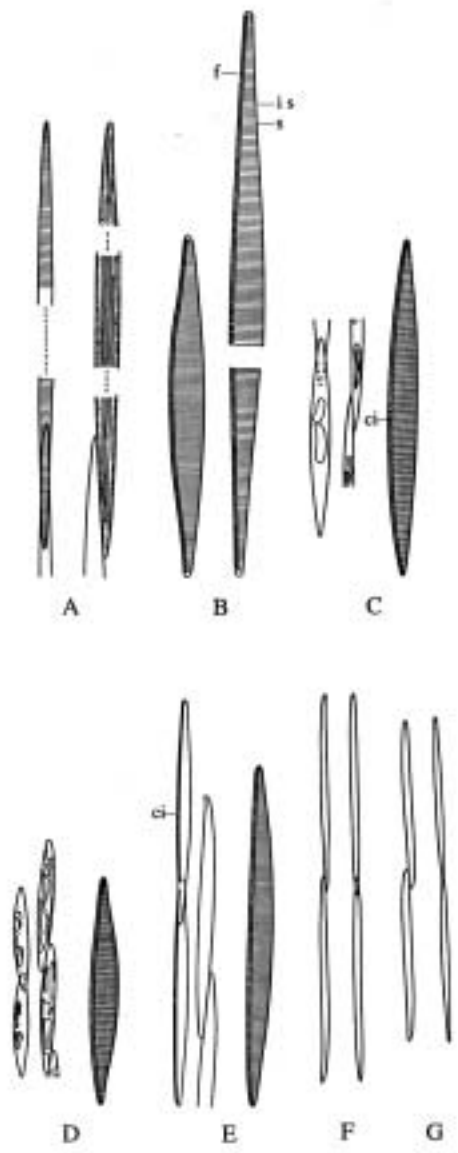

Figure 3. Line drawings of *Pseudo-nitzschia* species. (A) *P. pungens*/*P. multiseries*, valve (left), girdle (right); (B) *P. australis*, valve views; (C) *P. fraudulenta*, valve (left, right), girdle (middle); (D) *P. subpacifica*, valve (left, right), girdle (middle); (E) *P. heimii*, valve (left, right), girdle (middle); (F) *P. pseudodelicatissima*, girdle (left) valve (right); (G) *P. delicatissima*, girdle (left), valve (right). f = fibulae (silica bridges on either side of the raphe); is = interstriae (nonperforated siliceous strips between two striae); s = stria (lines of pores); ci = central interspace (space between the two central fibulae) (from Hasle 1972 and Medlin and Hasle 1990).

interstriae and fibulae; stria structure; the presence or absence and size of the central interspace; the shape of the valve ends in girdle and valve views; and the length of overlap of the cell ends. Unfortunately, some of these features are not visible when using light microscopy and either scanning or transmission electron microscopy is needed to resolve them. The use of electron microscopy to positively determine species is especially important when there is a question of whether a species is toxic or not. However, there are toxic and non-toxic strains, domoic acid production varies during the life cycle and with species, and the ability to produce toxin may decrease during the life of a culture. Further, morphological changes often occur in cultures with cells sometimes forming lobes on the valves. This can happen soon after a culture has been started, or not for several years. The cells never go back to having straight sides and it is not known if physiological changes occur with the lobing. Also in culture, cells may stop forming stepped chains and become solitary or they may form stacked or ribbon-shaped colonies (similar to *Fragilariopsis*) or balls with one end of the cell toward the center and the other toward the outside of the ball. Cells also become shorter.

Species known to be present in the Pacific Northwest are *P. pungens* (Grunow) Hasle, *P. multiseries* (Hasle) Hasle, *P. australis* Frenguelli, *P. fraudulenta* (Cleve) Hasle, *P. heimii* Manguin, *P. subpacifica* (Hasle) Hasle, *P. delicatissima* (Cleve) Heiden, and *P. pseudodelicatissima* (Hasle) Hasle (Fig. 3). Of these, *P. pungens*, *P. multiseries*, *P. australis*, *P. delicatissima*, and *P. pseudodelicatissima* are known domoic acid producers with *P. australis* and *P. pseudodelicatissima* currently being the most common toxigenic species.

The life cycle of *Pseudo-nitzschia* is also somewhat complex (Fig. 4). Like all diatoms, the cell wall of *Pseudo-nitzschia* is composed of two halves, one slightly smaller than the other and fitting inside the larger half, similar to a box. During vegetative cell division, the cell divides with one new (daughter) cell receiving the large half of the original (parent) cell and the other new cell receiving the small half of the parent cell. Each daughter cell then makes a new, small half. As a result, the cell size gets smaller and smaller, and in culture at least, the population may die out without undergoing sexual reproduction to regain cell size. Sexual reproduction in *Pseudo-nitzschia* was first described as a spontaneous occurrence in an Antarctic clone of *P. subcurvata* (Hasle) Fryxell (Fryxell et al. 1991)

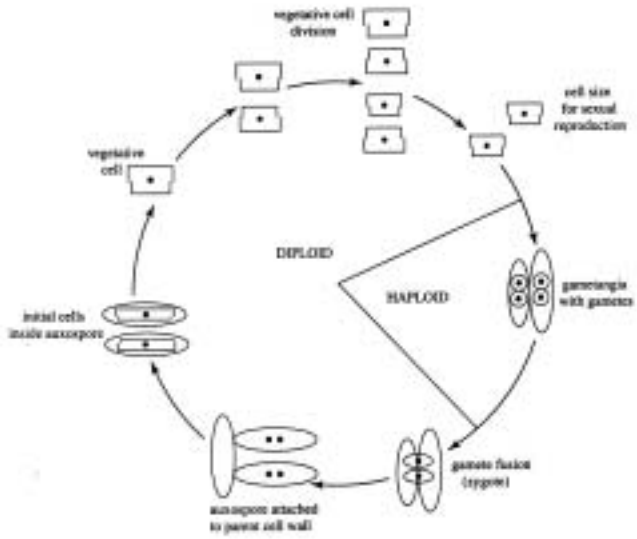

Figure 4. Generalized life cycle of *Pseudo-nitzschia* (redrawn from Hasle and Syvertsen 1996 and Davidovich and Bates 1998).

and was induced in cultures of *P. multiseries* and *P. pseudodelicatissima* where two mating types were required (Davidovich and Bates 1998). Cells of the two types line up valve to valve and the contents of the parent cells divide to produce two gametes each. The gametes have no flagella and those from one cell move by amoeboid motion to the other cell where fusion occurs, producing a zygote. The round zygotes (auxospores) remain attached to the parent cell wall, elongate, and form a large initial cell inside that looks like the old parent cell. It is the initial cell that produces the new population. However, sexual reproduction is rare in most diatoms and it may be years before it occurs in natural populations. Some diatoms also have resting spores, but these are not presently known for *Pseudo-nitzschia*.

TAXONOMY

Both genera have had a variable taxonomic history and this has caused confusion among scientists and the general public alike. Members of the genus *Alexandrium* were part of the genus *Gonyaulax* until 1979 when, based on morphological features, some species, including the toxigenic ones, were removed to (1) a previously described genus *Gessnerium* (Loeblich and Loeblich 1979) and (2) to a new genus *Protogonyaulax* (Taylor 1979); both papers transferred the same species and both were published in the same book. Nontoxic species remained in the genus *Gonyaulax* and it is still a valid genus today. However, investigators soon realized that the transferred species were morphologically similar to the genus *Alexandrium* described by Halim (1960), and, in 1985, Balech put them, along with seven new species, into *Alexandrium* (Balech 1985). It is now generally recognized that the toxigenic and morphologically related species formerly in the genera *Gonyaulax*, *Protogonyaulax*, and *Gessnerium* belong in *Alexandrium* (Balech 1995).

The genus *Pseudo-nitzschia* was first proposed (Peragallo and Peragallo 1900) for some pelagic pennate diatoms with pointed, fusiform valves and included two species that formed chains by overlapping of the cell ends (stepped colonies) and one species that did not form chains (Hasle 1965). Later, the genus was reduced to a section (group) in the very large genus *Nitzschia* (Hustedt 1958) and was studied as such by Hasle (1965). However, in 1987 when *Nitzschia pungens* f. *multiseries* was shown to produce domoic acid, attention again turned to the taxonomic features of species that formed stepped colonies, including *Pseudo-nitzschia australis* that Hasle (1965) had transferred to *Nitzschia* as *N. pseudoseriata*. In 1993, Hasle emended the description of *Pseudo-nitzschia* and raised it from a section of the genus *Nitzschia*, which is still a large and viable genus, to the rank of genus. Additional proof was provided based on transmission and scanning electron microscopy (Hasle 1994). Further, *Nitzschia pungens* f. *multiseries*, the form first shown to produce domoic acid, was elevated to specific status as *Pseudo-nitzschia multiseries* based on morphological (Hasle 1995) and molecular (Douglas et al. 1994, Scholin et al. 1994, Manhart et al. 1995) features.

CONCLUSIONS

Much remains to be learned about the biology of these two toxin-producing genera, particularly with regard to bloom origins and environmental controls. On the Washington coast, for example, blooms occur both on the open Pacific coast, possibly originating offshore, and in inland waters of Puget Sound, most likely originating in situ. If this is the case, are coastal and inshore populations related? Are they genetically the same? Why do blooms collapse? A fungal parasite has been seen in *Pseudo-nitzschia* spp. in Puget Sound embayments and on the open coast, sometimes infecting 5-10% of the population, while a parasitic dinoflagellate sometimes infects *Alexandrium*. Are these parasites enough to terminate blooms? Or are other physical

or biological factors needed? What induces toxin production? In *Pseudo-nitzschia*, there are toxic and non-toxic strains and, depending on species, toxin is produced during exponential growth (*P. pseudodelicatissima*), during stationary phase (*P. multiseries*), or over the whole growth cycle (*P. australis*), at least in culture. What causes the differences? Blooms of these organisms probably can't be prevented, but can they be predicted? What can be done to mitigate or control their effects on public health and local, often already stressed, economies? These are some of the questions that remain to be answered.

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FDA Initiatives in HAB and Marine Biotoxins

Sherwood Hall
Marine Toxin Laboratory, U.S. Food and DrugAdministration, Washington, D.C.

Introduction

First I'd like to acknowledge a very important contribution made by a few people to a key study done in the wake of the 1994 PSP outbreak in Kodiak, Alaska. Fortunately samples were obtained and made available to us for analysis, thanks to the good work of Brad Gessner and Dick Barrett. The samples were from shellfish and the human victims, and the analyses were very revealing.

Saxitoxin discovery

I'm going to give a perspective on seafood toxins, principally PSP, pointing out the role that Alaska has played in the worldwide development of an understanding of this issue. The largest outbreak in history is said to have occurred in 1799 along the Peril Strait, just northeast of Sitka. Work largely funded by the Army Chemical Welfare Service led to the collection of several hundred pounds of siphons from the butter clam *Saxidomus giganteus.* The butter clam siphons were a reliable source of the toxin then known as "shellfish poison." Based on those studies, the toxin was named "saxitoxin" after *Saxidomus.* Its structure eventually was determined in 1975, after about 15 years of work. This saxitoxin is the "parent" of the family.

Subsequent research, which contributed significantly to expanding this concept, occurred right here in Alaska, at the University of Alaska Fairbanks (UAF) Institute of Marine Science in Seward. I'd like to emphasize the importance of the work done in Alaska, with Sea Grant and University of Alaska funding, and the excellence of the facilities available in this state for doing the work.

Discovery of *Alexandrium*

In Alaska it was very difficult to find the PSP source organisms. Some cursory studies were done at places on the Alaska coast where shellfish were known to be toxic, but researchers failed to find any of the known toxigenic organisms. This led to a number of conjectures about unusual mechanisms by which shellfish might become toxic. In contrast to that earlier experience, a couple of chance observations of fairly large blooms demonstrated that *Alexandrium* (formerly *Gonyaulax)* were sometimes found in abundance. One chance observation, by a UAF Institute of Marine Science research crew out in Bristol Bay, was that the entire bay had an *Alexandrium* bloom. Another chance observation in Southeast found an intense bloom in one of the areas where PSP was known to occur, but they could not locate the organism. In fact, it's common to go to the coast and not find the toxic organism. It must be emphasized that these organisms are elusive and ephemeral in their occurrence.

Culture from benthic cysts

An interesting discovery was that sediments from virtually the entire Alaska coast, when treated appropriately by incubation on plates, gave rise to motile cells from cysts. Isolation of single cells from sediments gave rise to more than 80 strains, and they could be grown to large volumes. Study of these revealed substantial variations in toxicity depending on growth conditions, not associated with growth rate. This helped researchers understand the detection of fairly high levels of toxins in organisms taken from the environment, compared to low toxicity of organisms that people have dealt with in cultures. We simply need to optimize the culture conditions for toxin production. From the examination of extracts from these cultures, there was something interesting: the toxicity of non-hydrolyzed fractions was relatively low. Hydroylsis of the fractions, under appropriate conditions, profoundly enhanced the toxicity of some of the fractions, suggesting that some fractionation was occurring. Exactly what was going on was not initially clear.

Saxitoxin chemistry

It was the work in Seward that first led to producing the crystalline form of natural saxitoxins, although

two other derivatives of saxitoxin had been crystallized. This allowed chemical structures to be unambiguously determined, and the finding of precise coordinates for the atom centers. This Alaskan work substantially increased the number of known toxins.

The toxicities of this growing family of toxins vary over a wide range, in part due to the 21-sulfo group. This plays a significant role in the development of detection methods. A successful analytical technique allowed the resolution of the various known toxins, and made it possible to improve the world understanding of Paralytic Shellfish Poisoning with regard to toxin composition. Populations of organisms are homogeneous within a given region, but they differ dramatically from one region to another. We identified at least five entirely different regional populations of *Alexandrium*.

FDA MISSION

The mission of the FDA (U.S. Food and Drug Administration) is entirely how to ensure that the seafood available to consumers is safe—whether it's shellfish that people get off the beach, or shellfish from the market. We want to make sure that people can consume seafood with confidence. Our primary role is as a public health agency, so our obligation is to ensure that people don't get sick from what they eat.

But we also have a larger role. Instead of being a regulatory agency, a bunch of cops who are trying to keep bad stuff off the market, and are thus the bane of industry, we are a de facto industry advocate. It is only by ensuring safe seafood, and thus supporting consumer confidence in the product, that a consumer base is maintained that ensures the prosperity of the industry. So, while our goal is to make sure the seafood is safe, our largest function is to make sure that industry can make money selling good seafood. Within this, we have the goals of understanding the problems, anticipating future problems, and most important, developing and implementing effective management strategies. We are trying to understand exactly how the system works and what we can do to make sure that the shellfish that ends up on the dinner table is safe.

DETECTION OF SEAFOOD TOXINS

As mentioned before, there's more to seafood toxicity than just the saxitoxins, and PSP. In addition we have domoic acid, as well as a variety of lipophilic polyether toxins, and each of these presents a different detection problem. Of these, domoic acid is by far the simplest to detect by sophisticated instrumental methods. Slightly different extraction methods are necessary. The pharmacology of each toxin is quite different, although some share a binding site and differ only in intensity.

A very important underlying concept is that the detection of seafood toxicity globally is an extremely difficult task. It's difficult to imagine implementing a toxin detection method that is going to tell you whether your seafood is toxic. You will most likely have to employ a detection method suitable to each of the different categories of toxin, which becomes extremely tedious if you don't know which one you might be dealing with. The ability to anticipate what sort of toxicity is likely to be a problem is key to efficient management. It is only with some sort of notion of what to expect that you can focus your resources for toxicity detection on the right kind of toxin. Lacking that, you have to guess, and it gets pretty expensive.

Within the area of PSP and the saxitoxins, there are many different sorts of assays. Underlying consideration is the variety of toxins and specific potencies. Our best measure of human oral potency is the standard mouse intraperitoneal potency. The practical measure of the activity of the toxin is its ability to kill a mouse. There are also assays that are based on nerve cell receptor binding, the receptor that the saxitoxin binds to that causes toxicity. These assays involve a direct measure of binding; they involve cytotoxicity, as Jellett Biotek is doing. Another method is HPLC (high performance liquid chromatography), an instrumental technique that actually separates and identifies the various toxins. Both the receptor assay, which measures the total amount of toxin in a sample, and the HPLC method, which is used to detect each type of toxin molecule in a sample, are used to measure unknown amounts of toxin in seafood and seawater. Immunoassay has been very popular—numerous attempts have been made to develop effective immunoassays. Most of these attempts have stumbled on the diversity of toxins, and the need to develop an immunoassay that has response factors to each of the toxins corresponding roughly to the potency of that toxin to a person.

At the risk of being frightfully didactic, I'm going to try to go back through the foregoing and lay some groundwork, to clarify why an analysis is one thing,

and an assay is something else—why an assay is so tricky. I'm using these terms in a specialized sense. An analysis is something that resolves the components of concern and allows you to quantify them individually. An assay is a detection method that takes all of the components of concern and gives you a number. The mouse assay, for instance, takes all of the toxins and tells what the toxicity of that mixture is to a mouse. Now, if we're doing an analysis on a single toxin, we end up with a response. We don't know what that response meter deflection number on a readout means, but we get a response. To give that response a meaning, we need to have a response factor—the ratio between human oral potency and the unit response. We can multiply that by whatever our detection method gives us to end up with an indication of the toxicity or relative threat of the sample. If we have several different toxins, and we separate those toxins in an analysis, we have a relatively simple situation in which we need only the response factor for each toxin. This allows us to multiply the separate responses to each toxin present by the appropriate response factor. The sum of all those gives us a very good measure of the total toxicity of the sample.

John Sullivan at the University of Washington developed the HPLC method to resolve the different toxins, with materials from the University of Alaska. Sullivan has data showing the correspondence between mouse bioassay and HPLC, showing an entirely satisfactory correlation between the summed peak areas times response factors and mouse bioassays. This sort of thing works, although it is relatively expensive and it's very difficult to make it cost effective. It's absolutely the only tool that's going to tell you what toxins are present in what concentrations, but it's not a terribly cost effective tool for telling you how toxic a particular batch of clams is.

For an assay, if there's only a single toxin we get an assay response; we know a response factor, and we multiply the assay response by the response factor and we get a number that tells us how toxic that sample is. Sadly, we seldom have so simple a case to deal with. Instead, we have multiple toxins, and the assay is only giving us a single number. The various toxins present have different response factors. Only insofar as the ratio between the response of the system to human oral potency is constant for each of the toxins in the system, are we going to get something which is tractable. Only insofar as these magnitudes are in the same ballpark do we get an assay that allows us to provide a reliable measure of toxicity in natural samples where we do not have knowledge of the toxin composition. About the only way we can easily do that, aside from devising a cocktail of antibodies, is by using the native receptor binding. That is a simplification in a sense because there are actually different classes of native receptors.

It has proven reasonably practical to develop in vitro assays that exploit the selectivity of the receptor binding site—the root of the problem in mammals. We can compare mouse bioassay results for various pure toxins to results from binding assays using rabbit brain. In general, binding assay response corresponds to the hierarchy of mouse bioassay response. Thus we can say with reasonable confidence that a binding assay would provide an acceptable measure of human oral potency. A complication of the binding assay is the need for a labeled reagent toxin. Binding is measured generally by the amount of radioactivity left either in a supernatant or a bound component. The current reagent toxin is made by exchanging hydrogens for tritium, on saxitoxin. The tritiated saxitoxin method, developed by Strichartz 20 years ago, is technically accessible, although it has become something of a political issue because of the chemical weapons treaty. It is the basis for which the majority of binding assays have been done. The problem is with the disposal of tritium, which is radioactive. There are several technical issues that make it a suboptimal binding tool.

The regulatory limit is 80 µg per 100 grams. The detection limit is 40 µg per 100 grams, half that. The correlation of the results for samples of blood serum and urine from victims in the 1994 Kodiak outbreak is very high. There is an extraordinary correlation on down to the lower limit, suggesting the strong sensitivity and reliability of these detection methods. There's an awful lot to be said for the use of the binding assay as a detection method. It is desirable to somehow get around the limitations of the tritiated saxitoxin.

PHARMACOLOGY RESEARCH

One of the major research goals of the FDA has been to understand the pharmacology of these compounds, or the structural activity relationships, to find how we can decorate the saxitoxin molecule and still end up with acceptable pharmacology. We've done this through a collaboration with Ed Moczydlowski, now at Yale. He takes single activated sodium channels and incorporates them to a lipid bilayer, opens them with bitracotoxin, and observes the current, the flow

of sodium ions, through that single channel. The same sort of preparation can be done adding a very low concentration of saxitoxin. A single molecule of saxitoxin arrives at the binding site of the channel, resulting in a cessation of current. The current remains at zero until that single molecule of saxitoxin diffuses away. It is beautiful to be watching bimolecular kinetics of, literally, two molecules. For less toxic saxitoxin derivatives, the "dwell times" of the binding events are much shorter.

We record "on rates" (the rate at which the molecule goes on) and "dwell times" (how long the molecule stays) for each of the saxitoxin derivatives. We can evaluate the pharmacology of toxins with different potencies. Using tools like this we have attempted to get a better handle on how the toxins bind and how changes in their structure affect their binding. In general, in every case where we added an N-1 hydroxy, we got an increase in "dwell time" and decrease in "on rate."

TRACKING MARINE BIOTOXIN EVENTS

Guatemala

In addition to laboratory study, focused on trying to develop a detection method, we are continuing to be involved in episodes that occur worldwide where there is some sort of a marine biotoxin mishap. A tragic bloom occurred, of an organism that was easily visible with very primitive field observation tools. Its significance was not understood, and the result was 26 dead people in a few days.

New Zealand 1993

New Zealand has a very healthy shellfish industry. They have some of the finest shellfish I've ever seen, incredibly fine products. For years, New Zealanders thought of themselves as clean, green, insular, and unaffected by things that affected the rest of the world. We told them, "You might worry a bit about the possibility of marine biotoxins in your shellfish. If you're going to ship shellfish to us, you ought to involve a marine biotoxin component." They would point out to us how silly that was and a waste of their time because they'd never had any toxicity. This went on for years. Then on Christmas 1992, a large number of people became ill. The outbreak was not recognized until two cats, in a community north of Auckland, were taken to the vet with hind-limb paralysis. When asked what the cats had been fed, the owner said that they had been fed some shellfish. The vet had the wisdom to recognize it as shellfish toxicity and it was that event, despite more than 200 sick people, which cued New Zealand to the fact that there might be something wrong with their shellfish. On investigation a widespread bloom was found. It was principally an NSP-like problem (neurotoxic shellfish poisoning). Interestingly there were symptom reports like, "I went out into the sun, and it felt like there were champagne bubbles popping under my skin." Very interesting hypersensitizations. Also there was some PSP, DSP (diarrhetic shellfish poisoning), and domoic acid. All of a sudden they realized they had a fairly significant widespread problem, and since then they have been particularly responsible in developing a very sophisticated marine biotoxin management program.

Noteworthy was the number of people who had said, "I've been diving in this place for twenty years, and the water's always been clear, but this year I couldn't see my hand in front of my face." A large number of people were realizing that there were things that were different and strange about the environment, but nobody thought to correlate this and use it to direct their concerns.

U.S. West Coast domoic acid outbreak

A fundamental change in our thinking about how to approach this sort of thing came as a result of our concerns about domoic acid. Domoic acid had occurred in eastern Canada. We kept worrying that it would occur in the United States, but had no indication of it. We conducted surveys of seafood throughout the country and got no hits. The first indication was from observations where people noticed that brown pelicans were behaving strangely. It was found that they had consumed anchovies that contained *Pseudo-nitzschia* and that *Pseudo-nitzschia* in turn contained lots of domoic acid. The instrumental method for detecting domoic acid is as simple as one could wish; it could not be more detectable unless it smelled strongly or had a bright color. You put it into an HPLC and you get a beautiful single peak out of it. It's very easy. Compared to all of the other marine biotoxins, the stuff almost detects itself. The dogma that has prevailed for years is that only through the development of better detection methodology could we solve the problem. So, one was left to wonder, why in the case where we had excellent detection methods, did we still have a problem? The point here is that there is no prospect of eliminating the cost of sampling, and preparing a sample. There is a limit to how much improvement in detection methods can improve the cost-effectiveness of marine biotoxin management, particularly

when you don't have a clue as to which marine biotoxin you're necessarily looking at. So, it is extremely important to understand that, even the best detection methods in the world only take us so far toward a global solution of the marine biotoxin challenge. We need instead to be figuring out what other resources we can draw on. The great lesson of the Pacific Coast domoic acid outbreak was the need to look for whatever resources we can draw on.

Field plankton monitoring

One of the things that is terribly important to realize is that these phenomena do not respect borders. It is imperative that there be good communication across administrative boundaries within biologically related regions. Not much need for the East Coast to communicate with the west, but the West Coast needs to communicate with itself. Environmental monitoring to detect phytoplankton or other significant events can be extremely useful in helping to focus toxicity monitoring on the times and locations and toxins of greatest concern. It is important to do this to help make your employment of toxicity monitoring more cost effective.

The concept of field plankton monitoring that I'm encouraging you to contemplate was derived out of my attempts to do oceanography out of my pocket and out of my suitcase, as a grad student in Alaska without a lot of funding. The surveys that I did along the Alaska coast were mostly done with a flattened glass capillary and a hand lens, which works very nicely for detecting live *Alexandrium* because of their swimming behavior. A microscope is a luxury, while it does provide extraordinary images. Programs to try to incorporate volunteer effort and use volunteer observations of signal events and real time plankton observations have been most successfully promoted in California by Greg Langlois (California Department of Health Services). Volunteer efforts in Maine and Massachusetts have also been successful. We are trying to encourage people wherever they feel it might be useful to consider it and give it a try. It's useful to note that plankton observations and reports from citizens have been an essential component of the Florida marine biotoxin management program for a long time.

It's also important to realize that the techniques we use are not effective for sampling *Gymnodinium*. *Gymnodinium* lyses instantaneously when you show it a plankton net. Our attempts to employ this for *Gymnodinium breve* blooms have been notably unsuccessful. But for the majority of other toxigenic algae, the methods are remarkably effective, and a surprising number of toxigenic algae can be recognized even at the hand lens level because of their swimming behavior and profiles.

Physician education

I'd like to close with some thoughts about some episodes in Ketchikan with respect to human illness and what can be done about it. In Ketchikan, in May 1981, there was an episode following a low tide in which numerous people had collected shellfish. One fellow had consumed butters heavily. He ended up in the hospital in the evening, he went into respiratory arrest shortly after his arrival, and he walked out of the hospital the next morning. He would have been thoroughly dead had he not gone to the hospital. He was provided respiratory support and respiratory support alone. The toxins, although they can be lethal in sufficient concentrations if respiratory support is not provided, are very restricted in their effect, and they leave the body very quickly. The utility of an antidote needs to be evaluated in view of that option for case management.

In the 1994 Kodiak outbreak, there was one fatality of an older person at a village. There would have been a much larger number of fatalities had there not been effective therapy applied at the emergency room. So we have something here which has a significant potential to kill people, which isn't killing many because an effective therapy is being applied. I think the most important thing is to ensure that people who are in a position to encounter cases of illness due to marine biotoxins understand what the symptoms are. It is my understanding that the physicians who encountered the first three victims in Kodiak were not aware that what their patients were suffering from was PSP. So, physician education is terribly important.

Clinical samples needed

Our primary goal is to prevent human illness, but should human illness occur, it is extremely important for clinical samples to be captured. We can't go around giving people this stuff to see what happens. Our only opportunities for finding out how people respond to what level of toxins is by evaluating cases. The reason we got samples was that there was a succession of outbreaks due to people ignoring the warnings. But we only got a couple of samples. It would've been wonderful to have a time series. It would have helped a great deal to understand how the toxins move out of the body.

Warnings ineffective

In Alaska we have seen it necessary and expedient to simply say, the shellfish are probably toxic and you shouldn't eat them. No one monitors the entire coast to make sure the shellfish are safe and that people can eat the safe ones. Thus, the official position has been that you shouldn't eat the shellfish. Alaskans being Alaskans, a lot of shellfish get eaten, in spite of this warning. The result is that we have a population that has been trained to ignore the state's warning, as demonstrated in Kodiak. It is a devilish situation and I'm not suggesting there is a good answer to it. But we need to understand that our principal tool of telling people not to eat the shellfish because they're bad has been rendered ineffective. We've been saying, "Don't eat the shellfish because they're bad," for decades, yet most who ate the shellfish did so in relative safety and health.

Thank you very much for your attention, I appreciate the opportunity to be here.

The Alaska Science and Technology Foundation and HABs

Robert E. Chaney
Alaska Science and Technology Foundation, Anchorage, Alaska

This is a summary of what ASTF (Alaska Science and Technology Foundation) is, what we do, how we can participate in a HAB program, and how you can use us—because we're here to be used.

ASTF is part of the Department of Community and Economic Development of Alaska. We operate from an endowment. Back in 1988 they set aside $100,000,000 and put it in a fund in Alaska—it's the permanent fund that we get our PFD (Permanent Fund Dividend) from every year. We take the interest that we earn and fund projects to diversify the state's economy from oil and gas. One of the concerns people have is that 85% of the state's operating budget is from the oil industry taxes. Our purpose is to work in economic development. We have a telecommunications and a science and technology mission, but our main focus is on developing industry in the state. We're a little bit different from a lot of funding agencies. ASTF is more applied. The team of people brought together to make the project go forward is very important. It's got to have some direct impact in Alaska and it has to have some shared risk. When a team is sharing the risk with the agency, then we see much more commitment to the project and a much higher success rate.

Fisheries is probably our largest project topic area in terms of dollar value. We fund all kinds of salmon processing projects and aquaculture/shellfish culture projects including PSP. But in general, we have a broad range of projects from satellite communications to composting toilets and everything in between.

At ASTF the investigator is responsible for setting up the commercialization of the project. If you're working with an inventor you always try to get the business component lined in with the inventor so that there is a continuation of sequence from invention to commercialization. For many inventors and entrepreneurs that is difficult to do, but that's one of the things we stress. We do that also in PSP. We're looking at the end users, and we have the agencies and companys working together on projects.

The three areas of interest in our PSP program are improved beach monitoring programs, improved toxicity testing procedures, and development of antitoxins. Over the last year we have come to the conclusion that antitoxins is far beyond what we're able to do here in Alaska. If there are good antitoxin projects that can be proposed we would be interested in them, but right now we are focusing on the first two—they are most likely to have benefit here in the state.

ASTF has funded 11 shellfish projects through 1999 (Table 1) and we have funded five PSP projects (Table 2). Funding levels range from $20,000 to $120,000. When I worked with the Department of Energy I was always worried about constraints on what we could fund because of budgets. I think ASTF is in a situation now where we don't have to worry so much about what the budget is for a project, but whether it is a good project. If it is a really good project, then let the budget fall where it may. As long as we get cost share and collaboration, investigators proposing projects to us need not worry so much about funding levels. We have money for good projects.

As projects come up in your various regions—California, Washington, or Oregon—if there is an Alaska component and we can help, look at our agency as a co-funder for some of your other projects so you can get more synergism out of it than what you are really putting in.

Table 1. ASTF funded projects related to shellfish.

Topic	PI	Year	Budget ($1,000)
Shellfish hatchery development	Kaill	90-92	95
PSP toxin test	Smiley	90-95	119
Kodiak green sea urchin	Donohue	91-93	87
Blue heron mussels	Brainard	92-94	20
Kachemak Bay shellfish nursery	Bradley	96-99	68
Geoduck PSP depuration	Painter	97-98	50
PSP test kit field trials	Roberts	97-98	104
PSP beach monitoring program	Horn	98-	49
Seawater purification system	Sewell	98-	228
Alaskan shellfish broodstock development project	Sczswinski	98-	18
Application of new technology to detection of dinoflagellates	Plumley	98-99	20
TOTAL			$858

Table 2. PSP projects funded by ASTF, 1990-1998.

Topic	PI	Year	Funding ($1,000)
Alaskan field trials for PSP test kits	Roberts	98	91.7
PSP monitoring program (Alaska Peninsula)	Horn	98	49.0
Application of new technologies to detect dinoflagellates that produce saxitoxin	Plumley	98	19.8
Depuration of PSP from geoduck clams	Painter	97	49.8
Simplified tests for PSP toxins	Smiley	90	118.7

West Coast Harmful Algal Blooms: The Moving Target

John Wekell
NOAA Northwest Fisheries Science Center , Seattle, Washington

BACKGROUND

The entire Pacific region has a variety of very serious harmful algae blooms (HABs) that present both health and economic problems to the residents of this area. If we examine only the northeastern Pacific, where the U.S. Government has interests, we find that the coastline habitat is enormous, extending literally from the North Pole to the equator. Within this vast area, four HABs present serious, ongoing problems: **paralytic shellfish poisoning (PSP)**, **domic acid poisoning (ASP** [also known as amnesic shellfish poisoning]**)**, ***Heterosigma*** (impacting salmon aquaculture in Washington and British Columbia), and ***Ciguatera*** **poisoning** (or tropical fish poisoning). For the purpose of this talk, I will restrict myself to the area along the West Coast of North America and ignore the tropical areas (Hawaii, American Samoa, and other trust territories). These coastal states and British Columbia represent a population base of nearly 48 million people, most of whom live within 100 miles of a seawater habitat. Because of this close proximity to the sea, many in this population utilize the ocean or marine habitat as both a source of recreation and livelihood.

NORTH AMERICAN WEST COAST HABS

The continental Pacific Coast of North America commonly experiences blooms of two significant algae that can cause severe health and economic injuries to coastal communities: *Alexandrium catenella* and members of the genus *Pseudo-nitzschia*. *Alexandrium* is the causative alga for PSP, and members of *Pseudo-nitzschia* are responsible for ASP. So far, at least for the West Coast of North America, ASP is somewhat a misnomer since domoic acid appears to be vectored to its victims more commonly by finfish, e.g., anchovies and sardines, than shellfish. To date, on the West Coast of North America, the victims of domoic acid poisoning have been marine mammals and ocean birds. As far as we know, there have not been any confirmed illnesses in humans due to domoic acid on the West Coast, although high levels of this toxin have been measured in razor clams.[1] The razor clam fishery, in Washington state, is now largely a recreational fishery in which hundreds of thousands of people participate each year

Typically, when we speak of *blooms* of algae, we are referring to concentrations of millions of cells per liter. We rarely see these levels of *Alexandrium* in most West Coast waters; nevertheless, these lower cell concentrations are capable of generating very high levels of PSP toxins in shellfish. On the other hand, we do see million-cell levels of *Pseudo-nitzschia*, but we do not always see concomitant high levels of domoic acid in exposed shellfish. Part of the reason is that not all *Pseudo-nitzschia* species produce domoic acid or produce it at very high levels. To date, only a few species of *Pseudo-nitzschia* on the West Coast have been associated with domoic acid production and poisonings: *P. australis*, *P. multiseries*, and *P. pseudodelicatissima*. The mechanism for the production of domoic acid by *Pseudo-nitzschia* spp. is not clearly understood.

Another HAB species that occurs in British Columbia and Washington state is *Heterosigma akashiwo*, also simply referred to as *Heterosigma*. To date, *Heterosigma* has only impacted salmon aquaculture farms, where it has a lethal effect on penned salmon. The mechanism of this lethality is not known; some have suggested a toxin being released into the water column while others have proposed reduction in dissolved oxygen. Whatever the mechanism, salmon aquaculture farms have lost marketable salmon and brood stock to direct fish kills from this organism. Blooms of *Heterosigma* have been somewhat erratic in Puget Sound and the Strait of Georgia; nevertheless, their costs have mounted into the millions of dollars over the past 10 years.

[1] Recently (September 2001), we have heard of a possible domoic acid poisoning in Anchorage, Alaska, from the consumption of razor clams. The frozen clams were purchased from an unlicensed street vendor. It was not clear where these clams initially originated.

In addition to the costs of the direct fish losses, salmon farms incur considerable costs when they must use pre-emptive measures to mitigate the effects of *Heterosigma*. For example, many farms will barge their salmon pens into waters free of the harmful alga. In order to do this effectively, tugboats and spotter airplanes must be kept "on retainer" in operational standby status, for a quick response to a threatening bloom. So far as is known, *Heterosigma* impacts have been only economic with no known human health implications. Some recent work by researchers at the Northwest Fisheries Science Center and at the University of Washington appears to offer some hope of understanding the conditions that initiate *Heterosigma* blooms, perhaps leading to a predictive model within the next few years.

The West Coast

Along the West Coast of North America, the most severe and dangerous marine toxin problem is clearly PSP. The first recorded deaths due to PSP in Western tradition were during the exploration of what is now known as Puget Sound and the Strait of Georgia (1791-1792) by Capt. George Vancouver (1758-1798). Several members of his crew died after eating shellfish taken from a cove near modern-day Vancouver, B.C. Of course, the coastal Indian tribes were very aware of the lethality of the shellfish and had developed folkways of trying to determine whether shellfish were toxic. In some anecdotal stories, some Indian tribes in southeast Alaska and British Columbia may have practiced a form of "chemical warfare" by giving, as gifts, toxic shellfish to unwary rival tribes or villages.

In more recent times, Washington state has observed toxin levels in excess of 20,000 µg per 100 g, but the highest levels of PSP have been registered in British Columbia, typically in excess of 30,000 µg per 100 g of tissue. To place these figures in some perspective, it is estimated that a lethal dose in humans is about 2,000 µg; therefore, the contaminated shellfish would contain about 15 lethal doses in 100 g (about a quarter of pound) of meats. Since this was measured in mussels (*Mytilus edulis*), it would take only a few mussels to yield a lethal dose of this potent toxin. Because of the potential severity of this problem, the coastal states and provinces maintain very extensive and expensive shellfish monitoring and surveillance programs to ensure the safety of our shellfish resources. These programs are effective—no one has reported deaths from shellfish taken from regulated commercial operations. Deaths and illnesses have occurred in Alaska and other states from shellfish taken from non-regulated beaches, usually by recreational or subsistence fishers who have ignored or are unaware of the beach's status.

The causes for the explosive growth, or bloom, of these harmful algae are not well understood. In addition, we have even less understanding of what triggers toxin production within these blooms. We do know that algal growth and toxin production depend on nutrients (silicon, nitrogen, phosphorus, perhaps iron) and temperature. Our current sense is that the interplay of these factors is important in HAB events, but the precise mix, which might yield a predictive model, has yet to be developed.

Although the exact trigger mechanisms for these blooms are not known, we have a general understanding of the movement of HAB biotoxins through the food chain (Fig. 1). Some blooms may initiate offshore in the pelagic zone. In this area, algae increase in number as the bloom is moved toward shore, driven by wind and currents. The bloom is initiated and sustained by upwelling at the continental shelf, which brings up cooler water rich in basic nutrients required by the cells for growth. Herbivorous fish, such as anchovies and sardines, then consume algal cells, accumulate toxin if it is present, and they in turn pass it onto predator species such as marine mammals or humans. Furthermore, as some of the algae settle into the benthos, benthic filter feeders or scavengers (clams and crab) can also accumulate toxins. As the algal bloom moves on into the shore or intertidal area, filter feeding organisms such as clams begin feeding and accumulate the toxins. Within this model, the offshore benthic accumulation of toxins, for example by Dungeness crab, is speculative at present.

Brief history of domoic acid

The first confirmed and reported case of domoic acid poisoning occurred in 1987 from the consumption of mussels from Prince Edward Island, Canada. In this outbreak, three people died, six people were hospitalized, and over a hundred others suffered varying degrees of illness. These ranged from mild upset stomach to significant and devastating permanent short-term memory loss. This is the hallmark of this poisoning, hence the name "amnesic shellfish poisoning." This was the first time that a marine biotoxin was found to enter and directly damage brain function. The origin of the toxin was found to be the diatom *Pseudo-nitzschia multiseries*.

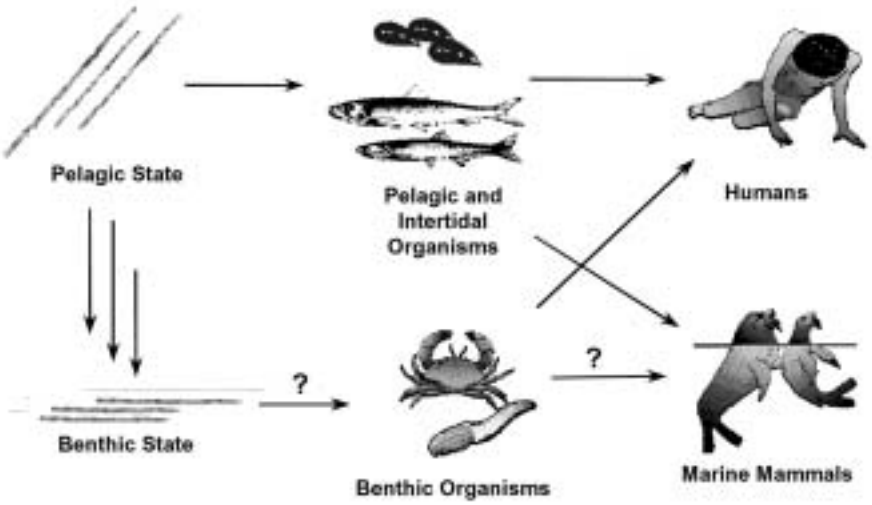

Figure 1. The movement of marine biotoxins through the food web.

West Coast domoic acid events

Four years later, in the summer of 1991, a Monterey Bay, CA, beach survey observed a large number of dead and dying pelicans and cormorants. Sick and dying birds exhibited peculiar symptoms and a poison was immediately suspected. Examination of the stomach contents indicated the birds had been feeding on anchovies and sardines from the bay. Examination of fish samples taken from the bay showed that they fed on the diatom *Pseudo-nitzschia*. Indeed, analysis of the bird stomach and anchovy gut contents showed very high levels of domoic acid. Interestingly, our laboratory obtained anchovies taken five months earlier in April 1991 from near Catalina Island, about 300 miles south of Monterey Bay, which also showed high levels of domoic acid. During the fall months of 1991, domoic acid began appearing in shellfish in Oregon and then Washington. In Washington state, domoic acid levels in razor clams began to climb in the late fall and reached a maximum in the first week of December 1991 (Fig. 2). Levels remained elevated at all of Washington's recreational beaches well into 1992. In 1991, the razor clam fishery, which is largely recreational in Washington and Oregon, was closed. This severely impacted the local economies in both states. In Oregon, the closure of a small commercial razor clam fishery contributed to the collapse of at least one razor clam processing company.

Recently, in 1998, very severe blooms of *Pseudo-nitzschia* occurred on the West Coast (Table 1). The first indication of this HAB event was a number of reports in May and June of sea lions hauling out onto land in southern California. These animals were extremely lethargic, and exhibited symptoms of head weaving and bobbing. California veterinarians and biologists immediately suspected some form of intoxication. Examination of stomach contents, serum, and fecal material indicated the presence of domoic acid. The vector was assumed to be anchovies. Anchovy samples taken from areas near the haul-out points had high levels of domoic acid (2,300 ppm).

In July, domoic acid was detected in razor clams in Oregon but at very low levels (41-57 ppm) (Table 2). In August, Washington state detected domoic acid in mussel samples from the coast (Table 3). This is somewhat unusual because mussels tend to clear the toxin very rapidly—it has a half-life in mussels of only a few days. In September, a significant bloom of *Pseudo-nitzschia* was observed at Kalaloch Beach by Mitch Lesoing of the Quileute Indian Tribe, working in partnership with our biotoxin program at the Northwest Fisheries Science Center (NWFSC). At Kalaloch Beach, domoic acid content in seawater was highly correlated with *Pseudo-nitzschia* counts (Fig. 3). About two weeks later, in mid-September, significant levels of domoic acid were detected in razor clams from Kalaloch (Fig. 4), reaching a maximum in October. Sample data from other beaches on the Washington Coast also indicated increased numbers of *Pseudo-nitzschia* in the water column.

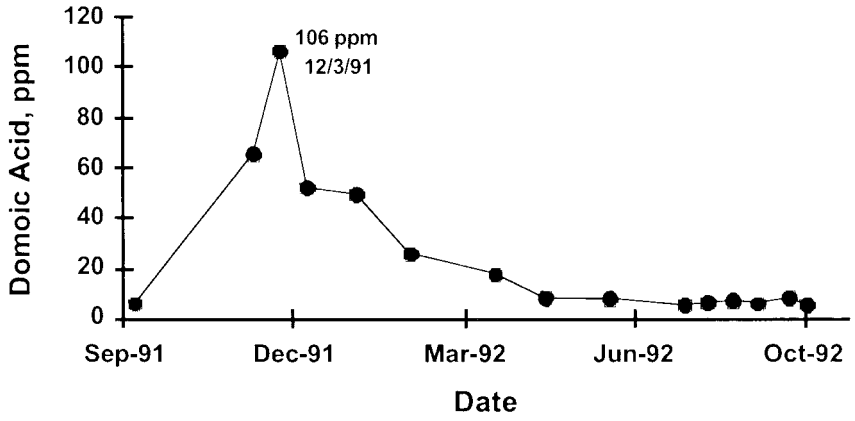

Figure 2. Average domoic acid levels in razor clams from all Washington state management area beaches, 1991 to 1992 (adapted from Wekell et al., 1994, *Nat. Toxins* 2:197-205).

Table 1. HAB events in California, spring-fall 1998.

Date	Event	Location	Notes
Late spring	Anchovies and sardines begin showing significant domoic acid levels (84-257 ppm)		
Early May	Viscera from anchovies contain domoic acid up to 2,300 ppm	Monterey/ San Francisco Bay	NMFS data
May 21-31	Nearly 80 adult and juvenile sea lions washed ashore, in physical distress	Coast from San Luis Obispo to Santa Cruz, California	
June 29	Seizuring sea lions	Off Monterey coast	*Pseudo-nitzschia* and domoic acid suspected
Summer	Dense blooms of *Pseudo-nitzschia australis* and *P. multiseries*	From Santa Barbara to San Francisco	

Information provided by Susan Loscutoff (California Dept. of Health Services, Food and Drug Branch) and Gregg W. Langlois (California Dept. of Health Services, Marine Biotoxin Program).

Table 2. HAB events in Oregon, summer-fall 1998.

Date	Event
July 2	All beaches closed due to PSP
July 28	Domoic acid at 41 ppm and 57 ppm in razor clams from Clatsop Beach
July 31	Southern and central beaches opened but northern beaches closed due to domoic acid
Sept. 18	Domoic acid declines, then increases to 65-82 ppm in razor clams

Information provided by Deb Cannon (Oregon Dept. of Agriculture).

Table 3. HAB events in Washington, summer-fall 1998.

Date	Event	Concentration
Late Aug.	Domoic acid levels rise in razor clams	8-12 ppm
Early Sept.	Domoic acid levels increase in razor clams	5-34 ppm
Late Sept.	Domoic acid levels accelerate in razor clams	52-94 ppm
Oct. 9	Domoic acid delays razor clam season	287 ppm (Kalaloch Beach)
Oct. 23	PSP in Puget Sound mussels, causes illness	9,000 µg/100 g

Contributed by Linda D. Hanson (Washington State Dept. of Health).

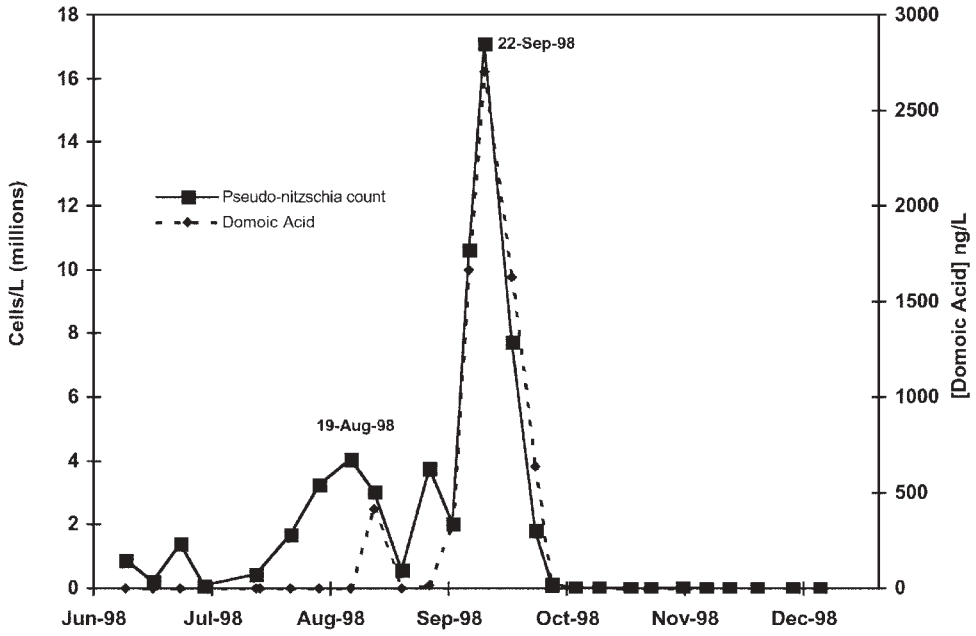

Figure 3. *Pseudo-nitzschia* counts and domoic acid levels at Kalaloch Beach, Washington (adapted from N.G. Adams, M. Lesoing, and V.L. Trainer, 2000, *J. Shellfish Res.* 19:1007-1015.)

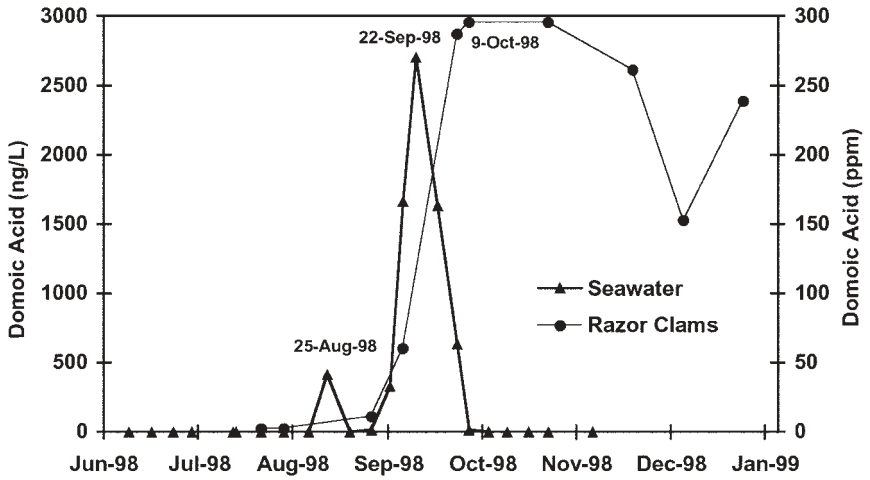

Figure 4. Domoic acid levels in seawater (ng per L) and in razor clams (ppm) at Kalaloch Beach (adapted from N.G. Adams, M. Lesoing, and V.L. Trainer, 2000, J. *Shellfish Res.* 19:1007-1015).

Average composite levels of domoic acid from shellfish taken at Kalaloch Beach reached record levels for Washington state, nearly 300 ppm (Table 4), and persisted well into 1999. A sharp decline in razor clam domoic acid levels was observed between April and May. Nevertheless, domoic acid was detectable in razor clams for nearly a year after the 1998 bloom. In addition, much like 1991, domoic acid was also detected in the hepatopancreas of Dungeness crabs (Table 5). At about the same time as these events unfolded in Washington state, British Columbia also experienced both domoic and PSP episodes (Table 6 and 7).

In the 1991 outbreak in Washington state, shellfish at Kalaloch were not sampled until March 1992 (Fig. 5), about 4 months after the highest levels of domoic acid were reached at the other Washington state beaches. The first analyses in 1992 of Kalaloch razor clams indicated levels of domoic acid (42 ppm) exceeding those observed at all the other Washington state beaches. Since that time, Kalaloch Beach appears to have higher levels of domoic acid in clams than other beaches in Washington. Interestingly, in 1998, razor clam levels reached their highest (287 ppm) in October of 1998 (about 2 months earlier than in 1991) and still contained about 140 ppm in March of 1999—over 3 times the levels seen in the same period for 1991. At about the same time that domoic acid had reached its maximum on the coast of Washington, PSP became a significant problem in Puget Sound. Several illnesses from shellfish required hospitalization. PSP levels of 9,000 µg per 100 g were reported from the shellfish beds that supplied the victims.

Can we compare the 1998 domoic acid outbreak in Washington state to what happened in Canada in 1987? During the Canadian outbreak, the best data indicated levels of domoic acid at about 790 ppm, possibly as high as 900 ppm in mussels from Prince Edward Island. While the levels in mussels that produced deaths in humans may not ever be known exactly, we could conservatively assume that the levels of 790 to 900 ppm were responsible for some of the lesser symptoms, such as memory loss. In 1998, Washington state razor clams averaged nearly 300 ppm domoic acid, and ranged from 140 ppm to over 400 ppm in some clams. The 1998 event produced shellfish at about half of the toxicity observed in Canada in 1987. Washington state beaches were closed for all razor clam harvests during the fall of 1998. However, had razor clams been taken and consumed from Kalaloch, some illnesses would likely have occurred.

ECONOMIC IMPACTS

While the primary focus of harmful effects are the health related issues in HAB events (i.e., death and illnesses), a less considered part of the "harm" equation is the economic impact. Most obvious, hospitalization and deaths have very definite associated cost burdens, i.e., direct medical and hospital costs,

Table 4. Domoic acid concentration in whole razor clams from Kalaloch Beach in Washington.

North Sample	Domoic acid ppm	South sample	Domoic acid ppm
1	144	7	294
2	236	8	252
3	296	9	343
4	300	10	277
5	406	11	334
6	373	12	279
Average	293		297
S.D.	94		35
C.V.	32		12

Table 5. Domoic acid concentration in Dungeness crab hepatopancreas (Destruction Island, Washington).

Sample	Domoic acid ppm
1	2.8
2	5.3
3	62.9
4	24.1
Average	15.7
S.D.	24.8
C.V.	156

Samples provided by Mitch LeSoing (Quilleute Nation).

Table 6. HAB events in British Columbia, summer-fall 1998.

Date	Event
Aug. 25	Domoic acid shows up in mussels, to 7 ppm
Sept. 4	Domoic acid spreads to west side of Vancouver Island, to 29 ppm
Sept. 10	Domoic acid declines slightly
Sept. 15	PSP appears in sardines and pilchards
Sept. 28	Domoic acid in razor clams, Queen Charlotte Island

Data courtesy of Klaus Schallié (Canadian Food Inspection Agency).

Table 7. Paralytic shellfish poison levels in shellfish from British Columbia, 1998.

Harvest date	Species	PSP µg/100 g
Sept. 9	Mussels (monitored)	960
Sept. 14	Sea mussels	870
Sept. 14	Varnish clams	77
Sept. 14	Manila clams	44
Sept. 14	Oysters	55
Oct. 21	Sea mussels	210
Oct. 21	Varnish clams	62
Oct. 21	Manila clams	<42
Oct. 21	Oysters	<42

Data courtesy of Klaus Schallié (Canadian Food Inspection Agency). Location is Okeover Inlet in subarea 15-4. PSP bloom started in early August.

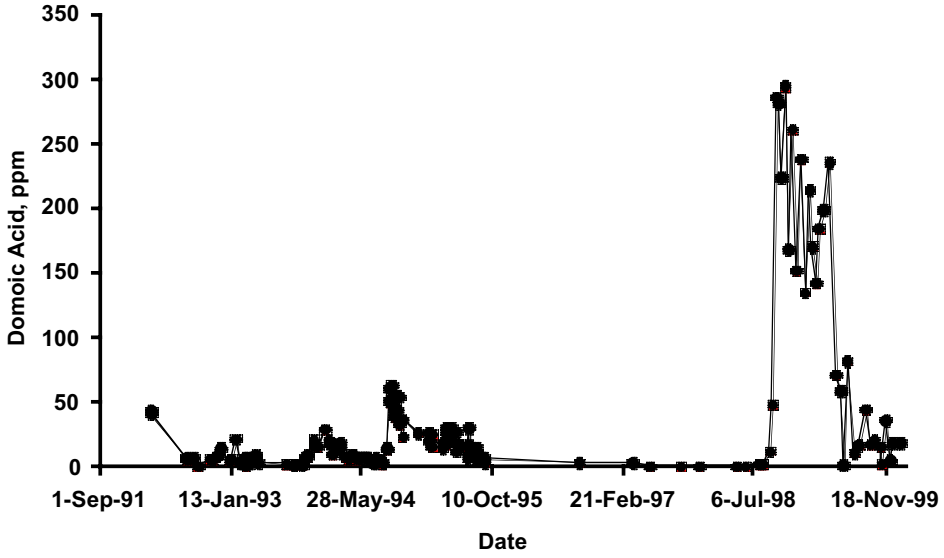

Figure 5. Domoic acid levels in razor clams at Kalaloch Beach from 1992 to 2000 (data from the Washington State Department of Fish & Wildlife (WDFW) Web site—http://www.wa.gov/wdfw/fish/shelfish/razorclm/levels/levels.htm, and pers. comm. D. Simons and D. Ayers, WDFW).

lost work days, etc. However, in addition there are indirect economic losses encountered by recreational and commercial fishery closures. When fishing grounds are closed, product is not collected and cannot move into commercial channels. In some cases, when a product is absent from a market for an extended period, other sources replace it. This usually requires a significant effort (i.e., cost) by the affected company to regain the previous marketshare when closures or embargoes are finally lifted.

In the recreational fishery, support industries, such as motels and restaurants, sport shops, service stations, and other tourist businesses, must close or severely reduce staff during the closures. These actions translate into higher unemployment rates in the affected area.

Economists tend to take a broad view when dealing with these "losses." Since many recreational fisheries are generally made up of within-state residents spending "disposable" income, their view is that the money is just spent elsewhere, perhaps closer to home. When examining it from this point of view, it is concluded that the impacts are minimal if not zero. However, locally where the HAB event occurred, the economic impact can be severe and substantial.

To give an example, in 1991 and 1992, during the domoic acid outbreak in Washington state, a complete closure of the razor clam recreational and commercial fisheries was made. The state of Washington closely monitors how many people come to the southwestern counties of the state to dig razor clams. Each visitation is counted as a "digger trip." The highest number of digger trips to the coastal communities was estimated at 900,000. However, for the purposes of our exercise, I used an average of 275,000 derived from averaging the previous 5 years (1986 to 1990). The local chambers of commerce estimated, in the mid-1980s, that each digger trip brought in about $25 to the local communities. This figure was an approximation that supposedly included gasoline, food, and lodging. Using the $25 figure, this resulted in a loss to the communities of approximately $7,000,000 (1980s dollars). The $25 figure should be modified upward to reflect increased costs.

The most significant industries in Washington's southwest counties, where razor clamming is practiced, were forest products and fishing. In 1991, these counties also suffered from relatively high unemployment rates (20-25%) due to changes in the forest product industry and declining fisheries off the coast. The razor clam recreational fishery provided many

jobs in the tourist support businesses in the area. Many of the diggers who travel to this part of the state come from outside the immediate region, most from the Puget Sound region; they and a good portion of that $7 million dollars stayed home that year. Was the money spent elsewhere? Most certainly!

In assessing these economics as applied to commercial fishing operations, there is a tendency to look only at ex-vessel prices, i.e., the money paid to fishermen at their boats. This usually underestimates costs. Crab or clams move into processing plants and other commercial and retail channels. All of these hire people and when crabs or clams are not coming in, people are out of work and processors are not selling. For many fishery products there are substantial multipliers, i.e., each $1 ex-vessel translates eventually into $4 to $10 (in some cases much higher than that!) at the final point of sale. Taken together, substantial losses can be removed from these local economies.

More difficult to assess economically are social and cultural losses that are encountered in ceremonial consumption of shellfish. For many of the coastal tribes in the Pacific Northwest, the consumption of shellfish is culturally very important. So important and central is the eating of clams to their culture that the Quinault Indians have a phrase *"ta'aWshi xa'iits'os"* [2] or "razor clam hungry" that is used when they go for an extended period without shellfish.

In addition to cultural or social impacts, many of the people living in this area (Indian and non-Indian alike), because of unemployment and other factors, depend on shellfish as part of their subsistence. Average incomes in this area, particularly in 1991, were low compared to statewide earnings. Having to turn to commercially prepared foods imposes a substantial burden on these populations, especially when most employment avenues are also reduced.

Conclusions

Since we have very limited data from 1991, we have only the 1998 *Pseudo-nitzschia*/domoic acid event to really examine closely In both of these events, we appeared to have an event that seemed to have begun in southern California and then spread north along the West Coast of North America. The question arises: was this one event with a single population of *Pseudo-nitzschia* or was it a spreading of an environmental condition that permitted local populations of the diatom to bloom? Since we were not able to collect offshore data during the 1991 event, we can't really say what happened then. However, in 1998 we were able to collect seawater samples from California and Washington. Off the California coast, *Pseudo-nitzschia multiseries* and *P. australis* were the predominant species present. Off the Washington coast, the predominant species was *P. pseudodelicatissima*. It would appear that what occurred was movement of an environmental condition, not a single population of *Pseudo-nitzschia* cells moving slowly up the coast. What exactly these conditions are is unknown at the present time.

Comments from the Audience

COMMENT (Vera Trainer): One of the hypotheses that people have put forward about some porpoise beachings is domoic acid poisoning. Porpoises also consume small fish—sardines and anchovies. In the last few years we have had some spectacular beachings of porpoises and small whales.

COMMENT: Historically, along the Gulf Coast there were a lot of reports back in the 1960s that when the red tides and the winds came in some people in the coastal communities had a hard time breathing, and they would literally have to move out of town for a short period of time. What the long-term effects of that are, no one really knows.

COMMENT (John Wekell): Follow-up on marine toxin outbreaks is a very difficult thing. This is a point that came home to us in the early 1980s. There was a consumer foods study, seemingly unrelated to marine biotoxins, which provides some insight as to why this kind of epidemiological investigation is so difficult. The purpose of the survey was to determine what people thought were the safest foods and which were the most dangerous. Most respondents said the safest was seafood—because it is absolutely clean! So when someone does get ill at a restaurant after eating shellfish or finfish, rarely do they associate it with the seafood. Usually other foods become the prime suspect, i.e., potato salad or the chicken. This makes epidemiology very difficult in assessing seafood-borne illnesses, particularly if you try to do a retrospective survey or analysis, i.e., go back and ask people if they were

[2] Xa'iits'os is the Quinault word for razor clams. The "x" is pronounced as a hard "h", the "W" is pronounced as a whispered long "u" or "o" sound and the "i"s as the English "ee" sound. The rest are pronounced as in English but with accents. I thank Joe Schumacker, shellfish biologist for the Quinault Indian Tribe, for this information.

ill during a particular time period. In 1991, an attempt was made to do exactly that in the state of Washington. After the initial domoic acid outbreak in razor clams, an epidemiologist attempted to survey people who lived in and around the southwestern Washington counties where razor clams are taken. He couldn't find any statistically significant occurrence of illnesses that could be attributed to the consumption of razor clams.

Does this mean that 1991 was the first time that domoic acid was on the coast of Washington (or Oregon or California)? Probably not! Our lab had the opportunity to analyze some "historical" samples, both canned and frozen razor clams that dated back into the mid-1980s. We found domoic acid in these samples. Although the levels did not exceed the 20 ppm guidelines, there were significant amounts present. Since we do not know how much of the toxin might have degraded during the processing or storage, we cannot estimate how much domoic acid was in the original razor clams. However, we think that domoic acid and *Pseudo-nitzschia* has been on the Pacific coast for some time.

Since the Canadian outbreak in 1987, has anyone been poisoned by domoic acid? We are not aware of any cases; however, because the symptoms are so similar to other dementia disease states, such as Alzheimer's disease, it is possible that cases have been missed or misdiagnosed. Based on the 1987 Canadian outbreak and some laboratory work with monkeys, the elderly appear to be more susceptible to domoic acid poisoning. Consider that if an older person goes to a family physician with symptoms of forgetfulness or confusion, the diagnosis might be a minor form of dementia. While there are some specific diagnostic techniques for identifying Alzheimer's disease, how many physicians apply them and would recognize a case of domoic acid poisoning? Combine this with the implicit assumption that seafoods are considered safe and wholesome—the chance of diagnosing domoic acid poisoning is small.[3]

In support of this latter point, in the 1987 Canadian outbreak, health authorities were alerted to domoic acid poisoning only because two elderly patients presented themselves at the same hospital on the same day and *happened to be treated by the same physician*. It was only after the second patient showed up with identical and severe symptoms that a very alert doctor *suspected* a foodborne poisoning. It is interesting to speculate what would have happened if these two patients had been taken to two separate hospitals. Perhaps we wouldn't know about domoic acid to this day.

[3] The patient mentioned in the first footnote perhaps represents what can happen to most victims of domoic acid poisoning. In his case, the hospital in Alaska initially diagnosed PSP, largely based on eating shellfish. A very reasonable assumption within the State of Alaska would be that he suffered a relatively mild case of PSP, based on his symptoms at the time. It was only later, when he began having very severe neurological symptoms (forgetfulness, confusion, headaches, etc.) that he suspected some other agent. Several consultations later and a trip to the University of Washington Medical School in Seattle, appears to confirm that he may have suffered a true case of domoic acid poisoning. At present (September 2001) his case and status are still under study.

Marine Biotoxin Monitoring in California, 1927-1999

Greg Langlois
California Department of Health Services, Berkeley, California

This is an overview of our program, how we operate, with background on what we see in terms of both PSP and domoic acid activity. I will also describe some of the things we're doing as we try to figure out the best way to manage these events.

We have a long history of PSP in California, dating back to the times of the coastal Indians. There are stories of Indians posting sentries along the coast to look for bioluminescence in waves.

PSP BACKGROUND

- Coastal tribes posted sentries
- First recorded deaths in 1903 (5)
- Most recent recorded deaths in 1980 (2)
- Last reported illness was 1991
- 510 reported illnesses
- 32 reported deaths

I think our numbers pale in comparison to the numbers reported by Alaska and perhaps even British Columbia. Our first recorded death was in 1903; there were five that year. Our most recent recorded death in California was in 1980. There were two deaths, one in Marin County and one in Sonoma, which are both north of San Francisco. The victim in Sonoma consumed two raw rock scallops, whole. He received immediate medical attention and was placed on a helicopter for transport to the nearest hospital, but died en route, less than two hours after consuming the scallops. Our most recent reported illness was in 1991. The victim was transported by helicopter to the nearest hospital and placed on life support. They were released the next day with apparently no ill effects. There have been 510 illnesses and 32 deaths since records were first kept.

PSP MONITORING

- Primary monitoring tool is mussels
- Commercial growing areas
- Coastal monitoring
- Quarantines
- Annual mussel quarantine from May to October
- Special quarantines as needed
- Public education
- Phytoplankton monitoring

We still rely on mussels as our primary monitoring tool. They're ubiquitous along the California coast and are common in all of our commercial shellfish growing areas. We do routine monitoring in all of our commercial areas on at least a weekly basis and the frequency is increased as needed. We use our coastal monitoring program both as an early warning system for our growing areas, which are all inside bays and estuaries, and for coastal sport harvesters. Because coastal monitoring of shellfish alone cannot provide a guarantee that each mile of shoreline is free of toxins, we implement an annual quarantine that goes into effect for sport harvesting of mussels from May 1 to October 31. We issue special quarantines on other species as needed. Mussels are monitored year-round despite the quarantine. If a bloom is detected or a PSP toxicity increases at our mussel monitoring stations, monitoring is expanded to include other species in the area, e.g., clams or rock scallops. In more extreme circumstances a quarantine will immediately be issued for additional species in that region.

One aspect of most monitoring programs that is vitally important and terribly underfunded is public education. Although local agencies have different outreach programs for their communities, there is a need for additional educational materials such as multi-language literature and warning signs. Another component that California has added recently, as a result of our domoic acid episode in 1991, is phytoplankton monitoring, which will be discussed later.

Our primary sites for shellfish monitoring are at commercial harvesting and commercial growing sites, scattered along the entire state coast. Our secondary sites are monitored a maximum of twice

per month during the spring and summer months. Tidal conditions do not permit a greater frequency of sampling. Sentinel mussel stations consisting of bags of mussels suspended from piers or moorings are employed wherever possible. Other sites are monitored infrequently or on an as-needed basis to help define the parameters of a bloom.

SHELLFISH SAMPLING LIMITATIONS

- Tide dependent
- Weather dependent
- Transportation times
- Analytical throughput
- Each toxin requires different assay

As is typical for most monitoring programs, limitations exist. One limitation is that access to shellfish resources is limited by tides and weather. Poor weather, even with a good minus tide, can restrict access to intertidal sampling stations. Transportation also presents difficulties—certainly not nearly the problems as in Alaska—but lag times are introduced by transportation from remote sites, adding at least a day between the time the sample is collected to the time it's analyzed. Toxin levels above 80 µg require an immediate response for public health protection, and any delay means there is less time to respond. Analytical throughput can also be a limiting factor, although we find sometimes that our ability to get the samples is more limiting. The cost involved in analyzing for different toxins is prohibitive, a further limiting factor.

PHYTOPLANKTON MONITORING

- Phytoplankton sampling is not dependent on tides or weather.
- Observations are done in the field.
- One observation works for all toxin-producers.
- Volunteers enable low cost per analysis.

Phytoplankton monitoring certainly has its place and its own limitations, but it has helped California's monitoring program to overcome some of the limitations with mussel monitoring alone. Phytoplankton monitoring is not intended as a replacement, but as a supplemental approach. Sampling frequency and resolution of data can be improved because plankton sampling is not dependent on tides or weather. The benefit is that the observations are done in the field, so there is not the lag time associated with mussel monitoring. The other advantage is that a water sample can be examined for the presence of all the toxin-producers. The phytoplankton monitoring program is also cost effective because it relies on volunteers. The interaction with volunteers is rewarding and helps to establish a connection between the community and the agency.

Plankton stations

California's primary phytoplankton monitoring sites, monitored once a week, include all commercial shellfish growing areas and numerous coastal sites. Some sites are sampled by volunteers who also do field identification work for us. There are over 45 people coastwide collecting samples for the program, with a core group doing the field identifications, and phoning or emailing in results. All volunteer samplers also ship samples to the state lab for confirmation of their observations.

PSP MONITORING DATA

From 1992 through 1998 there were erratic occurrences of PSP toxicity. During 1989 and 1991 we experienced exceptionally high concentrations of PSP toxins over a wide geographic range. In 1992 California initiated the phytoplankton monitoring program, looking for the presence of *Alexandrium* and *Pseudo-nitzschia*. It would be worthwhile to combine all of the West Coast data for the past 10 or 20 years to look for large-scale geographic patterns. Anecdotal evidence suggests that, during a year of low PSP activity in California, there is increased toxicity farther north along the coast of Washington and British Columbia.

Data from the phytoplankton monitoring program is qualitative. A relative abundance is assigned to each species present in a sample; quantitative cell counts are not performed because of the prohibitive amount of time required to process each sample. The complication with qualitative assessments is that a sample can have a very high relative abundance of a given species, but a very low cell mass. Other methods are employed, which I won't frighten you with, to keep track of the relative cell mass in each sample. Results of the phytoplankton monitoring program have been encouraging: PSP toxicity is almost always associated with the presence of *Alexandrium*. For every major PSP event *Alexandrium* has been observed in the area. Small numbers of *Alexandrium* cells have been observed in the absence of PSP activity.

By plotting all of California's toxicity data by calendar year (i.e., on the Julian calendar from days 1 through 365), an interesting pattern emerges. A PSP toxicity peak is seen in March, and later in late July and in September . This pattern corresponds to the seasonal patterns experienced in California. California has an upwelling-dominated coastline, particularly from San Francisco northward. There are two major upwelling periods—one in early spring and the other in late spring/summer . After the early spring upwelling, a relaxation event frequently oc curs that is associated with the onset of PSP toxicity.

If an environmental pattern exists that is associated with PSP toxicity events, then there is the potential to develop an environmental cue that could easily be monitored. The NOAA Coastwatch node in San Diego provided access to the satellite imagery of sea surface temperatures, and California' s biotoxin program has been examining this imagery relative to phytoplankton and toxicity data.

A stretch along the Marin coastline is the hot spot for PSP toxicity in California. PSP events occur at greater frequency and in higher concentrations in this region than anywhere else in the state. One possibility is that dinoflagellate populations are associated with offshore warm water masses. A relaxation event causes advection of the warm surface water and the associated dinoflagellates to the nearshore area. Examination of sea surface temperature imagery in conjunction with toxicity data and phytoplankton observations have confirmed this possible pattern. There have been occasions when the patterns in sea surface temperatures developed but PSP toxicity was not observed. Although this environmental pattern may be indicative of a change to a dinoflagellate dominated phytoplankton assemblage, it does not necessarily predict that *Alexandrium* will be present or will have a competitive advantage over other species. Nonetheless, the use of satellite imagery provides a simple cue to the environmental conditions conducive to a PSP event, allowing the program to focus monitoring efforts in these regions of higher risk.

DOMOIC ACID MONITORING

In 1991 the first documented domoic acid episode in Monterey Bay occurred. Mussel samples analyzed at the time had a high of 47 ppm in the Monterey region and low level activity at other sites throughout the bay . Domoic acid was detected in every county north of Monterey . There was no domoic acid activity south of Monterey at that time. In 1992 a very low level of domoic acid was detected in the southern counties.

Pseudo-nitzschia is ubiquitous along the California coast. It appears to be around more frequently than not, although in very small numbers. *Pseudo-nitzschia* peaks were observed in 1995 and 1996. In 1995 a bloom lasted well over a month along the San Louis Obispo coast south of Monterey Bay . Crab samples collected by the state' s Food and Drug Branch contained low concentrations of domoic acid. A similar pattern was observed in 1996.

Domoic acid in Monterey Bay, April-May 1998

In 1998 *Pseudo-nitzschia* was first observed in April in samples from southern California—Los Angeles, San Louis Obispo, and near Santa Barbara. Cell densities were not extremely high but greater than observed in samples from stations farther north. While others have observed that PSP events frequently follow domoic acid events, in 1998 we observed just the opposite. PSP toxicity occurred along the Monterey coast and north through April until a strong upwelling event occurred, with *Alexandrium* disappearing and being replaced by a bloom of *Pseudo-nitzschia*.

In May-June there were 70 marine mammal strandings from San Louis Obispo through San Mateo. They were exhibiting neurologic symptoms associated with domoic acid poisoning. The Marine Mammal Center in Marin County reported that domoic acid was detected in serum, urine, and feces samples from the animals. At that time there was an increase in *Pseudo-nitzschia australis* according to the Monterey Bay Aquarium Research Institute (MBARI).

When *Pseudo-nitzschia* began appearing in our volunteers' samples and observations, the department began collecting samples of anchovies and sardines from the local bait fishery in Monterey Bay. High concentrations were detected in anchovies but only low levels were detected in mussels from the area, which is a bit problematic relative to the usefulness of mussels as a monitoring tool for domoic acid. Sardine data exists for domoic acid concentrations from April 30 through June 14. A peak was observed around May 15. The Santa Cruz wharf was the focal point for *Pseudo-nitzschia* cell densities according to MBARI biologists. Cell counts were reported as high as 200,000 cells per liter (which does not necessarily indicate a dense bloom).

An important aspect of this event is that our volunteer -generated data illustrates a peak that coincides very nicely with the anchovy toxicity peak. It was quite heartening for us to know that the work our volunteers were doing gave us a pretty accurate representation of what was going on in the environment. In fact, this data set agreed nicely with the more rigorous and quantitative data generated by researchers in the area.

Future needs

- *Improved Analytical Methods.*
- *Indicator Species for Domoic Acid*. In California, there is a potential problem in that a good indicator species for domoic acid does not exist. Mussels are common along our coast but don't seem to be a very good indicator for the early stages of a domoic acid event.

  This brings to mind Emerita, a filter-feeding crab. Some work has been done on this in the past relative to PSP toxins. Our program conducted some exploratory work a couple summers ago, looking at Emerita as a possible indicator species for PSP toxicity. The crab is buried in the sand, and follows the tide so it's easy to collect regardless of the tidal prism. It is unclear how effectively it accumulates PSP toxins or domoic acid. Our preliminary work indicated that it did accumulate PSP toxins in roughly equivalent concentrations to nearby mussels. A research project ongoing at California State University at Monterey Bay is looking at PSP in these crabs. The standard acid digestion procedure has apparently created some difficulty because the large amount of calcium carbonate in the crab's shell serves as a wonderful buffer, making it difficult to lower the pH adequately.
- *Expanded Phytoplankton Monitoring.* We've been heartened by some of the results we've obtained over the past few years with phytoplankton monitoring, but it does have some limitations.
- *Relationships between Environmental Processes and Blooms.* One limitation to phytoplankton monitoring is the same one experienced with shellfish monitoring. A high frequency of sampling is required to understand and follow trends. It may be that in some cases even weekly sampling isn' t good enough to detect a change in environmental conditions. Monitoring for another environmental cue as part of a matrix of tools is the most promising approach. The best approach to routine monitoring may be established by confirming the relationship of sea surface temperature and circulation patterns of water masses, plankton species composition, and the distribution and magnitude of toxicity .
- *Technology.* SeaWIFS [satellite images] is inaccessible for state monitoring programs at present. It would be nice to establish some linkages so that the people doing the research, who can interpret these data, could provide some input to the state monitoring programs, which are trying to monitor on a real-time basis.

Questions from the audience

QUESTION: With regard to using the crab for monitoring purposes, you might want to homogenize the entire crab—they are just little crabs—and extract the toxin with water. Forget about the buffering for the time being, and take a water extract. See if that works, because, at least for PSP, the toxins are very water soluble.

G. LANGLOIS: So you don't think you need the acid?

QUESTION: Well, it would be nice to have it but you've got so much calcium carbonate that you'd have to dump in tons of acid. Carbon dioxide is a great buffer. The other thing is that SeaWIFS and other images are available, but they're about $1,000 a pop. They're really great for following chlorophyll—not necessarily telling you where to go, but where not to go, which can be just as important. It is a great technology but it's just too expensive, especially if you need to do it on a day-to-day basis, in real time. It would be just absolutely prohibitive at that cost.

The Marine Toxin Problem in Washington State

Frank Cox
Washington Department of Health, Olympia, W ashington

What I'm going to talk about is by no means exhaustive of the problems that we have in Washington. But because of the short time we have to cover this topic, I've focused on the most recent, most noticeable toxin problems, starting with 1996. We most certainly had major problems well before that, but this workshop is about current trends, and I've focused on current problems here.

The biotoxin program covers the entire state of Washington. We work from the Canadian border all the way down to the south end of Puget Sound and Hood Canal, the Strait of Juan de Fuca, Grays Harbor, and outside coastal beaches where razor clams are found.

1996 MARINE TOXIN EVENTS

In 1995 we had a very quiet year as far as PSP is concerned, and then in 1996 we had a lot of events. The first significant one was in Port Gamble, at the north end of Hood Canal. Hood Canal as a whole generally has minimal or no problems with PSP from year to year. In Port Gamble the Indian tribe had started harvesting geoducks. Some geoducks that were tested reached toxin levels over 2,000 micrograms per 100 g meat. That event influenced a change of plans with our department. Up until this time geoduck harvesting in Washington was primarily in South Puget Sound. Geoducks were harvested by commercial companies who bid for a particular tract of subtidal land, to harvest a specific poundage, which Department of Natural Resources put out for bid. In 1996 various Indian tribes began geoduck harvesting businesses in Kitsap County on the mainland side of Puget Sound, and also in the north end of Hood Canal and up along the Strait of Juan de Fuca. Until that time our department detected low incidence of PSP in South Puget Sound. We were harvesting samples and testing body meat and neck meat. We were ignoring what was in the gut of the geoducks.

Our Canadian counterpart, in British Columbia, and some local health department folks brought to our attention the fact that members of the Asian and tribal communities were eating the gut of the geoduck in soups and in other ways. In the past we thought that just the neck and the body was being consumed. So we changed our procedures and we started looking at the guts in earnest. These two events, (1) switching to the middle and north parts of the state where we started having chronic problems with PSP, and (2) finding out about gut consumption, really impacted our department.

The second 1996 event that we had was in Kilisut Harbor, a narrow harbor inside two islands. There we had mussels reach 4,818 micrograms in October. This caused a closure that lasted through March of 1997. About 10-20 commercial shellfish companies were shut down and unable to conduct their business. The mussels had the highest levels, but other species of shellfish exceeded the limit as well.

In 1996 we did not have any illnesses or deaths from PSP. We had six recalls and, as you know, recalls don't impact just one company—they impact all the companies in the state because recalls affect many markets. Many companies see loss of sales, and they see the price actually soften up for their products. The first year we started looking at the gut of geoducks, 11 geoduck tracts had to be closed due to PSP.

1997 MARINE TOXIN EVENTS

In 1997, although we had no illnesses or deaths in Washington due to PSP, we had some very significant PSP blooms. We had a bloom starting in November-December down in Case Inlet, in South Puget Sound. Levels reached 6,799 micrograms at the peak of the bloom, setting another new record as had the blooms the year before. This bloom did something that has never happened before in the history of our program in Washington. It affected other parts of South Puget Sound that previously had been PSP-free. The bloom affected quite a large number of companies and had a huge impact on our laboratory. As we watched the bloom expand it was like dropping a rock in a pond. You could follow

toxin as it moved farther beyond Case Inlet. For example, one week an area would be fine, then the next sample would come in under 80, and the next sample over 80, and some went clear up into the thousands before it was over. The area was closed from November through January and set new records for South Puget Sound, and new records for the state (except for the 1978 bloom that we had in Whidbey Island).

We saw a major bloom in Willapa and Grays Harbor in November and December. At the same time we had a razor clam recreation season going on at the Twin Harbor beach. We did not pick up PSP toxin in the razor clams at Twin Harbor. This situation was a flip-flop from what we previously had seen in this area. Normally we would see razor clams along the outside coast start to show some PSP, and then it was not uncommon to see some in the Grays Harbor area. This time, most of the blooms were in the harbors with nothing on the outside, a complete reversal. These blooms occurred at a time when it caused significant damage to the industry, because it was right before Thanksgiving when the industry markets a huge amount of their oyster products. For many companies, 60% of their annual sales are done within a few weeks in that time of year. In Grays Harbor we got numbers up as high as 286 in Pacific oysters. In the Elk River area and in Willapa and the Bruceport area we had numbers up to 341. All told for 1997 we had four recalls, plus two recalls where the product was in trucks and hadn't made it to market yet. We had 10 geoduck closures in 1997.

1998 MARINE TOXIN EVENTS

We thought that after the 1997 struggle, maybe 1998 would be better. It wasn't. In 1998 we had five illnesses from commercial mussels harvested by a company in South Puget Sound. This set new records; as far as I know this has never happened. In the north end of Carr Inlet, people from two different families ate mussels that they had bought from a company in Seattle. Fortunately, thousands of pounds of this product were not sent out. But this oyster harvesting company really surprised me. No one in our program knew that he had chosen to start marketing mussels that were an incidental catch hanging on his oyster culture. He only harvested 75 pounds so he had three bags of them. Two went to market and one his wife was cooking at home when we called him to give him the test results. This is how close we came to having more illnesses. We were looking at levels over 10,000 micrograms in the mussels. South Puget Sound was once the sacred cow that was not affected by PSP. Obviously that has changed.

In the history of our program the highest levels were during the 1978 bloom in Penn Cove and Holmes Harbor, when numbers got up to 30,000 micrograms in mussels. The Carr Inlet bloom affected the oysters and it affected all of Carr Inlet. And what was unusual about it is that same inlet had a bloom earlier in the year when we recorded 2,442, which at that time was a record high. We have never seen a place where a bloom went over 1,000, and then later in that same year again went to a second very high level. To me this is extremely alarming. First that it happened at all, and second that it was in South Puget Sound where it followed right on the heels of the inlet next door the year before.

During the first bloom that happened in August 1998 in Carr Inlet we also had a geoduck tract closure in Pitt Passage. This was significant because the geoducks were over 1,000. A bloom in Carr Inlet caused quite a large recall, and quite a bit of difficulty. The company that had sold the mussels also was involved in a clam recall that went to several states. All the publicity that goes with it, plus the illnesses, did not have a good effect on the industry.

On top of all of this in 1998 a domoic acid bloom occurred on the outside coast at Kalalock, which was a significant event. For domoic acid since 1992, we hadn't had a lot going on. But starting in July 1998 we set new records for domoic acid in Washington. If 30,000 people had been out on the beach harvesting razors, and we were unaware of this toxic bloom, we would've had health problems of major proportions.

The major difficulty with geoducks is that it is very hard to manage a tract that in a period of one year oscillates so much. We've wrestled with it and I don't think we've come up with an answer. We can increase the sampling, but it just doesn't help. It's so unpredictable. It's what I call the light switch resource management plan. It opens and closes like a light switch.

Another geoduck tract, Skiff Point, has done the same type of thing—great oscillations. It just plays havoc with the tribal activities and the non-tribal harvesters.

Trying to manage geoducks is difficult. In 1998, when there was a bloom in South Puget Sound, we measured over 1,000 micrograms in areas near the

geoduck tracts. Even though we sampled the geoduck tracts every day, they didn't show any PSP. We don't know why we saw levels go very high over the closure limit and then back down again, when the intertidal shellfish do not show those fluctuations. At Jameston near Sequim, along the Strait of Juan de Fuca, again we have seen extreme fluctuations, which make it a very difficult resource to manage.

SUMMARY

What makes the harmful algal blooms in Washington unique? One factor is the unusual time of year—we had these blooms in October-November-December, which we haven't seen before. We used to say our red tide or PSP season was from April to October. There's just about no time anymore that is a safe time in Washington state, when you can breathe easy and reduce the sampling. If anything I think we're going more and more to sampling year-round in more locations.

Second, long closures. The domoic acid in particular has a tremendous impact on the recreational fishery and I think it's going to continue. That means a lot of dollars are being lost to coastal communities. Because of the domoic acid trend, I don't believe that this spring or even next fall [1999] will be safe to allow a razor clam recreation season to occur. A record number of clams could be harvested, if they would become safe.

In new areas, particularly in South Puget Sound, PSP has become extremely unpredictable in time of year, location, and how high the toxin level will go. We saw new records over the last several years. I happen to think that those five illnesses that we had last year were very fortunate. We could have just as easily had 10 or 20 deaths. If we had high levels in mussels, we would have had illnesses all over the country because that's where the company ships to. And there's a good chance a lot of them would have been in places where they wouldn't even have known what the problem was. Thankfully in Washington, particularly Seattle, the hospitals are a little more aware of this problem.

Multiple blooms: We had two major blooms in Puget Sound in one year, which hasn't happened anywhere in Washington, let alone South Puget Sound.

Management: The geoduck has the light switch syndrome. There doesn't seem to be any pattern that we can relate this to that helps us manage it.

Increase in cost: A few years ago I was trying to do a cost reduction for the biotoxin program. I spent many hours trying to come up with cost reduction to see where we could cut back. That's all been thrown out the window. We've increased our budget just about yearly. It's also having a tremendous impact on our labs. They're working weekends, holidays, and nights.

Economy: Algal blooms affect the overall economy of the state of Washington.

Marine Toxin Monitoring Program: British Columbia

Klaus Schallié
Canadian Food Inspection Agency, Burnaby, British Columbia, Canada

There have been some changes in the regulatory structure with regard to the Canadian Shellfish Sanitation Program. Rudy Chaing and all of us in fish inspection were previously part of the Department of Fisheries and Oceans. Then the federal government reorganized the federal food inspection system and put us under one umbrella. The Canadian Food Inspection Agency has taken in all of the food inspection components from Agriculture Canada, Health Canada, and the Department of Fisheries and Oceans. Also some people who were with a previously existing department, Consumer Affairs, had already been amalgamated with Health Canada at the time. What follows is a brief overview of our program.

In British Columbia shellfish is a rapidly growing industry. We've had a lot of problems with El Niño and environmental effects. Our finfish fisheries that were traditionally the bread and butter for our processors have gone into a tremendous decline. Ocean survival has really been affected for salmon species for whatever the reason, whether it's overfishing, environment, El Niño, sea survival, or all of those. In 1996 the farmed shellfish were worth 14.5 million dollars, and the total value of British Columbia shellfish harvest, wild and farmed, is 163 million dollars. It's a growing contributor to our economy in British Columbia. About two months ago the provincial government announced the Shellfish 2000 Initiative, a program that will double the amount of the land under tenure in British Columbia for shellfish aquaculture. Some of these are going to be intertidal but the majority of them will probably be deepwater leases.

Canadian shellfish sanitation program

The Canadian Shellfish Sanitation Program (CSSP) is very similar to the National Shellfish Sanitation Program in the United States. A major difference is that in the U.S., the Food and Drug Administration (FDA) audits the individual state programs while in Canada, one federal program applies to all of our producing provinces. We have a CSSP manual, similar to the Shellfish Model Ordinance, which outlines the program and provides guidance. It is administered by three federal agencies, the Canadian Food Inspection Agency, the Department of Fisheries and Oceans, and Environment Canada. Fisheries and Oceans are the fish managers. They are responsible for enforcement of closure regulations; enacting, opening, and closing areas; resource issues; and stock allocation—all fisheries issues. Environment Canada does the growing water classification, shoreline sanitary surveys, and growing water quality. They recommend classification of shellfish harvest areas. We use the same criteria that the FDA applies in the National Shellfish Sanitation Program, and we are responsible for food safety. We are in control of handling, storage, transportation, processing, labeling, and the marine toxin control program. We are also the federal contact for exchanges with foreign governments, and we make recommendations for harvest openings and closures to the Department of Fisheries and Oceans. They have the regulatory framework to carry out the opening and closing. When I was part of that department I was telling them to close it; now since I am not part of that department I recommend opening and closing, but of course it's just a rubber stamp. The relationship between fish inspection and the Department of Fisheries and Oceans is unchanged.

A provincial requirement mandates that all bivalve shellfish must go through a federally registered plant in British Columbia, which makes our work easier. We don't have "direct from the harvester" or "back of a pickup sales" or anything like that. If it's a bivalve shellfish, it has to go through a federally registered plant. And many of our plants are on the Interstate Certified Shellfish Shippers List, which means that they can ship product into the United States without any additional inspection. We've had the bilateral agreement with the United States since 1948 or 1949.

Marine toxin monitoring

We test for paralytic shellfish poisoning, using the same action level, 80 µ g per 100 g meat. And we

also look for amnesic shellfish poisoning (ASP) in our samples, using high performance liquid chromatography (HPLC), again the same action level of 20 ppm. We have the capability within our agency to do diarrhetic shellfish poisoning (DSP), but we have yet to identify any outbreak. If we have suspicions about a sample it is sent to our Halifax Laboratory where it can be run for the DSP, etc.

Biotoxin monitoring

We use mussels as the sentinel species because they acquire the biotoxins more quickly than the other species of shellfish. We have plastic mesh sacks filled with sea mussels, *Mytilus californianus*. It's a larger animal than our native *M. edulus* mussel, so it really reduces the amount of time our technicians have to spend in getting their 100-150 grams of meat to run the sample.

Monitoring sites

We have approximately 70 monitoring stations on the south coast. Samples are shipped weekly to our lab from May 1 to October 30, which we consider the higher risk times of the year. Samples are shipped every two weeks from November to April. From November 1 to April 30 there can be weather considerations. We could have nasty weather, and never ask anyone to go out and risk their lives to take a shellfish sample. In the greater Vancouver area there are no monitoring stations. From there to the U.S. border everything is classified as prohibited, because of urban runoff, agricultural runoff, industry, etc. Greater Vancouver has more than 2 million people. No one can harvest for any purpose. Just across the line, in Washington, there's still some harvesting going on I believe.

In northern British Columbia we have a similar number of monitoring sites, but there are no roads to the north and central coasts. The only way you get samples in and out is by air or by water, so it's very expensive. It's just like the Alaska coastline—it's very convoluted with a huge number of bays and islands and inlets. So, we don't monitor all of the sites continuously; we only monitor the sites when there's a harvest in the area. For example, if the geoduck fleet is in a certain area we establish a mussel monitoring station in that area. We also presample the geoducks prior to the opening of the harvest. During that time I recommend that DFO lift only the prohibition on the geoducks. All the other species remain closed because I'm only monitoring for the geoducks. I can't vouch for the butter clams or any of the other intertidal bivalves. Areas are monitored only when the shellfish are harvested. Afterward I close it down again because the samples stop coming in.

On the central coast the Heiltsuk First Nation has a manila clam harvest. They have specific harvest locations and we've arranged for monitoring sites. When that fishery is going I lift only the prohibition for the Manila clams in that area, and I leave the butter clams and the other species closed.

Some years ago our federal government had to balance the budget. We were running a huge federal deficit. We had gone through three or four rounds of resource cuts, including staff cuts. So we went to the industry and said, "Look, we've been paying over $100,000 Canadian every year to contract people to provide us with the mussels from these 70 monitoring sites, and we can no longer do it. The money's not there. What would you like us to do?" We gave them two options. (1) The industry could give us the money, the 110K, and we'd roll over the contracts and reissue them, or (2) the different sectors in the industry could divide up the coast and provide us with the samples. They chose the second option. So the shellfish industry and First Nations are involved as partners, and they provide us with the samples. We analyze the samples, we administer the program, and we provide the toxin analysis records to the industry. "User pay, user say." They're providing us with something, and for our part we agreed to improve communications. I have a 24 hour phone-in line that they can call day or night to find out what the status of their area is. Also, when there are changes to the status of an area, whether it's openings or closures, I have a fax fan-out to all the partners and to the processors. Currently the B.C. Shellfish Growers Association in cooperation with CAIA, the Canadian Aquaculture Industry Alliance, are trying to get some government funding for looking at different ways of getting cost recovery for these various programs. The industry would like to have one-stop shopping. Environment Canada has been similarly impacted with cuts, as has DFO.

What happens is this: we say, "We need samples from you. Environment Canada says we can no longer survey the water unless you chip in." Then DFO says, "We can't do any more stock assessments unless you pay, therefore we won't open the fishery." Now, they would like to be able to have an equitable cost recovery program from the federal government. But it's proving to be a real challenge, because

of the high profile of the B.C. Shellfish Growers Association. Next, Environment Canada or I say, "OK, time to cough up for some funding here." They don't represent all the growers. You could be a member of the BCSGA and your next door neighbor with an oyster lease may not be a member. Thus you're being asked to contribute to the management program and your neighbor isn't paying a nickel but derives the same benefit. There has to be some way to develop an equitable cost recovery system to the industry to help pay for the programs that are necessary for them to continue.

Burnaby laboratory

The annual number of PSP mouse bioassays at our Burnaby laboratory is approximately 3,000 to 3,600. We are having problems getting adequate supplies of mice. We've got a lot of underweights these days, which means we have a mouse hotel. We have to hold them over and feed them until they come up to weight. We get 350 mice in a shipment and we can only use 50 of them. So I spend a lot of my time telling the lab to prioritize samples. I assign the samples from areas open in commercial harvest the highest priority. If I saw a blip in the previous sample I'll give a higher priority to a particular area. For ASP HPLC (domoic acid), we do about 2,800 samples a year. DSP all goes to the Halifax lab.

Marine toxin closures

If an analysis exceeds the action level, I review the data for all the surrounding monitoring sites. If necessary we consult with our fishery managers. The industry would like us to close as small an area as possible so it has a minimal impact on their income. The Department of Fisheries Conservation and Protection officers would like to close the whole statistical area, because they feel that it's a better safeguard for the recreational and First Nations harvester because they're often not aware of where the subareas are. So there is that bit of a tug-of-war going on there between the two sides.

We determine the extent of the area to be closed and then I recommend a harvest ban to the regulations unit of the Department of Fisheries and Oceans. Their part is to rubber stamp it—the director general, who signs the closure order, is not going to say, "No, I'm not going to close that" and take the risk of somebody becoming ill. The regulations unit then prepares the closure order and it's signed by the regional director general for DFO. Signs are then posted by DFO at prominent locations, at public wharves and beaches. But like in Alaska, the coastline is so extensive that we don't even pretend that we are getting adequate coverage with this kind of signage.

Those who have recreational harvest licenses, which they are required to have to dig shellfish or knock them off the rocks, are supposed to call the 24-hour number before they go. They can get information about sanitary closures and marine toxin closures. But people still go out without bothering to check, and they have gone out and harvested in closed areas and have suffered because of it. When there is a closure I send a fax fan-out to all the shellfish processors and the monitoring partners. DFO notifies all of their offices and they either e-mail or fax any changes in openings and closures to their subscriber list of industries. The local DFO office may notify the local media—radio, television—and if there's a sport fishing resort or provincial park where people are camping and likely to be harvesting, DFO also makes them aware. Department of Fisheries Conservation and Protection officers patrol the closures. But the officers are limited by night tides, in the winter especially, and they are limited by how much overtime they can work. I don't pretend the system is 100% effective at keeping people from harvesting in closed areas, whether it's for sanitary reasons or for biotoxin closures.

Marine toxin monitoring summarized

We use mussels as a sentinel species; it's our preferred approach. Our coastline is so vast, with so many inlets, so many bays, and so many remote areas with very few inhabitants, and there is a large tidal influence with very strong currents. These factors plus the inability to predict when and where HABs will occur make plankton monitoring impractical.

The advantage to using mussels as sentinel species is that they accumulate toxins much faster and to higher levels than other bivalves. When mussels are found to have levels of toxin above the action level, oysters and clams are normally safe to eat. This provides a safety margin when samples are shipped to our laboratory from remote harvest areas.

In 1998 there were fewer PSP blooms than in 1997, and they were less intense. The highest result in 1997 was 8,800 µg, while the highest result in 1998 was 1,400 µg. No ASP was detected in 1997, except residual amounts in razor clams from the Queen Charlotte Islands. In 1998 there was a minor ASP

outbreak on the west coast of Vancouver Island, but it was much less intense than in Washington, Oregon, and California.

Marine toxin research in British Columbia

Fiscal restraints to balance research budgets have resulted in cuts in staff and funding in most programs. The research emphasis has been on finfish due to a crisis in the salmon fishery caused by El Niño and other factors. Dr. Ian Whyte, with DFO's Pacific Biological Station, has been doing some limited research. He has been trying to obtain funding for HPLC for saxitoxin research, and also is doing some research into ASP. There is some indication that ASP may severely affect scallops, weakening or even killing them. Dr. Max Taylor, marine algae researcher with the University of British Columbia, also has done some marine toxin research.

Harmful Algal Blooms: The Economic Consequences for Alaska

Raymond RaLonde
University of Alaska Marine Advisory Program, Anchorage, Alaska

INTRODUCTION

Harmful algal blooms (HAB) are a worldwide problem and increasing in frequency and intensity, causing economic hardship, severe episodes of illness, and death. In Alaska, the most damaging HAB is *Alexandrium* dinoflagellate blooms that cause paralytic shellfish poisoning (PSP). A persistent problem for Alaska, PSP fatalities date back to 1799 when the crew members of Alexander Baranof of the Russian American Trading Company ate tainted blue mussels at the now notorious Poison Cove in southeast Alaska. Since 1990, two fatalities, a number of illnesses, and economic losses to shellfish fisheries have occurred.

The state of Alaska has not conducted an economic impact study of PSP. This initial examination attempts, with some quantitative support, to describe the major effects PSP has on Alaska's commercial fishery, aquaculture industry, and recreational/subsistence users.

Alaska has the largest, most productive fishery in the United States, contributing 54% to the total U.S. landings. With an annual revenue of approximately three billion dollars, commercial fishing is second only to oil as Alaska's most important industry. The fishing industry is Alaska' s largest employer supplying over 10% of the Alaska jobs, while seafood processing provides 63% of the employment to the manufacturing sector (Alaska Department of Labor 1997). Although the finfish and crab fisheries are enormous, PSP hinders expansion into underutilized shellfish fisheries. Even in regions of the state where PSP does not generally occur, regulatory requirements for testing (1) increase the costs and financial risks of investing in new fisheries and (2) prevent maximization of income from existing shellfish harvest.

The Alaska Department of Environmental Conservation (ADEC) operates a PSP testing program for commercially harvested shellfish from its single testing laboratory located in Palmer, 45 miles north of Anchorage. The ADEC regulations require strict compliance with a tiered uniform sampling program that decreases sampling requirements after set time periods of PSP-free samples. Not only do the fishery and aquaculture operations pay for the collection and shipping of samples, a dry, temperature-controlled holding area is required to store the harvest out of water until the laboratory results are completed. Consequently, PSP not only causes direct economic impact during toxic events, but the cost of shipping, testing, and storing commercially harvested shellfish also increases the cost of doing business.

THE COMMERCIAL CLAM FISHERIES

Published estimates place Alaska's sustained annual harvest of bivalve shellfish at over 50 million pounds (U.S. Department of Interior, Bureau of Commercial Fisheries 1968). Clam fisheries, with the exception of Lower Cook Inlet and Kachemak Bay, remain underdeveloped, lacking fisheries management plans, water quality certification, and a PSP testing program. ADEC's PSP testing requirements for remote fisheries prevent developing a new fisheries because of the expense of testing.

Alaska's current bivalve shellfish fishery consists of the native littleneck clam (*Prototheca staminia*), razor clam (*Siliqua patula*), and geoduck clam (*Panopea abrupta)* (Table 1). The littleneck clam fishery is located in Kachemak Bay. Razor clams, found throughout Alaska, are harvested commercially only in Lower Cook Inlet while a dive fishery harvests geoduck clams only in the southeast Alaska region (Fig. 1).

The magnitude of the economic impacts caused by PSP varies for each shellfish fishery based on remoteness, dispersion of the fishery, history of PSP toxicity in the region, and the value of the final product of the fishery.

Table 1. Commercial clam harvest and income, 1990-1999. Income is based on ex-vessel price paid to the fishermen and is averaged over the fishing season price.

	Littleneck clam				Razor clam		Geoduck clam	
	Southeast Alaska		Kachemak Bay		Cook Inlet		Southeast Alaska	
Year	Harvest	Income	Harvest	Income	Harvest	Income	Harvest	Income
1990	0.24	$.76	35.74	$48.25	324	$174.96	271	$609.75
1991	4.95	15.86	38.73	52.29	201	108.54	248	558.00
1992	11.78	25.54	54.63	73.75	297	160.38	195	438.75
1993	9.70	26.10	63.68	85.97	310	167.40	209	470.25
1994	9.41	25.93	44.29	59.79	355	191.70	147	330.75
1995	9.41	33.67	67.00	90.45	248	133.92	261	587.25
1996	0	0	53.52	72.25	355	191.70	NA	NA
1997	0	0	31.53	42.56	367	198.18	200	510.00
1998	0	0	23.47	31.69	372	200.88	180	720.00
1999	0	0	18.53	25.02	353	190.62	110	380.00
Ave.	7.58	21.30	50.68	68.42	289	156.06	222	499.50

Source: Frenette et al. 1997. Harvest is thousands of pounds, and income is thousands of dollars.

Littleneck clam fishery

The littleneck clam fishery is very small, compared to the total size of the resource. Although littleneck clams seldom have PSP above the 80 µg regulatory limit, three primary factors restrain commercial fishery expansion. These restraints are:

1. The complexity and cost of managing a fishery.
2. Water quality classifying of fisheries areas.
3. The logistics and cost of implementing a PSP testing program.

These problems confine the fishery to a small number of classified beaches where ADEC-required lot sampling for PSP testing can be performed with relative ease. The lot sampling program requires that clams from every harvested lot be tested under the scheme in Table 2.

For reasons previously stated, the southeast Alaska littleneck clam fishery ended in 1996, and was replaced with modest aquatic farming ventures intensively managing natural populations on beaches leased from the state of Alaska. By 1997, three small farms in southeast Alaska harvested 35,014 pounds of littleneck clams worth $67,580.

In Kachemak Bay, environmental variability and overfishing have depressed littleneck clam populations and the current management plan limits the harvest to 30,000 pounds annually. In 1997, Kachemak Bay experienced an unusual episode of PSP which suspended the littleneck clam harvest (M. Ostasz, ADEC, pers. comm.).

Razor clam fishery

The west coast of lower Cook Inlet is the major harvest area for razor clams. The area has no record of PSP problems, and lot testing is required throughout the harvest period. Of concern, however, is a positive domoic acid sample (11.5 ppm) obtained in 1995 from razor clams at Homer, Alaska, on the lower east side of Cook Inlet (D. Barrett, ADEC, pers. comm.). Consequently, the Cook Inlet fishery is being closely watched and only very low levels, <1 ppm, have been recently been recorded (M. Ostasz, ADEC, pers. comm.).

Figure 1. Locations of the major invertebrate fisheries in the state of Alaska.

Geoduck clam fishery

The southeast Alaska geoduck clam dive fishery began in 1989 and continues to operate under emergency order since no fisheries management plan has been developed. Under emergency order, Alaska Department of Fish and Game limits the harvest quota to 2% of the standing stock biomass of surveyed clam beds. The harvest is expected to increase as more stocks are located and surveys conducted. Aquaculture of geoduck clams is currently under investigation.

The meat of geoduck clams does not accumulate PSP toxin. However, the visceral ball can accumulate PSP above regulatory limit. Viscera toxin concentrations vary significantly between individuals harvested from the same location. As an example, during a single harvest day at Gravina Island near Ketchikan, toxin levels from individual clams varied from 41 to 559 µg. In Alaska, unacceptably high PSP concentrations are found year-round, leading to suspicion that toxin-bearing cysts in the sediment are causing the visceral toxicity. PSP in geoduck viscera appears to be regional with the highest levels recorded in the southern fishery near Ketchikan, while Symonds Bay near Sitka seldom exceeds the regulatory limit.

Geoduck clams sell as live or processed product. Processing geoduck removes the visceral ball, and the cleaned meats are separated into neck and steak portions. Divers receive premium price of approximately $7.00/lb. for live clams while income to the diver for clams destined for processing yield only $2.00-4.00/lb. Asian markets use the visceral ball for making soup broth. Because the visceral ball is a specialty product, ADEC tests the visceral ball separately from the meat, and if PSP concentration exceeds 80 µg per 100 g, the geoduck must be processed, and the visceral ball cannot be sold for human consumption.

To test for PSP in harvested geoduck ADEC requires divers to submit three geoduck visceral balls each day in the harvest for testing, and if one sample fails the test, the entire harvest is processed to remove viscera. The Alaska sampling protocol for geoduck viscera differs significantly from the state of Washington, which combines the three visceral

Table 2. Lot sampling requirements for PSP testing commercially harvested and farmed clams.

Lot (harvest) size	No. of samples (Each sample = 150 grams tissue)
≤1,500 clams	2
1,501-6,000 clams	4
≥6,001 clams	6

From ADEC, Uniform shellfish sampling plan for PSP.

samples into a single composite sample before testing. Alaska divers assert that the individual clam testing program required by ADEC unfairly causes greater risk of failing than the Washington state composition sample testing program. For the more southern southeastern Alaska fishery, there is little merit for this argument since 75.4% of the samples are above the regulatory limit; but in the more northern fisheries, like Symonds Bay, only 2.1% of the samples fail (ADEC sampling records 1992-1997).

Excessive visceral PSP prevents the fishery from shipping live geoduck. However, even in areas like Symonds Bay where PSP is not a problem, geoducks are still processed. The decision to process is often based on the shipping cost samples, ranging from $500 to $1,000 per shipment, and on the logistics problems of shipping samples from remote locations. Holding shellfish live and obtaining the results in a timely manner disrupt an often intensive fishery. The economic bottom line is that if the PSP test is negative, the higher price received for live product offsets the cost of testing. However, a harvest lot that fails the PSP test is a loss for the diver. Divers and processors are also reluctant to risk the potential dead loss incurred while holding the clams in refrigerated dry storage pending the PSP test results. The net result is that most of the Alaska geoduck harvest is processed and income is lost to both the diver and the processor (Table 3).

In search of a solution to the visceral PSP problem, the Alaska Science and Technology Foundation funded a project in 1997 to determine if geoduck clams could detoxify when held at a location known to be free of PSP. The specific question addressed by the project was that, since only the viscera contain PSP, could clams purge themselves of PSP during a short (less than 5 week) holding period? The result of the experiment was not encouraging. Although visceral PSP levels dropped 22.5%-55.7% over a four-week holding period, more that 50% of the clams still retained PSP levels above the regulatory limit. Also, the mortality rate from holding clams was unacceptably high at 25.5% (Painter 1998).

To the processor or wholesaler selling geoduck to Asia, PSP in the viscera devalues the product. In the 1998 fishery, if geoducks could have been sold live, the value of the fishery would have been over $1.2 million (Table 3). However, since processing was required gross value of the final product was $507,600, or a loss of $779,175.

The Bering Sea surf clam fishery

The potential problem of PSP and the testing requirements are major factors preventing development of a surf clam (*Spisula polynyma*) in the Bering Sea. A 1977 study, conducted by the NOAA Northwest and Alaska Fisheries Center, found the annual sustained harvest of the Bering Sea surf clams to be 28,773 metric tons (Hughes et al. 1977). Using the ex-vessel price for shellstock of U.S. East Coast surf clams, a developed fishery with this sustained harvest level would be annually worth approximately $8.8 million. Studies on surf clams found PSP to be consistently below the U.S. Food and Drug Administration regulatory limit in populations north of the Aleutian Chain; however, lot sampling is still required to comply with state regulations. An interesting feature of the Aleutian Chain is that shellfish south of the chain often have high toxin concentrations whereas north of the chain are often toxin free.

Crab fisheries

Alaska has four major categories of commercially harvested crabs (Table 4). Within the categories, each species has its own range and fishery management plan.

PSP affects the crab fisheries if viscera toxin concentrations exceed the FDA regulatory limit. All the major species—Dungeness, Tanner, and king crab—are affected. PSP in the viscera results in the crab being sold only as sectioned product by removing the viscera and breaking the crab into two pieces. The profit margin for sectioned crabs is less than for live or whole cooked product. King crab, even if they have no PSP problem, are sold primarily as sectioned product because the fisheries are distant

Table 3. Value of the entire 1998 geoduck clam harvest to the wholesalers as processed or live product.

	Total weight	Meat weight	Income/lb.	Total value
Live (95%)	171,000	171,000	$7.20[a]	$1,286,775
5% processed		9,000		
Steak[b]		1,980	$3.00	$5,940
Neck[c]		1,080	$18.00	$19,440
Processed	180,000			
Steaks		39,600	$3.00	$118,800
Neck		29,600	$18.00	$388,800

Summary of value loss as a result of processed versus live sales

Live geoduck	$1,312,155
Processed[d]	$507,600
Income lost	$779,175

[a]Processed is neck and steak meat combined
[b]22% recovery from live weight
[c]12% recovery from live weight
[d]Price assumes:
60% = 1 grade at $8.70
25% = 2 grade at $7.40
20% = 3 grade at $5.50
5% = Processed

from live holding facilities, and the sectioned market is lucrative and stable. The composition of live and processed sales of the other crab species varies by region.

Tanner and snow crab fishery

Opilio (snow) crab are found in exploitable numbers only in the Bering Sea while bairdi Tanner crab are found from southeast Alaska to the southern Bering Sea (Otto 1982).

The southeast Alaska bairdi crab fishery occurs over an intense two-week period in February, and sold 230,849 pounds of live crab in 1996. Ex-vessel prices are $1.25/lb. for product destined to processing while live product brings a price exceeding $2.50/lb. Live bairdi crabs bring $17-$20.00 prior to shipping and can reach $35.00 per pound on the Asian market (B. Paust, University of Alaska Marine Advisory Program, pers. comm.).

The southeastern Alaska bairdi fishery has not been impacted by visceral PSP, but live marketing from the Bering Sea was attempted in 1992 and discontinued when testing found PSP in the viscera. The 1995 bairdi crab fishery from Kodiak to Bering Sea harvested 4.15 million pounds; however, high visceral PSP allowed marketing of only sectioned crab. The Bering Sea bairdi fishery has been closed since 1996.

The Dungeness crab fishery

The Dungeness crab fishery is located in southeast Alaska and the Kodiak/Aleutian region. The Kodiak/Aleutian fishery harvested 769,729 pounds of Dungeness crab in 1997. Since 1992, visceral PSP in Kodiak area crab has been consistently high and the fishery has not been able to sell whole live or whole cooked crab. Processing live crab into a sectioned product caused a $204,747 loss to the Kodiak/Aleutian crab fishery in 1997.

Unusual occurrences of PSP in the viscera of Dungeness crab disrupted the southeast Alaska Region 2 Dungeness crab fishery in 1992. PSP contaminated viscera from an isolated section of the fishery caused the entire summer fishing season to shift from whole (live or cooked) to sectioned crab with an estimated loss to the fishery of between $411,550 and 507,500. If a PSP closure occurred for the entire southeast, based on the 1997 harvest of 2,330,000 pounds during the summer months (69% of the

Table 4. Species, harvest, and product types of Alaska commercially harvested crabs

Crab category	Species	1995 harvest by species[a]	Product types[b]
King	*Paralithodes camtschatica* (red)	12,074	Live, meat, whole cooked, sectioned
	Paralithodes platypus (blue)	34	
	Lithodes aequispina (golden)	4,070	
Tanner	*Chionoecetes opilio* (opilio or snow crab)	194,200	Live, sectioned, meat
	Chionoecetes bairdi (bairdi or Tanner crab)	4,800	
Hair	*Erimacrus isembeckii*	230,000	Live, whole cooked
Dungeness	*Cancer magister*	3,200	Live, whole cooked, sectioned

[a] Harvest in thousands of pounds.

[b] Whole cooked and sectioned crab is sold fresh or frozen.

Source: ADFG 1999 Statewide harvest statistics.

Table 5. Cost of PSP testing at the ADEC laboratory for 1997.

Industry sector and product	Sample number	Total sample cost for PSP
Aquaculture lot samples	1,012	$126,500
Razor and hard clam fisheries	230	28,750
Geoduck fishery	467	58,375
Bering Sea snail fishery	415	51,875
Crab fishery	444	55,500
Total	2,568	$321,000

Source: D. Barrett, ADEC, pers. comm.)

crab harvest), the total impact could be a loss of between $619,000 and 1,071,000.

Cost of lab testing

ADEC performs mouse bioassay tests on commercially harvested shellfish and aquaculture product at no cost. However, geoduck harvesters and samples from recreational/subsistence harvest areas must pay a $125.00 fee per sample. In 1997, ADEC tested 2,568 samples of shellfish for PSP at a cost of $321,000 (Table 5).

The recreational clam fishery

Despite the threat of PSP, recreational and subsistence fisheries for bivalve shellfish are active throughout Alaska. The official policy of ADEC is that no recreationally harvested shellfish are safe to eat except those harvested from "certified" beaches. Officially, the only certified beaches are located in lower Cook Inlet and Kachemak Bay where commercial fisheries and shellfish aquaculture regularly test shellfish samples. Since ADEC approves the recreational fishing in these areas, harvest and effort data are available. No recreational harvest and effort information is available for the remainder of Alaska.

Kachemak Bay has an intensive recreational fishery on littleneck and butter clams, although ADEC warns harvesters to leave the butter clams on the beach. While PSP warnings are a relatively rare occurrence in Kachemak Bay, one did occur in summer of 1997. The 17 oyster farms in that area suspended sales until the required three weeks of toxin-free samples were achieved. The farms experienced no significant economic hardship because lost

income was recovered from oyster sales after the closure period ended.

Table 6 indicates a significant reduction in the 1997 clam harvest from Kachemak Bay because of the reduced effort days caused by a PSP warning.

Lower Cook Inlet sustains the largest recreational bivalve fishery in the state where over 1 million razor clams are harvested annually (Table 7). To date, the Cook Inlet razor clam fishery has not experienced a PSP episode. If a PSP episode were to occur, the public health and economic impact to the region could be disastrous. As indicated previously, Alaska ADEC is closely monitoring domoic acid levels in Cook Inlet razor clams.

Protecting human health is a concern for the ADEC and Alaska Division of Health and Human Services. Since 1973, 176 incidences of PSP from 66 outbreaks have been documented. An outbreak is defined as two or more people from the same harvest time and location becoming ill. In 1997-1998, reported PSP illness occurred on Kodiak Island, the Aleutian Peninsula, and near Juneau. In 1997, nine cases of illness occurred and one death. Although most PSP illnesses occur during the summer months, the season for toxin conditions appears to be expanding. In the spring of 1999, another death occurred on Kodiak Island, and an illness requiring emergency attention was reported in February 2000. The state Department of Epidemiology, however, estimates that the actual cases of illness may exceed the reported incidences by 10- to 30-fold (Gessner and Schloss 1996).

Alaska Native consumers have a significantly greater risk of a PSP encounter. They are ten times more likely to contract PSP than the average resident of Kodiak (Gessner and Schloss 1996). Although it is a persistent and often serious human health problem, no studies on the human health costs of PSP have been conducted for Alaska.

A clam rehabilitation project is under way in Prince William Sound, initiated by the Chugach Regional Resources Commission with funding from the *Exxon Valdez* Oil Spill Settlement Fund. The project intends to rehabilitate clams for subsistence and development of an aquaculture industry for the villages around the Prince William Sound. The project will have spent over $1 million by the end of 1999 to rehabilitate beaches damaged by the *Exxon Valdez* Oil Spill and to re-establish clam populations in areas where beaches were uplifted during the 1964 Alaska earthquake. Dealing with the potential of PSP will be a major concern to Alaska Native corporations in Prince William Sound and Kodiak Island, requiring development of local PSP monitoring programs to assure safe recreational and subsistence use of shellfish. Alaska Native corporations are also pursuing aquaculture and commercial sales of littleneck clams.

Table 6. Harvest and effort from the recreation hard clam fishery for Kachemak Bay, Alaska.

Year	Participants	Days of effort	Harvest in numbers
1993	7,252	13,534	459,250
1994	8,528	17,318	727,815
1995	7,391	16,693	1,088,560
1006	6,225	13,729	924,055
1997	4,502[a]	1,820	315,755
Average	6,779	12,618	703,087

[a]Number of permits issued and not actual participation.

Table 7. Harvest and effort from the lower Cook Inlet razor clam recreational razor clam fishery.

Year	Participants	Harvest in numbers
1984	29,880	1,044,307
1985	31,195	1,068,340
1986	32,507	1,124,728
1987	25,427	979,020
1988	30,905	1,171,308
1989	22,658	832,155
1990	29,427	950,974
1991	31,899	1,166,787
1992	44,335	1,174,240
1993	31,095	963,054
1994	36,775	1,286,614
1995	31,834	1,180,958
1996	30,810	1,270,868
Average	31,494	1,078,540

AQUACULTURE COSTS

The direct economic impact of PSP for the aquaculture industry is the costs involved to prepare and transport shellfish samples to the ADEC testing lab

in Palmer. Often in remote locations, farmers must send shipments to the ADEC lab by the fastest means available, which often includes express mail or airfreight. The actual cost of the mouse bioassay test is borne by ADEC as part of their regulatory responsibility and at no cost to the farmers. The estimated cost of processing samples and shipping to the state lab ranges between 5% and 8% of harvest value of the entire lot. The cost of testing for Pacific oysters is estimated to be about 5% of the harvest value, and approximately $29,000 in testing services was supplied by ADEC in 1998.

SUMMARY

The cost of PSP to the commercial fishery, recreational harvest, and aquaculture surpasses $10 million annually. Because little information is available, the state of Alaska should conduct a more thorough study to better define the PSP problem in economic terms and use the information to develop solutions to the problem.

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PSP Toxin Concentrations in Alaska

Mike Ostasz
Alaska Department of Environmental Conservation, Anchorage, Alaska

Table 1. Concentration of PSP toxin in Alaska, and number of samples tested, 1992 to 1999 unless otherwise stated.

	No detectable toxin	Detectable toxin	Total tested	Toxin minimum µg/100 g	Toxin maximum µg/100 g	Toxin average µg/100 g	Location of toxin	Notes on toxin maximum
Blue mussels	583	153	726	32	20,606	1,900	Kalsin Bay, Kodiak area	05/27/94 epidemiology
Butter clams	202	159	361	32	8,532	399	Sturgeon River, Kodiak area	06/09/97 epidemiology
Cockles	36	31	67	31	737	84	Chignik Beach, Aleutian/Bering area	08/22/98 survey sample
					2,252[b]		Bridget Cove, Juneau area	07/86
Horse clams	86	10	96	35	342	81	Mud Bay, Aleutian/Bering area	07/20/98 survey sample
Littlenecks	2,719	149	2,868	32	1,374	70	Sturgeon River, Kodiak area	06/09/97 epidemiology
Oysters	4,825	417	5,242	30	535	56	Peterson Bay site, Seldovia area	08/19/97
					1,755[b]		Minterbrook Oysters, Washington	10/88
Razor clams	416	36	452	33	3,294	793	Humpback Bay, Aleutian/Bering area	04/05/95 epidemiology
					1,334		Bridget Cove, Juneau area	07/86
Red necks	38	1	39		37		Middle Bay, Kodiak area	Epidemiology
Scallops[a]	54	3	57	242	806	431	Akhiok site, Kodiak area	08/12/92 survey
					11,945[b]		Alaska Peninsula, Kodiak area	07/87
Softshell clams	6	1	7		37		Gravina West, Ketchikan	
					47[b]		Bare Island, Kodiak area	07/87
Surf clams	49	10	59	32	816	273	Mud Bay, Aleutian/Bering area	07/07/98 survey
Geoduck viscera	417	1,097	1,514	31	1,088	94	Gravina West, Ketchikan area	11/19/88 attempted live shipment lot sample
					1,526[b]		Grant Cove, Ketchikan area	03/89
Geoduck tissue	906	33	939	31	49	35		
					61			1991
					86[b]			< 1991

[a] Some of the scallop samples were viscera only, and some were whole animal.

[b] Highest level in this species ever detected by DEC.

Alaska Field Trials for MIST™ Diagnostic Kits for Detecting Paralytic Shellfish Poisoning

Joanne Jellett
Jellett Biotek Ltd., Dartmouth, Nova Scotia, Canada

EXECUTIVE SUMMARY

The purpose of this trial was to demonstrate the effectiveness of the MIST™ diagnostic test kits for detecting paralytic shellfish poisoning in field situations, regional testing labs, and at the Alaska Department of Environmental Conservation (DEC) regulatory lab.

Field-collected shellfish samples were split, with half of the sample going to the DEC regulatory lab in Palmer and the other half of the tissue homogenate used to test for PSP at the field site. Participating sites included the Sheldon Jackson College, Sitka (regional test site), the University of Alaska Fairbanks Fishery Industrial Technology Center, Kodiak (regional test site), Bristol Bay Health Corporation, Dillingham (field site), Elfin Cove Oysters (field site), and the Ketchikan General Hospital (regional test site). All trial participants received a two-day training session in the AOAC (Association of Official Analytical Chemists) extraction method for extracting toxins from shellfish tissues and in the use of the MIST™ kits. Field and regional participants performed the yes/no qualitative Mini-MIST™ kits, except for the Kodiak site which did both fully quantitative and Mini-MIST™ tests. The DEC regulatory lab performed both the fully quantitative MIST™ kits, some Mini-MIST™ tests, and the standard AOAC regulatory mouse bioassay as a control. Data was generated with a total 867 tests using a variety of shellfish species.

The fully quantitative MIST™ Quanti kits detected toxicity in 99% of the tests where the mouse bioassay detected toxicity. Overall agreement of the MIST™ Quanti to the mouse bioassay was 82%, which increased to 88% if natural (±20%) variability in the mouse bioassay and MIST™ technology is taken into consideration. After improvements were made to our kit packaging during the trial, the agreement between the MIST™ Quanti and the mouse bioassay increased to 91% if the variability is taken into consideration.

The overall agreement of the Mini-MIST™ (yes/no) kits with the mouse bioassay was 85%, although this increased to 90% if the poorest data from Bristol Bay is excluded.

The Mini-MIST™ kits used in the field and at regional testing sites had less favorable results because of a "matrix effect" in the shellfish tissue extract and due to kit damage in shipping to remote sites. To overcome the "matrix effect," which was something in the sample extract that destroyed the cells in the Mini-MIST™ tests kits, thereby giving erroneous results, the kit was redesigned to allow for additional dilution of the sample. The resulting new test kit, known as MIST™ Screen, dilutes out the matrix effect and provides three ranges of toxicity indication, rather than only yes/no.

Improvements to kit packaging that were implemented during the trial substantially improved the performance of later kit shipments and reduced the false positive and negative tests to 6.8% and 2.7% respectively.

Although not part of the original project, HPLC (high performance liquid chromatography) was used as a third technology to test some of the false positive and false negative samples as well as some that agreed. Two unusual peaks occurred in the false positive samples, which could not be further identified at this time. The HPLC also elucidated three false negative mouse bioassay results among the 25 samples tested by HPLC.

From data gathered from the DEC lab we demonstrated that the use of the MIST™ technology for prescreening for PSP could reduce state testing costs by approximately 28%.

Although all field participants with access to samples were able to perform the MIST™ tests successfully, some found the MIST™ kits somewhat complex to use in a field environment. Tempera-

ture sensitivity, limited shelf life, the requirement to do eight samples at once, and the need to pipette reagents and incubate the test plates detracted from using the tests as a harvest management tool at aquaculture sites. These detractions were not as serious at regional testing sites or in the DEC lab where higher volumes of testing and less primitive working conditions would be expected.

The ability to test at the field level was considered important by most participants from both economic and product safety perspectives. Jellett Biotek is already in the process of developing a second generation of qualitative (yes/no) immunochromatography field tests, similar to commercially available home pregnancy tests. These will be inexpensive, single-use tests, which will require only a drop of shellfish extract on the membrane and within minutes provide a color indication of positive test results. Jellett Biotek plans to submit a proposal to ASTF to help fund and validate these innovative tests for field use.

Research goals and benchmarks

The goals of the project were:

1. To conduct parallel trials with the Maritime In Vitro Shellfish Test (MIST™) test kits and mouse bioassay to demonstrate that the MIST™ kits are an accurate, cost effective method for screening for paralytic shellfish poisoning (PSP).
2. To demonstrate the utility of screening for PSP with MIST™ technology as a harvest management tool at the aquaculture site or regional testing centers and as a beach monitoring tool.
3. To develop a MIST™ technology transfer/commercialization strategy for Alaska.

Research goals met

Trial partners were selected to ensure the MIST™ kits were used in a variety of applications. The Department of Environmental Conservation Lab (DEC) in Palmer demonstrated the kits in a regulatory food safety application by performing the fully quantitative MIST™ kits in parallel with the mouse bioassay. The DEC lab also did some Mini-MIST™ tests. The Ketchikan General Hospital (Ketchikan), University of Alaska Fairbanks Fishery Industrial Technology Center (Kodiak), and Sheldon Jackson College (Sitka) used the Mini-MIST™ qualitative kits as a screen for PSP, and demonstrated the potential of the kits in a regional PSP screening application. The Bristol Bay Health Corporation (Dillingham) demonstrated the kits as a beach monitoring tool, and Elfin Cove Oysters, Elfin Cove, demonstrated the kits as a harvest management tool for the aquaculture industry.

The Fishery Industrial Technology Center (Kodiak) also performed some fully quantitative MIST™ kits to demonstrate regional testing for PSP.

The Alaska trial sites are as follows:

Site 1. Alaska Department of Environmental Conservation, Palmer

Site 2. Sheldon Jackson College, Sitka

Site 3. University of Alaska Fishery Industrial Technology Center, Kodiak

Site 4. Bristol Bay Health Corporation, Dillingham

Site 5. Elfin Cove Oysters, Elfin Cove

Site 6. Ketchikan General Hospital, Ketchikan

A training session of all Alaskan participants was held by Jellett Biotek staff at the DEC lab in Palmer, where the AOAC PSP toxin extraction method and fully quantitative MIST™ and Mini-MIST™ kits were demonstrated and practiced by all participants.

During the trial, shellfish samples were collected by trial participants, and the tissue was homogenized and then split, with half the sample being sent to the DEC lab for a regulatory (Association of Official Analytical Chemists) toxin extraction, a fully quantitative MIST™ test, and a mouse bioassay (MBA). The DEC lab staff also performed a number of Mini-MIST™ kits. An acid extraction was performed by the trial participants on the other half of the sample, and the MIST™ assay was performed. The DEC lab also tested shellfish samples submitted under the ongoing, normal regulatory program using the fully quantitative MIST™ kits with the mouse bioassay as control. The trial ran from June 1998 to January 1999.

Technical report

This technical report provides the details of the trial and its findings.

As stated earlier JBL shipped 1,372 tests to our six trial partners, consisting of fully quantitative MIST™ Quanti tests, Mini-MIST™ (yes/no kits), and later a few MIST™ Screen tests. This resulted

Table 1. Overall agreement of MIST™ bioassay with mouse bioassays.

Type of MIST™ Tests	number	±20% Error	%Agreement	%Disagreement
All Quanti	602	No	82	18
	602	Yes	88	12
Original Packaging	445	No	81	19
	445	Yes	87	13
Improved Packaging	157	No	86	14
	157	Yes	91	9
All Quanti + Mini	732	No	85	15
Excluding Site 4	732	Yes	90	10

in 558 Quanti test results, 250 Mini-MIST™ results, and 44 Quanti tests conducted in-house by JBL.

Summary of results

Table 1 provides a summary of the agreement between the MIST™ Quanti data and the MBA. In the overall trial (558 + 44 = 602 tests), the Quanti agreed with the MBA 82% of the time. If we factor in the ±20% error for the two technologies, the agreement rate rises to 88%. After our improved packaging was implemented, our agreement rate increased to 86%, and factoring in the ±20% variation, the overall agreement increased to 91%.

Distribution of shellfish samples tested

An objective of the trial was to demonstrate the efficacy of the MIST™ technology with a broad range of shellfish species. Figure 1 shows the distribution of tissue types among the samples tested. A total of 372 shellfish samples were tested by the various methods used in this trial. The majority of tissue types tested during the trial were oysters or butter clams, followed by blue mussels, littleneck clams, horse mussels, and snails. No particular tissue type gave different results for all tissues analyzed together with respect to variability in results, agreement level with the mouse bioassay, or matrix interference in the MIST™ tests.

Toxicity ranges during the trial

The trial exposed the MIST™ technology to a wide range of toxicity. Figure 2 shows the distribution of MBA and MIST™ Quanti results into a number of toxicity ranges. It can be seen that the distribution of detection is fairly even for both the MBA and MIST™ kits. These ranges were selected as: <80 µg per 100 grams is below the regulatory limit, 80-150 is the area where people are unlikely to become sick, 150-400 µg is a range that can be dangerous to those particularly sensitive to PSP, and >400 µg is generally considered a dangerous level for most people.

Trial results

Figure 3a and b demonstrate the agreement between the MBA and MIST™ technology. An agreement of over 80% was achieved in the 0-80 µg per 100 gram range, and 40% agreement within the 80-400 range, followed by 61.4% agreement in the 400+ range. The agreement improves when the ±20% variability is factored into the results. All categories improved agreement significantly; however, the most important is around the regulatory limit, where agreement with the mouse increased to 75.4%.

We did experience some differences in agreement level with the MIST™ Quanti and the MBA, depending on site. Both the DEC lab and FITC (Site 3) in Kodiak performed the MIST™ Quanti tests, and JBL also did 44 samples in-house from shellfish extracts shipped from the DEC lab. Figure 4 shows the agreement by site and includes separate graphs including the ±20% error. It is interesting to note that the JBL samples (performed under the best conditions by the most experienced staff) agreed 100% with the MBA at the regulatory limit and 400 µg+ range, and the ±20% error factor significantly improved the agreement with the MBA.

False positive and false negative samples

Figure 5 shows the distribution of false positive or false negative samples. A false positive occurred when the MIST™ technology detected toxicity above the regulatory limit while the MBA indicated it was below the regulatory limit. A false negative occurs when the MIST™ technology indicated that the toxicity was below the regulatory limit when the mouse indicated the toxicity was above the regulatory limit. Obviously from a regulatory and human health perspective, the false negative is a much more serious situation than a false positive.

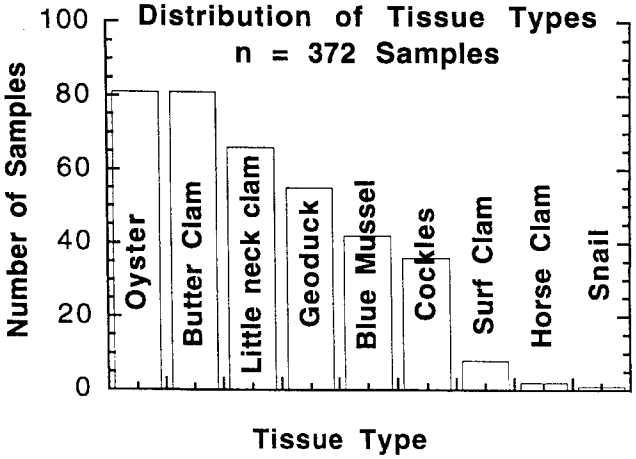

Figure 1. Distribution of tissue types among shellfish samples tested. Note that many of the samples were tested more than once by different participants.

In 602 tests, the MIST™ Quanti test produced 34 false negative samples (given ±20%), or 5.58%. We demonstrated later that the MBA also produced false negatives.

Tables 2 and 3 provide the samples that registered as false negative and false positive respectively. In addition the tables show the lower number of false negative and false positive tests once the ±20% variation is considered. The number of false positives was 60 (10%), which reduced to 18 (3%) once the variation factor was included.

Table 4 contains the six cases (1%) where the MBA detected toxins but the MIST™ technology failed to identify any toxin in the sample. It should be noted that the highest amount of toxicity in any of these "missed" samples was 201 µg per 100 grams. It is unlikely that this amount of toxicity would pose any health risk.

Mini-MIST™ qualitative (yes/no) data

The Mini-MIST™ achieved good agreement with the mouse bioassay, although the outcome of the trial varied from site to site (Fig. 6), and in general was not as good as agreement of the MIST™ Quanti with the mouse bioassay results. Site 1 (DEC lab) performed the highest number of tests with Mini-MIST™, and achieved good agreement with the mouse bioassay in lower ranges (0-80, 80-400 µg per 100 g), but less agreement with the higher toxicity samples (Fig. 6a). Good agreement in all ranges was seen in data from Sites 3, 5, and 6 (Figs. 6c, e, f). However, very poor agreement was observed in data from Site 4 (Bristol Bay). We have compared the results obtained by Site 4 with the same samples tested in the MIST™ Quanti at Site 1 (DEC lab). We had a great deal of difficulty with shipments to this location, with quite a number spending greater than 24 h holding in unheated conditions. This resulted in a loss of many plates that were sent to this site and could not be used. We attribute the disagreement among tests performed at Site 4 to some unusual features of the samples taken from that site, as well as the more difficult shipping logistics which posed a greater risk of cold exposure and damage to the plates than any of the other sites. The poor agreement at higher toxicity levels at Site 1, and the unusual features of the shellfish samples tested from Site 4 and their impact on the efficacy of the Mini-MIST™ will be discussed in the later section entitled "Mitigation of problems encountered."

HPLC analyses

Although this was not part of the trial, we were concerned with the false positive and negative values that were obtained using the MIST™ technology, and decided to run some of these samples on a third technology, HPLC, to confirm the results. Unlike the mouse and MIST™ bioassays that give results of total toxicity, the HPLC analysis gives the amount of each of the toxin analogs present in each sample, or a "profile" of the toxin analogues.

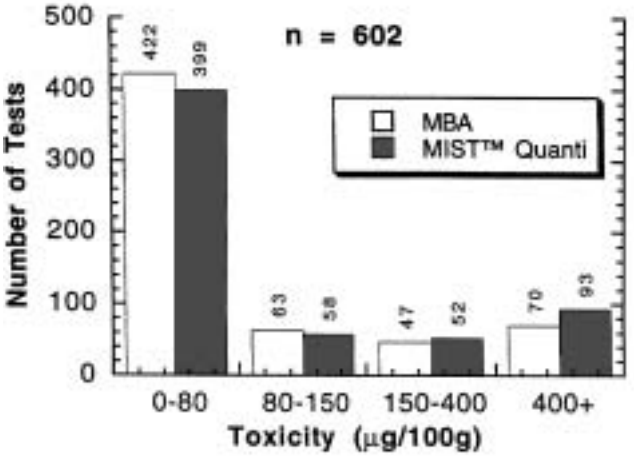

Figure 2. Distribution of mouse bioassay (MBA) and MIST™ Quanti results into four toxicity ranges of 0-80, 80-150, 150-400 and >400 µg per 100 g. The number above each bar represents the number of tests which gave results in that range for that technology.

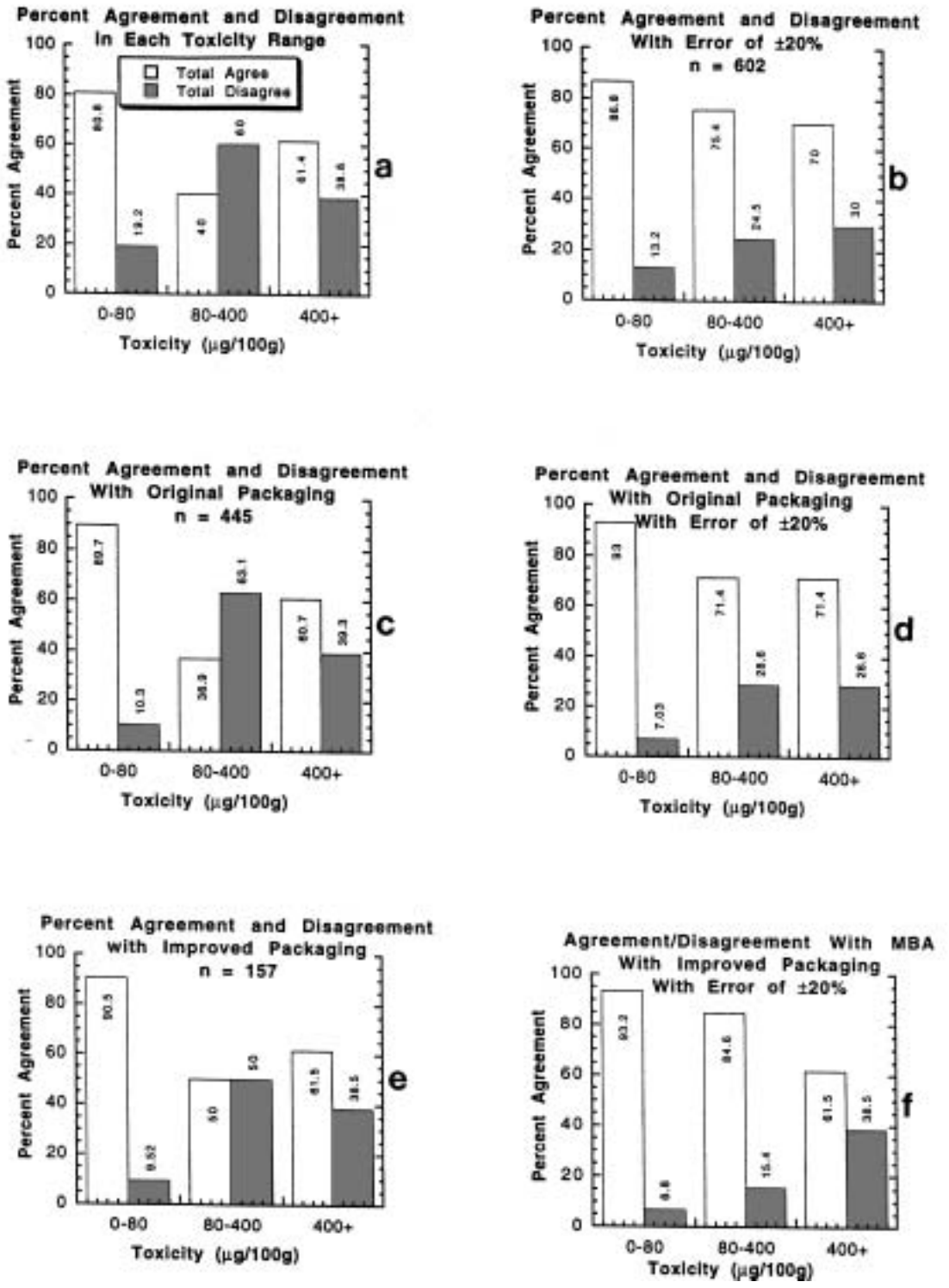

Figure 3. Percentages of agreement and disagreement with the DEC mouse bioassay in three ranges of toxicity for all MIST™ Quanti tests done during the Alaska trial. This includes 489 tests done at DEC, 56 at Site 3, and 44 at Jellett Biotek. Figures 4a, c, and e are derived using direct comparison of each MIST™ Quanti test result with the corresponding mouse bioassay result for the same test. Figures 3b, d, and f are derived similarly but after ascribing an error of ±20% to each technology. Figures 3a and b show the total MIST™ Quanti results. Figures 3c and d show the results from 445 tests performed at all locations with the original packaging. Figures 3e and f show the results from 157 tests that were done after the packaging was upgraded for better thermal protection.

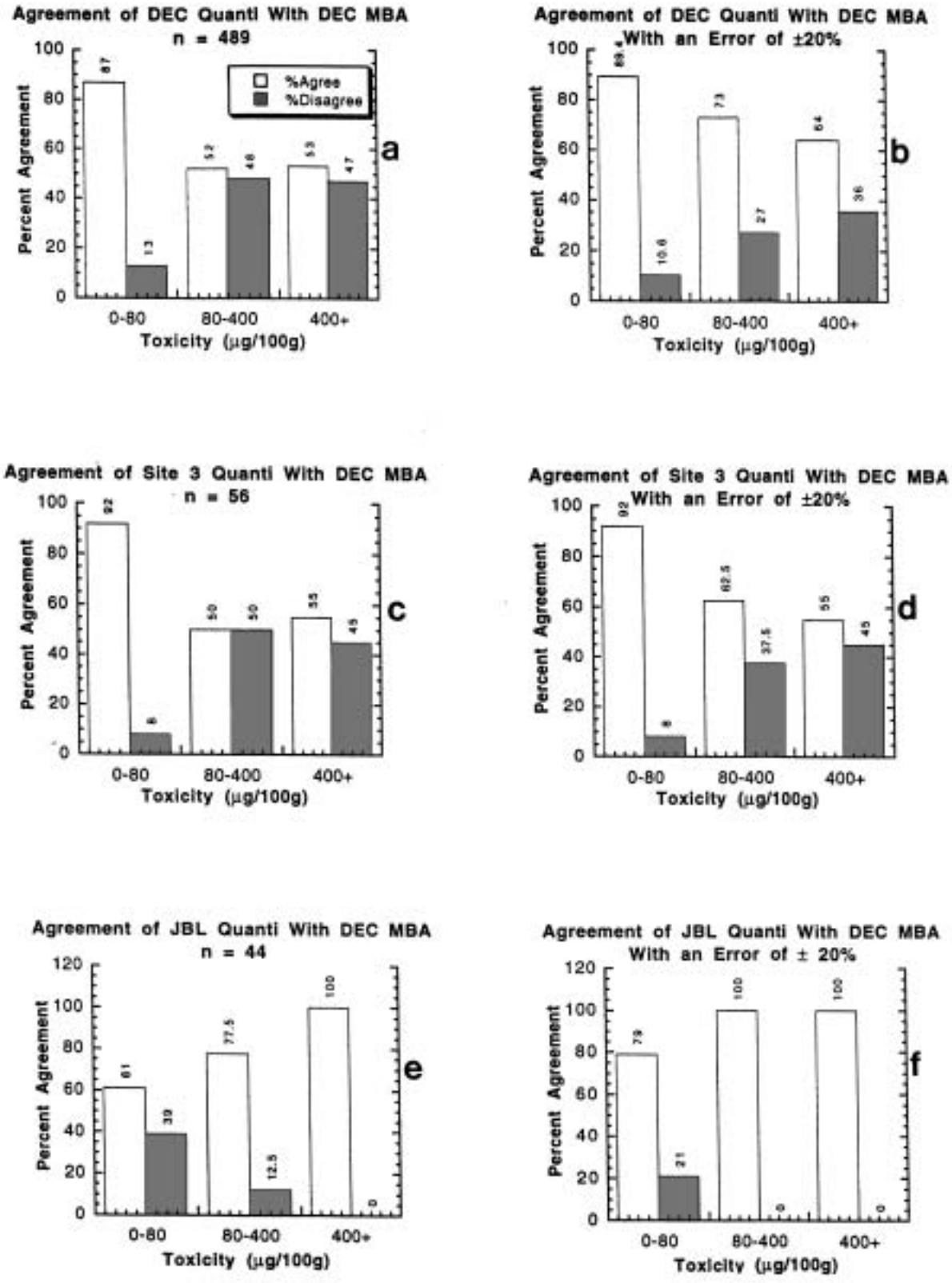

Figure 4. Percentages of agreement and disagreement with the DEC mouse bioassay in three ranges of toxicity for all MIST™ Quanti tests done during the Alaska trial. They are separated into three sets of MIST™ Quanti data which includes 489 tests done at DEC, 56 at Site 3, and 44 at Jellett Biotek. Figures 3a, c, and e are derived using direct comparison of each MIST™ Quanti test result with the corresponding mouse bioassay result for the same test. Figures 4b, d, and f are derived similarly but after ascribing an error of ±20% to each technology.

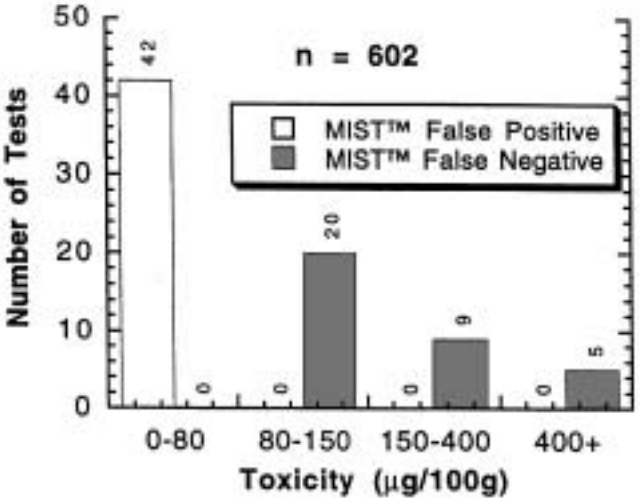

Figure 5. Distribution of 602 MIST™ Quanti and mouse bioassay test results into four ranges of toxicity: 0-80, 80-150, 150-400, and >400 µg per 100 g.

The toxicity is then inferred by multiplying the amount of each analogue by the relative toxicity of that analog as previously determined in the mouse bioassay. The HPLC analysis would allow us to determine if there were particular or unusual profiles responsible for the discrepancies between the mouse and MIST™ bioassays. The HPLC analyses were performed at the National Research Council of Canada by our employee using their equipment and methodology (the Oshima method).

We used the HPLC to verify that the field extracts being made by the trial partners was similar to the AOAC extract being made in the DEC lab. The two extracts are almost identical, and therefore the field extracts should be valid.

We obtained some interesting findings through the HPLC analyses. We were able to determine that there is no specific toxin profile or analogue associated with false positive or false negative samples. The profiles were surprisingly diverse and were not specific to any site. These are among the first in-depth profiles that have been performed on shellfish from Alaska waters. Sample 04-0021 (littleneck clam) had a very unusual profile and also caused the highest recorded false negative with the MIST™ technology.

Comparisons of the toxicity values obtained by the three methods are shown in Table 5. In most cases the HPLC corroborated the mouse bioassay results, but in three cases, samples 05-0007, 01-0050, and

Table 2. MIST™ Quanti false negatives.

Sample #	Tissue Type	DEC MBA	MIST™ Quanti
01 0489	B	98	ND
03 0009	B	108	ND
03 0046	B	201	ND
03 0056	LN	87	ND
04 0092	LN	160	ND
06 0010	Gv	86	ND
04 0085	SC	143	6
04 0093	LN	184	8
04 0084	RC	1610	11
03 0011	B	206	14
04 0041	Ck	110	18
04 0094	B	659	18
01 0088	O	127	20
03 0002	BM	132	20
04 0086	SC	120	24
03 0383	Gv	130	26
03 0047	B	125	27
04 0081	SC	100	29
03 0047	Bc	125	34
04 0082	SC	123	37
03 0046	B	201	41
01 0475	Gv	112	42
04 0077	LN	799	43
01 0028	B	133	45
04 0087	RC	1414	46
04 0021	LN	749	47
03 0003	BM	227	48
04 0083	SC	162	48
01 0318	B	101	61
03 0023	BM	116	61
04 0029	LN	218	61
01 0224	O	116	63
04 0013	Ck	131	65
01 0017	GV	123	68.5
Not False Negatives with +/- 20 % Error			
04 0015	Ck	86	18
01 0048	O	92	25
04 0002	RC	88	33.1
01 0087	O	98	41
01 0051	O	87	44.7
03 0080	B	92	46
04 0040	Ck	80	48
01 0084	O	83	52
01 0017	GV	123	55
03 0004	B	347	67
01 0262	O	87	67
01 0424	LN	108	68
01 0032	O	151	74
04 0041	Ck	110	76
01 0473	B	150	76

Table 3. MIST™ Quanti false positives.

Sample #	Tissue Type	DEC MBA	MIST™ Quanti
04 0064	Ck	42	105
04 0068	Ck	33	107
01 0001	B	43	113
05 0001	O	36	117
01 0476	Gv	66	120
06 0017	Gv?	50	122
01 0105	O	ND	123
04 0060	Ck	31	128
03 0025	LN	ND	129
04 0049	Ck	65	133
05 0005	O	47	137
01 0080	O	ND	139
05 0007	O	63	139
01 0023	O	41	140
01 0490	LN	ND	144
04 0088	Ck	ND	146
05 0005	O	47	148
03 0034	BM	55	150
04 0058	Ck	ND	160
05 0026	O	33	168
01 0049	O	63	173
04 0062	Ck	33	178
01 0445	Gt	ND	188
05 0006	O	39	264
05 0007	O	63	264
01 0116	O	ND	405
03 0052	B	ND	482
01 0099	B	63	506
01 0461	O	ND	516
01 0454	LN	ND	552
01 0460	O	ND	618
05 0038	O	ND	>115
04 0010	B	33	>118
01 0109	O	ND	>137
04 0012	B	48	>141
04 0012	B	48	>150
01 0365	O	32	>158
06 0005	Gv?	38	>187
01 0456	O	ND	>200
01 0089	O	ND	>236
01 0115	O	ND	>1124
04 0039	Ck	60	>1484
Not False Positive with +/- 20 % Error			
05 0028	O	ND	81
05 0001	O	36	84
05 0006	O	39	85
01 0221	O	57	>87
01 0223	O	71	>90
01 0077	O	ND	>90
01 0001	B	43	92
03 0038	BM	33	93
01 0239	BM	ND	94
01 0109	O	ND	95
04 0056	Ck	36	95
01 0478	Gv	ND	98
01 0016	GV	67	101
04 0048	Ck	75	>113
01 0050	O	78	121
01 0097	B	77	140
01 0050	O	78	193
01 0016	GV	67	210

01-0080, the HPLC corroborated the MIST™ suggesting that the mouse bioassay may not always be "correct" either. These were two cases identified in which the MIST™ and HPLC both indicated that the samples were toxic above the closure limit, while the mouse bioassay gave results less than the closure limit, clearly mouse false negatives. In two cases the mouse result was very close to the closure limit (78 and 63 µg per 100 g), while the MIST™ and HPLC indicated the result to be around 97 and 133 µg per 100 g (average of two repeats). In the second case where the mouse did not detect any toxicity, the HPLC gave a result of 492 µg per 100 g while the MIST™ result was 100 µg per 100 g (average of two repeats). Table 5 also shows there were three MBA false negatives and three MIST™ false negatives.

Mitigation of problems encountered

It was mentioned earlier that difficulties were encountered in shipping the MIST™ test kits to our trial partners. Kit shipments were stopped for about three weeks to investigate the problem, which we found was chilling of the cells in the kits during transit. The packaging was modified to improve the thermal protection of the kits with the inclusion of phase change gels designed to keep the kits at 20 to 25ºC, and the addition of a thicker polystyrene shipping container which offered more insulating value.

Figure 7 shows the effect of the improved packaging compared to the original packaging on the performance of the MIST™ Quanti kits. Figures 4c and d demonstrate the agreement with the mouse bioassay with in old packaging and Figs. 4e and f clearly show agreement in all toxicity levels with the improved packaging.

Figure 7 further shows the reduction in false negatives from 5.7% to 2.7% when the new packaging was incorporated. No change in the incidence of false positives was detected; it remained around 7%. Figure 8 illustrates the levels of agreement with the MBA achieved after the implementation of the improved packaging.

Conclusions

- The MIST™ Quanti and Mini-MIST™ were successfully performed at the regulatory laboratory (Site 1) and at all regional testing sites and field sites that were able to obtain samples.

Table 4. Cases where MBA detected toxin and MIST™ detected no toxin.

Cases Where MBA Detected Toxin and MIST™ Detected No Toxins	
Sample No.	MBA µg/100g
06-0010	86
03-0056	87
01-0489	98
03-0035	139
04-0092	160
03-0046	201

- The MIST™ Quanti detected toxicity 99% of the time that the mouse detected toxicity, showing its effectiveness at screening for PSP.
- The MIST™ Quanti results agreed quantitatively with the mouse bioassay greater than 90% of the time, after the packaging was upgraded to withstand greater exposure to cold.
- The MIST™ Quanti had a false negative rate of 2.7% and a false positive rate of 6.7% after improvements to packaging.
- The MIST™ Quanti was equally effective at testing all tissue types encountered in the trial.
- Using HPLC analysis as a third, corroborative technology, the chemical analysis agreed with the mouse bioassay in most cases, but there were three samples where the mouse did not detect any toxicity while both the MIST™ Quanti and the HPLC detected PSP toxins. In one case, the toxicity level was high enough to cause illness. The HPLC also detected toxicity in one sample where none was detected by either the MIST™ or mouse bioassays, and in another sample the HPLC was unable to detect toxicity found by the bioassays. This indicates that none of the three methods, the mouse bioassay, the MIST™ bioassay, nor the HPLC, are perfect methods for PSP detection all of the time.
- Some unidentified peaks were found by HPLC in the false positive samples and not in others, which may be related to our higher rate of false positives than the mouse.
- The mouse bioassay proved superior in some aspects (e.g., the false positive rate) but this may reflect the many decades of optimization of the animal test compared to the first large scale application of the MIST™ technology.
- Field extractions were found to be as effective as those performed at the regulatory laboratory.
- The Mini-MIST™ performed well at some sites but not at others. Poor performance was mainly due to shipping logistics to the more remote field locations (greater risk of cold exposure to the temperature-sensitive plates), as well as a "matrix effect" (a component of some shellfish tissue) which caused false negative results and was much more pronounced at Site 4 (Bristol Bay) than other sites. We have produced an improved version of the qualitative test called MIST™ Screen, which gives an indication of toxicity in three ranges and which completely mitigates the matrix effect problem encountered in the Mini-MIST™ bioassays.
- An upgrade of the packaging used to ship the MIST™ kits designed to withstand greater exposure to cold greatly improved our ability to deliver plates successfully to the sites, as well as improving the performance of the MIST™ bioassays, i.e., agreement with the mouse bioassay increased and incidence of false negatives decreased significantly. Only a few plates of the new MIST™ Screen were evaluated in the trial, but they performed well.
- Limitations in the application of the MIST™ screening technology to remote field sites have stimulated us to begin the development of a rapid diagnostic test for PSP which would be an inexpensive, rugged, simple, single-use device that would perhaps be used without an extraction procedure and which would produce results in minutes. This device would not be temperature sensitive and could be stored at room temperature for a year or more, eliminating the problems associated with providing testing technology to remote sites for beach monitoring or other field applications.

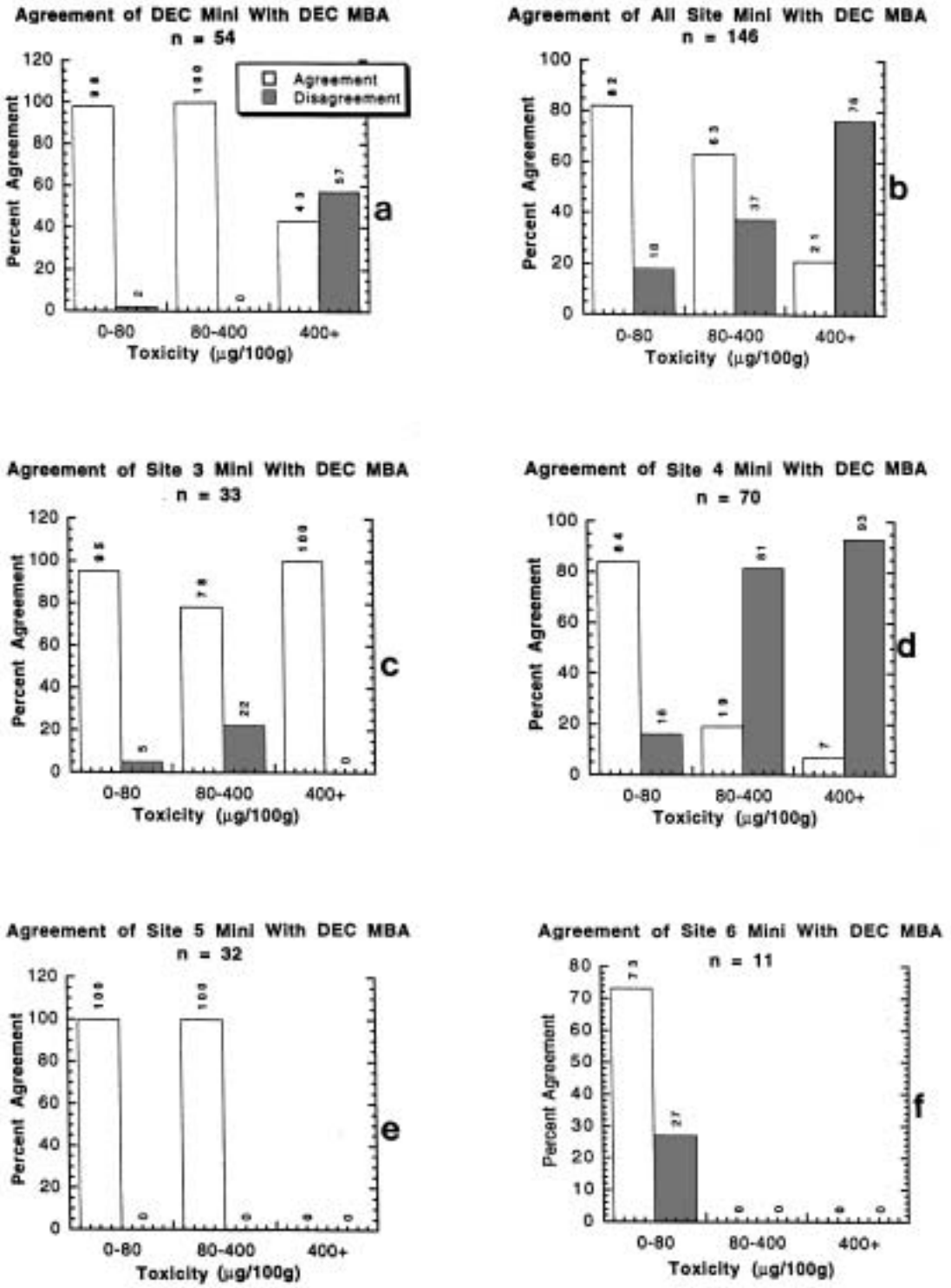

Figure 6. Percentages of agreement and disagreement with the DEC mouse bioassay in three ranges of toxicity for all Mini-MIST™ tests done during the Alaska trial. Since the Mini-MIST™ are qualitative, the error of ±20% was not taken into account.

Table 5. Comparison of MBA, MIST™ Quanti, and HPLC.

Sample #	T.T.	DEC MBA	MIST™ Quanti	NRC HPLC	MIST™ POS/NEG	MBA POS/NEG
01 0077	O	ND	56.2	ND		
01 0079	O	ND	30	ND		
05 0004	O	ND	63.05	17		
01 0023	O	41	121	17	(+)	
05 0001	O	36	100	39	(+)	
05 0006	O	39	174.5	46	(+)	
05 0005	O	47	142.6	47	(+)	
01 0001	B	43	113	58	(+)	
01 0016	GV	67	101	62	(+)	
01 0041	O	ND	ND	64		
01 0017	GV	123	55	91	(-)	
05 0007	O	63	201.7	97	(+)	(-)
01 0050	O	78	156.9	133		(-)
01 0051	O	87	130.4	135		
01 0028	B	133	228.5	140		
01 0068	O	187	137.5	160		
04 0002	RC	88	63.6	165	(-)	
01 0080	O	ND	100.5	492		(-)
04 0023	SC	816	800.5	969		
04 0020	B	1103	657.2	1401		
04 0069	RC	1596	>210	1765		
04 0070	RC	1322	>300	1894		
04 0021	LN	749	47	1919	(-)	
04 0071	RC	1327	161	1946		
04 0019	B	1936	2810	2462		
04 0018	B	2142	3304	2841		

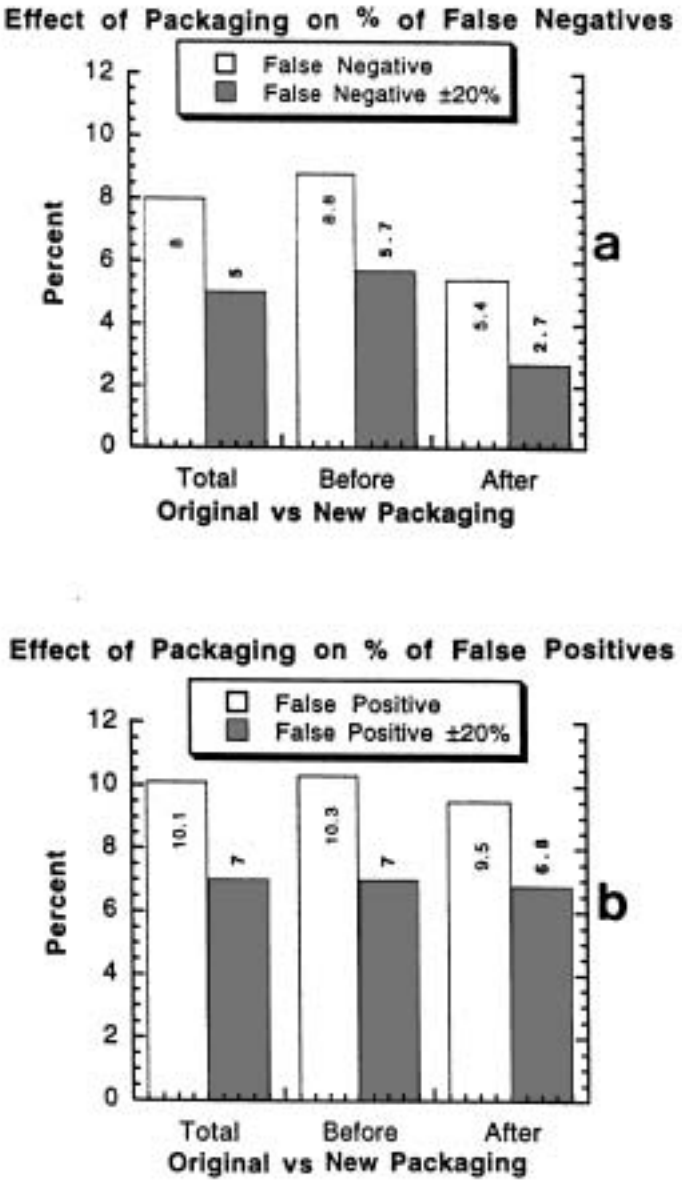

Figure 7. The effect of improvements in packaging on the percentage of false positives and false negatives recorded by the MIST™ Quanti tests. The light bars represent a direct quantitative comparison with the mouse bioassay result for the same sample. The dark bars show reduced incidence of both false negatives and false positives after taking ±20% error for each of the technologies into account. Data is given for the total number of false positive or false negatives in all 602 tests performed (Total), as well as for 445 tests performed with the original packaging (Before) compared to the 157 tests performed after the packaging was improved (After). Figure 7a shows the effect of improved packaging on the percentage of false negatives recorded. Figure 7b shows the effect of improved packaging on the percentage of false positives recorded.

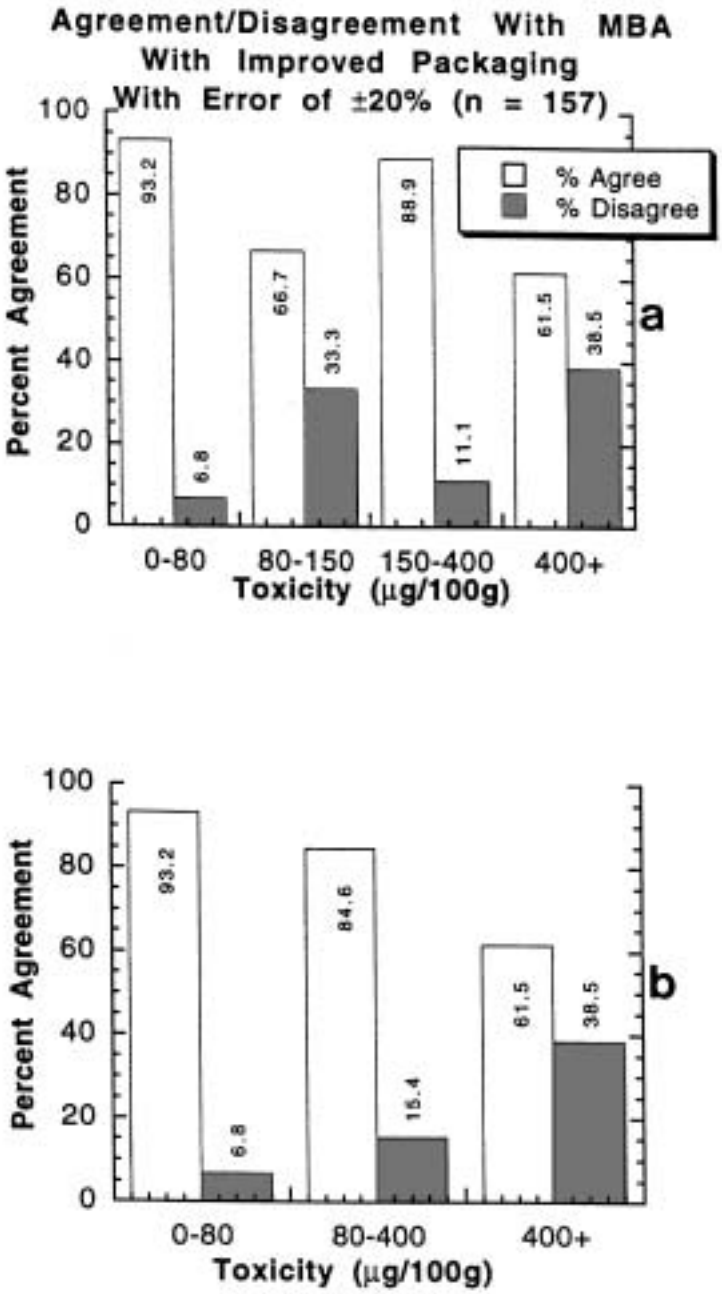

Figure 8. The percentage of agreement between the MIST™ Quanti and the mouse bioassay results after the improvement in packaging of MIST™ kits. This analysis was performed on 157 samples that were tested on plates sent in the new packaging after resumption of the trial. Figure 8a shows the agreement in each of four ranges of toxicity: 0-80, 80-150, 150-400, and >400 µg per 100 g. Figure 8b is similar but the results are distributed over only three ranges, 0-80, 80-400, and >400 µg per 100 g.

Acknowledgments

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Appendix A

Dear Ray [Ray Roberts, Jellett Biotek, Dartmouth, Nova Scotia, Canada]

. . . The mini-mist test was very easy to do on my own. The results were absolutely clear-cut. The negative control as well as the negative sample I obtained were completely clear. There wasn't even the slightest hint of color in any of the negatives. The positive control wells were a uniformly dark purple color and the positive samples were all colored. The degree of coloration in the positive samples matched the level of PSP in the control samples.

—Dr. Joseph Marcello, Sheldon Jackson College, Sitka, Alaska

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