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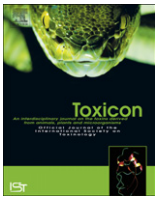

Paralytic shellfish poisoning: Seafood safety and human health perspectives

Stacey M. Etheridge*

U.S. FDA, Center for Food Safety and Applied Nutrition, Office of Regulatory Science, Division of Analytical Chemistry, 5100 Paint Branch Parkway, HFS-707, College Park, MD 20740, USA

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ABSTRACT

Paralytic shellfish poisoning (PSP) is the foodborne illness associated with the consumption of seafood products contaminated with the neurotoxins known collectively as saxitoxins (STXs). This family of neurotoxins binds to voltage-gated sodium channels, thereby attenuating action potentials by preventing the passage of sodium ions across the membrane. Symptoms include tingling, numbness, headaches, weakness and difficulty breathing. Medical treatment is to provide respiratory support, without which the prognosis can be fatal. To protect human health, seafood harvesting bans are in effect when toxins exceed a safe action level (typically 80 μg STX eq 100 g^{-1} tissue). Though worldwide fatalities have occurred, successful management and monitoring programs have minimized PSP cases and associated deaths. Much is known about the toxin sources, primarily certain dinoflagellate species, and there is extensive information on toxin transfer to traditional vectors – filter-feeding molluscan bivalves. Non-traditional vectors, such as puffer fish and lobster, may also pose a risk. Rapid and reliable detection methods are critical for toxin monitoring in a wide range of matrices, and these methods must be appropriately validated for regulatory purposes. This paper highlights PSP seafood safety concerns, documented human cases, applied detection methods as well as monitoring and management strategies for preventing PSP-contaminated seafood products from entering the food supply.

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1. Paralytic shellfish poisoning toxins and sources

Paralytic shellfish poisoning (PSP) is a common seafood toxicity problem with worldwide distribution, and typically this illness is due to the consumption of contaminated molluscan bivalves and other shellfish. A similar seafood-related syndrome involves puffer fish contaminated with the same family of toxins. To distinguish these puffer fish poisonings from those caused by tetrodotoxin, this food poisoning syndrome is becoming known in the literature as saxitoxin puffer fish poisoning (SPFP; Landsberg et al., 2006; Deeds et al., 2008a). The toxins responsible for both of these seafood-borne illnesses are the neurotoxins known collectively as the saxitoxins (STXs), also referred to as PSP toxins (or PSTs). At least 24 saxitoxin-like congeners have been identified (Fig. 1), with a range of hydroxyl, carbamyl, and sulfate moieties at four sites on the backbone structure. These substitutions result in congeners varying more than three orders of magnitude in potency (Oshima et al., 1993). The carbamate toxins are the most potent, and they include saxitoxin (STX), neosaxitoxin (NEO), and the gonyautoxins (GTX1–4). The decarbamoyl toxins (dcSTX, dcNEO, dcGTX1–4) have intermediate toxicity and are reported in certain bivalves, but are not commonly found in toxic dinoflagellates. The N-sulfocarbamoyl toxins (B1 [GTX5], B2 [GTX6] and C1–4) are less potent. There is a fourth group known as the deoxydecarbamoyl toxins, but their potency has not yet

* Tel.: +1 301 436 1470; fax: +1 301 436 2624.
E-mail address: Stacey.etheridge@fda.hhs.gov

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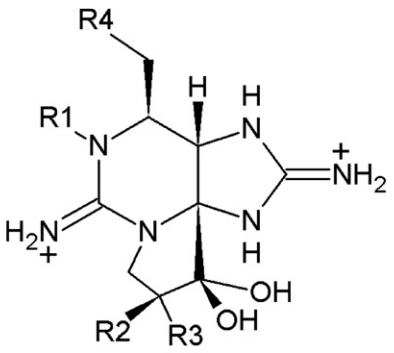

R1	R2	R3	Carbamate Toxins	Decarbamoyl Toxins	N-Sulfocarbamoyl Toxins	Hydroxybenzoate Toxins
H	H	H	STX	dc-STX	B1	GC3
OH	H	H	NEO	dc-NEO	B2	
OH	H	OSO_3^-	GTX 1	dc-GTX 1	C3	
H	H	OSO_3^-	GTX 2	dc-GTX 2	C1	GC1
H	OSO_3^-	H	GTX 3	dc-GTX 3	C2	GC2
OH	OSO_3^-	H	GTX 4	dc-GTX 4	C4	

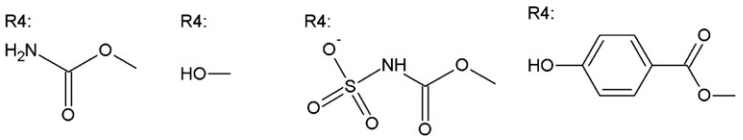

Fig. 1. Molecular structure of saxitoxin congeners.

been adequately assessed. A review of the chemistry, origin and distribution of saxitoxins can be found in Hall et al. (1990). PSP-related toxins known as GC toxins have also been specifically identified from the dinoflagellate *Gymnodinium catenatum*, and the molecular structure of GC1–GC3 was found to contain a hydroxybenzoate moiety instead of the carbamoyl group (Negri et al., 2003, 2007). The binding affinity of GC3 was reported to be similar to the GTXs, and GC1 and 2 epimers were similar to the C toxins (Llewellyn et al., 2004). It has been proposed that the hydroxybenzoate moiety on this particular group of toxins may result in an increased lipophilic nature that could enhance uptake and elimination in victims and shellfish (Llewellyn et al., 2004).

There is a substantial knowledge base on the sources of PSP toxins. Major toxin sources include certain species of microalgae, notably marine dinoflagellates of the genera *Alexandrium* (formerly *Gonyaulax*), *Gymnodinium* and *Pyrodinium* (see reviews by Cembella, 1998; Landsberg, 2002; FAO, 2004 and references therein). More specifically, *Alexandrium tamarense*, *Alexandrium fundyense*, *Alexandrium catenella*, *G. catenatum*, and *Pyrodinium bahamense* are responsible for most reports of PSP (Shumway, 1990, 1995). There are also numerous reports of STXs being produced by certain freshwater and brackish cyanobacteria, as well as calcareous red macroalgae (see review by Deeds et al., 2008a and references therein). These species include *Anabaena circinalis*, *Anabaena lemmermannii*, *Aphanizomenon gracile*, *Aphanizomenon issatschenkoi*, *Cylindrospermopsis raciborskii*, *Lyngbya wollei*, *Planktothrix* sp., and *Rivularia* sp. Regardless of which specific source, each species contains a suite of the toxin congeners, and both the composition and concentration of which determine its overall toxicity. An extensive list of source species can be found in Deeds et al. (2008a), along with their reported toxin profiles. It is noteworthy that toxin composition and concentration for given species have been found to vary with geographic region and environmental factors (e.g. Cembella et al., 1988; Anderson et al., 1990, 1994; Etheridge and Roesler, 2005).

The GC toxins described above are produced by strains of *G. catenatum* (Negri et al., 2007). Negri et al. (2007) reported that these toxins are produced in strains isolated from Australia, China, Portugal, Uruguay and Spain which demonstrate the globally widespread nature of the GC toxins. The discovery of these toxins highlights the need for shellfish monitoring programs to include them in surveillance in areas where *G. catenatum* serves as the toxin source. However, more research is needed on this toxin

group to fully understand the overall threat they pose to shellfish consumers in particular regions.

Bacterial origins of STXs have also been proposed (e.g. Doucette et al., 1998), and while these theories remain to be proven, there are reports that bacteria may at least play a role in dinoflagellate production of STXs (e.g. Doucette et al., 1998; Kodama et al., 1990a,b, 2006; Silva, 1990). A study by Baker et al. (2003), however, found that the fluorescent compounds isolated from two bacterial strains associated with toxic *Alexandrium* spp. cultures thought to be toxins were actually naturally occurring fluorescent compounds that co-eluted with GTX4 during high-performance liquid chromatography (HPLC) analysis, a finding that further challenged the theory of a bacterial origin.

Our understanding of the sources of PSP toxins and the nature of their production continues to expand. Such *a priori* information aids in determining the threat of PSP in specific locations, which allows more appropriate monitoring and management strategies to be implemented to protect public health.

2. Paralytic shellfish poisoning vectors

While PSP toxins are transferred through the food web and accumulate in many organisms ranging from zooplankton (Doucette et al., 2005) to whales (Geraci et al., 1989), this review focuses on vectors that are known to be consumed by humans that may pose a public health risk. The traditional pathway for PSP toxins in the food web involves the ingestion and concentration of toxic dinoflagellates by filter-feeding mollusks, in particular bivalves (Shumway, 1990). There are numerous reviews available describing traditional vectors (Shumway, 1990; Shumway et al., 1990; Shumway and Cembella, 1993; Bricelj and Shumway, 1998; Landsberg, 2002), including their uptake, accumulation, metabolism and depuration.

Explanations for sporadic paralytic shellfish poisonings were first provided by Sommer and Meyer (1937) and Sommer et al. (1937); both studies reported the occurrence of toxic *Gonyaulax catenella* near mussel beds off the coast of California, USA that were associated with outbreaks. Saxitoxin was first isolated from butter clams, *Saxidomus giganteus*, harvested from the west coast of the US (Schantz et al., 1957; Schantz, 1960). Since that time PSP toxins have been found in a range of molluscan bivalves that are consumed by humans. A brief review is provided by Deeds et al. (2008a) and a summary of uptake and elimination can be found by FAO (2004). An extensive list of invertebrate species with potential natural toxin hazards such as STXs can be found in the US Food and Drug Administration's Fish and Fisheries Products Hazards and Controls Guidance (http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/Seafood/FishandFisheriesProductsHazardsandControlsGuide/default.htm [accessed 13 October 2009]).

Toxin profiles in these molluscan bivalves may differ from the toxin source profiles due to conversion during digestion (e.g. Bricelj et al., 1991). In particular, the less potent, labile toxin congeners (N-sulfocarbamoyl toxins) may be biotransformed into the more potent carbamate forms, thus enhancing the potential toxicity to humans. Other studies report biotransformations that involve removing sulfate groups to form STX (Schantz et al., 1957) and carbamate side chains to form dc-toxins (Sullivan et al., 1983). Mollusks may also selectively retain certain congeners (e.g. Okumura et al., 1994; Cembella et al., 1993) and for prologed periods. The organism most well known for its ability to retain toxins is the butter clam (*S. giganteus*), a species considered permanently toxic in parts of the Pacific Northwest and Alaska (Quayle, 1969). For more information regarding metabolism in seafood, refer to the recent review by Kodama and Sato (2008).

Shellfish bed harvesting closures for PSP are based solely on toxin levels in the seafood (see more information below), unlike closures, for example, for neurotoxic shellfish poisoning (NSP). The dinoflagellate (*Karenia brevis*) responsible for the toxins associated with NSP has been found to be an adequate predictor of shellfish toxicity such that closures, at least in the US for this seafood-borne illness, can be made based on shellfish toxicity or *K. brevis* concentrations. Specifically, in the National Shellfish Sanitation Program's Guide for the Control of Molluscan Shellfish it states that shellfish harvesting is banned when toxins (brevetoxins) are in excess of 80 μg 100 g^{-1} or when *K. brevis* concentrations are greater than 5000 cells L^{-1} (http://www.fda.gov/Food/FoodSafety/Product-SpecificInformation/Seafood/FederalStatePrograms/NationalShellfishSanitationProgram/ucm046988.htm [accessed 13 October 2009]). Unfortunately, distributions and concentrations of the toxic algae responsible for PSP do not currently support closures based upon algal concentrations. A better understanding of the coupling between surface toxic algal blooms and subsequent shellfish toxicity is needed to assess the feasibility of using algae as predictors for closures and this is currently the focus of an NOAA-funded project through the ECOHAB (Ecology and Oceanography of Harmful Algal Blooms) program (http://www.whoi.edu/sbl/liteSite.do?litesiteid=13193 [accessed 9 January 2009]). Given the complexity of the link between *Alexandrium* spp. and shellfish toxicity, it will be unlikely that algal concentrations alone could be used to determine closures for PSP in the way that they are for NSP. Challenges include the nature of the algae, the toxin profiles of the algae, toxin transfer through the food web, potentials for biotransformation and varying degrees of depuration among shellfish species.

Although filter-feeding molluscs are the usual vectors for these toxins with mussels and clams being dominant, there are increasing reports of non-traditional vectors (Shumway, 1995). These non-filter feeding organisms include marine gastropods (carnivorous and grazing), crustaceans, and certain fish (see review by Deeds et al., 2008a and references therein).

Certain molluscan gastropods that feed by scavenging, predation and grazing are also known to accumulate PSP toxins through the food web (Shumway, 1995). Whelk, moon snails, and abalone are among those that have been studied to understand whether they pose a threat to humans. Gastropods tend to depurate slowly, remaining toxic for extended periods of time (Shumway, 1995; Deeds et al., 2008a). A thorough account of gastropod vectors can be found in the review by Deeds et al. (2008a).

There have been several documented cases of PSP toxins in Crustaceans (e.g. Shumway, 1995; Negri and Llewellyn, 1998), and a new report by Linares et al. (2009) demonstrated that shrimp may also serve as a vector for PSP toxins to humans. The most notable crustacean vectors from a seafood safety perspective are crabs (Kotaki et al., 1983; Gessner and Middaugh, 1995; Oikawa et al., 2004; Deeds et al., 2008a and references therein) and lobsters (Watson-Wright et al., 1991; Lawrence et al., 1994; Cembella and Desbiens, 1994; Deeds et al., 2008a and references therein). A review of crabs as PSP vectors was covered thoroughly in Deeds et al. (2008a). Lobsters, such as the American lobster *Homarus americanus*, are also known to be vectors of PSP toxins but have not received as much attention in the literature. Higher PSP levels have been consistently found in the hepatopancreas (also known as lobster tomalley) tissues (Watson-Wright et al., 1991; Cembella and Desbiens, 1994), with low levels detectable in the tail meat and gills (Sephton et al., 2007). Lobster hepatopancreas toxicity became such a concern that in 1990 the Canadian Department of Fisheries and Oceans extended its existing PSP monitoring program to include lobster (Lawrence et al., 1994). Cembella and Desbiens (1994) reported that a Canadian public health advisory was issued to warn against eating more than two lobster hepatopancreas per day after PSP levels were found to be in excess of 1500 μg STX eq 100 g^{-1}. In July 2008, hepatopancreas PSP levels in lobster harvested off the coast of Maine, USA were sufficiently high that advisories were made by the State of Maine Department of Marine Resources (http://www.maine.gov/dmr/rm/public_health/shellfishadvisory.htm [accessed 9 January 2009]) and the US Food and Drug Administration (http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2008/ucm116927.htm [accessed 13 October 2009]). These notices advised consumers to not eat tomalley from American lobster, regardless of where the lobster was collected.

Given that lobsters are primarily scavengers, they could be acquiring the toxins from varied sources. It has been suggested that toxic sea scallops, such as *Placopecten magellanicus* off the coast of Canada, serve as the source (Cembella and Desbiens, 1994), and it has been demonstrated in the laboratory that PSP toxins can be transferred from the scallop *Chlamys nobilis* to the spiny lobster *Panulirus stimpsoni* (Jiang et al., 2006). Due to PSP risks associated with scallop gonads and viscera, it is commonly a required practice in certain regions such as off the northeastern US coast to harvest the adductor muscle only and discard the viscera and gonads overboard. It has been recently hypothesized that this practice may play a role in the subsequent enhanced toxicity observed in lobster hepatopancreas. However, the timing and overlap of scallop and lobster harvesting do not fully support the idea that discarded scallop tissues could be solely responsible for lobster toxicity. Lobster harvesting occurs throughout Maine coastal waters, with highest concentrations around Penobscot Bay in central Maine, and in this region lobsters have been found to have an extremely diverse diet (Steneck and Wilson, 2001; Grabowski and Gaudette, 2008). Lobster hepatopancreas toxicity has been found along the coast with highest concentrations in areas such as off Jonesport and Cutler (D. Couture, pers. comm.). Scallop harvesting, however, is localized with approximately 90% coming from Cobscook Bay near the Canadian border (Schick and Feindel, 2005). In addition to inconsistencies with respect to spatial distributions, temporal differences exist as well, with scallop landings generally being limited to winter months. While it is possible that scallops may play a role in lobster toxicity, it is likely that there are multiple sources involved. Further, laboratory studies by Desbiens and Cembella (1997) demonstrated that lobster hepatopancreas toxicity depurated slowly, suggesting that PSP risks from this vector may persist awhile after the toxic algal bloom and initial uptake in lobster. Based on the complex nature of lobster toxin accumulation and apparent slow depuration, the best management strategy at this point appears to be issuing advisories warning the public about consuming the hepatopancreas until a greater understanding has been determined.

PSP toxins also accumulate in zooplanktivorous fish such as Atlantic salmon, *Salmo salar* (Sephton et al., 2007), herring, *Clupea harengus harengus* (White, 1981), and mackerel, *Scomber scombrus* (Castonguay et al., 1997). Toxins generally do not accumulate in the muscle thereby minimizing the risk to human consumers; however, those who consume whole fish are at greater risk of exposure. Reports of toxin transfer to finfish (including overall toxicity and toxin profiles) and associated human illnesses in some of the cases are summarized by Deeds et al. (2008a).

As stated earlier, saxitoxins can also accumulate in certain puffer fish (Family Tetraodontidae). Most puffer fish poisoning events are attributed to tetrodotoxin (TTX), a toxin that differs structurally from STX but also binds to the same site of the voltage-gated sodium channels and yields similar pharmacological responses (Narahashi et al., 1967; Kao, 1986; Hille, 1992; see below for more information). Saxitoxin was first reported in *Takifugu pardalis* livers from Japan (Kodama et al., 1983), and subsequently STX was confirmed as a component in other puffer fish *Takifugu poecilonotus* and *Takifugu vermicularis* (Nakamura et al., 1984), as well as *Arothron firmamentum* (Nakashima et al., 2004) from Japan. Additionally, STX-containing freshwater puffer fish were found in several locations such as the Philippines, Bangladesh, Brazil and Cambodia (see review by Deeds et al., 2008a and references therein). In 2002 the first confirmed STX-related puffer fish poisoning event occurred in the US (Bodager, 2002; Quilliam et al., 2004; Landsberg et al., 2006). Originally, the events in 2002 were thought to be caused by TTX; however, liquid chromatography–mass spectrometry analyses identified STXs as the contaminants rather than TTX (Quilliam et al., 2004). From 2002 to 2004 there were 28 confirmed cases of PFP caused by STXs in the US (Landsberg et al., 2006; Etheridge et al., 2006). It was determined that the STXs first originated in the dinoflagellate *P. bahamense* and was then transferred through the food web via small molluscan bivalves to puffer fish, particularly from the east coast of Florida, near the Indian River Lagoon, resulting in a risk to human consumers (Quilliam et al., 2004; Landsberg et al., 2006). Whereas TTX is commonly observed in the liver of puffer fish, STX can also be elevated in the muscle (e.g.

Deeds et al., 2008b). *Takifugu* is generally considered safe for human consumption if the puffer fish has been prepared such that the liver is successfully separated; however, when STX is found in puffer fish, it is generally located in the muscle, which is the portion typically consumed. Given this risk, the Florida Fish and Wildlife Conservation Commission placed a ban on commercial and recreational harvesting of puffer fish from waters along 5 counties in Florida (http://myfwc.com/marine/regulation.htm [accessed 29 December 2008]) and a monitoring program has also been established (Abbott et al., 2009) for the management and monitoring of this public health threat.

In addition to the freshwater puffer fish described above, there are also reports of STXs in other freshwater vectors. For example, Pereira et al. (2004) demonstrated toxin uptake by the freshwater mussel *Anodonta cygnea* when fed the toxic cyanobacterium *A. issatschenkoi*. Negri and Jones (1995) also described toxin uptake by the Australian freshwater mussel *Alathyria condola* when exposed to the toxic cyanobacterium *A. circinalis*. Although these studies show that freshwater bivalves can be a vector for STXs, they are not commonly harvested for human consumption. The potential exception in the latter case is that freshwater mussels may serve as a portion of the diet for aboriginal Australians. More recently it has been demonstrated that STXs can accumulate in tilapia raised in freshwater aquaculture (Galvão et al., 2009). The authors of this study reported significant depuration in their 5-day experiments and suggested that depuration with clean water could lead to elimination so that levels are safe for consumption. Another potentially emerging risk in Mexico is the freshwater snail, *Pomacea patula catemacensis*, known as "tegogolos" (Berry and Lind, 2010). The recent discovery of PSP toxins in this species suggests that this organism should be studied further to understand the threat it may pose to human health given its widespread local consumption and its significance as a major fishing product from the State of Veracruz.

There are other means by which STXs can pose a human health risk which cannot be ignored. The threat of drinking water as a source of toxins to humans has been considered (Falconer, 1993; Westrick, 2008). A study investigating cyanobacterial toxins in two Australian drinking water treatment plants found as much as 17 $\mu g\ L^{-1}$ PSP toxins in the plant's raw water source (Hoeger et al., 2004); however, following water treatment only traces ($<1\ \mu g\ L^{-1}$) were detected in the final product and tap water. Another example of drinking water posing a threat to public health was found by Molica et al. (2005), who reported the presence of STX in a Brazilian water supply. A more recent investigation of PSP toxins in a Brazilian reservoir (Clemente et al., 2009) found PSP toxicity to be 5.15, 43.84, and 50.78 ng STX eq L^{-1} in the spring, summer and autumn. Toxins, detected by mouse bioassay, were found to be present (1.36 MU mg^{-1} dry weight) in a drinking water and fish farming source in Lake Dianchi in southwestern China (Liu et al., 2006), further demonstrating the potential human health risks of STXs in freshwater resources. Despite observations of toxins in numerous water resources, no PSP-related human illnesses have been reported due to the consumption of drinking water; however, Negri et al. (1995) described fatalities in sheep that ingested water contaminated with PSP-producing *A. circinalis*. A review by Smith (2008) emphasized the global increase in aquaculture practices and suggested that this shift warrants concern about potential public health risks due to cyanobacterial toxins. It has also been suggested that dietary supplements made from potentially hazardous cyanobacteria may also be a source of STXs to humans (Carmichael and Falconer, 1993). Lastly, human fatalities occurred as a result of using a cyanobacterial toxin-contaminated (in this case microcystins) municipal water supply for dialysis (e.g. Yuan et al., 2006), raising the concern of this potential route of exposure as well. For more information on cyanobacterial sources of toxins and their relationship to human health, refer to WHO (1999).

3. Human illnesses and fatalities

Voltage-gated sodium channels are the molecular targets of these neurotoxins. These channels have a common structural motif and consist of one principal α subunit of 220–260 kDa and one or two auxiliary β subunits of 33–36 kDa (Catterall, 1984, 2000; Catterall et al., 2007). A number of neurotoxins are known to bind to receptor sites of sodium channels, effecting ion permeation or voltage-dependent gating. In the case with STXs, the functional effect is blocking sodium conductance (Hille, 1968, 1975; Ritchie and Rogart, 1977). The STXs bind to site 1 of the α subunit (Catterall, 1986), though it was reported that binding requires both the α and β_1 subunits (Catteral, 1989). Amino acid residues forming receptor site 1 are located in the pore loop and are believed to form the ion selectivity filter (Noda et al., 1989; Terlau et al., 1991). It has been demonstrated that mutations in the ion selectivity filter in the softshell clam *Mya arenaria* can result due to selective pressure from the presence of STXs in the natural environment (Bricelj et al., 2005). Such selective mutations could be responsible for enhanced toxin accumulation in vectors, increasing the risks of these toxins. For additional information on STX blocking in sodium channels, see the recent review by Wang (2008).

Symptoms resulting from these toxins binding to voltage-gated sodium channels include tingling sensation of the lips, mouth and tongue, numbness of extremities, paresthesias, weakness, ataxia, floating/dissociative feeling, nausea, shortness of breath, dizziness, vomiting, headache, dysphagia, and dysarthria (e.g. Halsetead, 1978; Kao, 1993 and references therein; Gessner et al., 1997). Gessner et al. also reported diastolic and systolic hypertension in almost all patients. Symptoms can generally occur within 30 min of consuming contaminated seafood, although Mons et al. (1998) indicated that symptoms can even ensue within a few minutes if high enough toxin concentrations are present in the food. Death has been reported as soon as 3–4 h following consumption (García et al., 2004). Medical treatment consists of providing respiratory support, and fluid therapy can be used to facilitate toxin excretion. For patients surviving 24 h, with or without respiratory support, the prognosis is considered good (Schantz, 1969; FAO, 2004). In fatal cases, death is due to asphyxiation.

The actual levels of toxicity that have resulted in illnesses and fatalities are highly variable, and appear to be mainly dependent on individual differences in sensitivity, with observable differences in mortality rates between children and adults (see FAO, 2004 and references therein). For example, the Australia New Zealand Food Authority documented that a concentration as low as 120 μg STX eq per person elucidated symptoms in humans, whereas values ranging from 400 to 10,000 μg STX eq per person had been found responsible for deaths (ANZFA, 2001).

The number of locations reporting intoxications rose by 27 from 1970 to 1990 (Hallegraeff, 1993) and greater than 20 countries have proposed regulatory limits for these toxins (van Egmond et al., 1992). A global review of PSP events can be found in FAO (2004), with PSP reports throughout Europe, Africa, North America, Central and South America, Asia and Oceania. Numerous fatalities have been reported worldwide (Shumway et al., 1990). For example, PSP-related fatalities have occurred in South Africa (IPCS, 1984), Canada (IPCS, 1984), Chile (Lagos, 1998; FAO, 2004), Guatemala (Rodrigue et al., 1990) and Mexico (IPCS, 1984; Orellana-Cepeda et al., 1998). Despite the numerous reports of events and fatalities, successful monitoring programs have been implemented in many countries and have minimized health risks and reduced illnesses and fatalities.

Even though there have been outbreaks, illnesses and fatalities, there is still a fairly limited knowledge base on PSP toxin metabolism in humans. Information on toxins in human urine and serum was obtained following four PSP outbreaks on Kodiak Island, Alaska (Gessner et al., 1997). Detectable levels (nM) were found in both urine and serum, though urine was the clinical matrix with clearly higher toxin loads. Upon comparing toxin profiles in the mussels consumed by the patients with those in the urine and serum, human metabolism of PSP toxins was supported. Metabolism was further indicated based on results from a post-mortem analysis of samples from a victim that consumed contaminated crab, *Zosimus aeneus* (Llewellyn et al., 2002). Toxins were detected in the adult male's gut contents, blood, urine and liver. Toxin profiles in the crab, victim's gut content and urine demonstrated the process of conversion from GTX2/3 and STX in the crab to NEO, dcSTX and STX in the urine. García et al. (2004) also described post-mortem toxin composition and concentration in human samples, this time in tissues and body fluids after the fatal consumption of mussels (*Aulacomya ater*) with concentrations reaching 8575 μg STX eq 100 g^{-1}. Toxins were found in the gastric content, urine, bile, cerebrospinal fluid, liver, kidney, lung, stomach, spleen, heart, brain, adrenal glands, pancreas, and thyroid glands. Toxin profiles differed among tissues/fluids and the oxidation of STX to NEO and GTX2/3 to GTX1/4 was apparent. The authors concluded that toxin metabolism does occur in humans following ingestion, and excretion occurs through urine and feces. In a later publication (García et al., 2009), the authors provided evidence of enzymatic biotransformation of GTX2/3 when incubated with healthy human liver microsomes. They suggested that metabolism occurs via a glucuronidation reaction, an important detoxification pathway in humans that involves the metabolic conversion of xenobiotics to water-soluble metabolites that can be easily removed from the body through urine and/or bile.

These limited, but important, clinical samples have provided snap shots of information about the transfer of toxins from seafood vectors to humans indicating metabolism and biotransformation. Time series of toxin data for clinical samples have been mostly lacking however, leaving the details about toxin excretion elusive. In 2007 four individuals became ill after eating mussels collected from a floating barrel off the coast of Maine. Upon arrival at the hospital, time series samples were collected of the patients' urine and serum for toxin analysis (Etheridge et al., unpublished data) and provided confirmation of the earlier reports regarding the metabolism and excretion of toxins in humans.

4. Action levels and regulatory perspectives

To protect public health, an action level or regulatory limit (the specific terms used tend to vary based on the legal ramifications of each country) is defined and it is unlawful to harvest seafood when toxins exceed the established limit. A review of regulatory limits for a range of countries can be found in FAO (2004). Typically it is defined as 80 μg STX equivalents (eq) 100 g^{-1} tissue, which is the case for the action level in the US. Action levels for marine toxins in the US can be found in the Food and Drug Administration's Fish and Fisheries Products Hazards and Controls Guidance (http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/Seafood/ucm091782.htm [accessed 29 December 2008]). The origin of an action level for STXs was described by Wekell et al. (2004). Briefly, it was established based on detection of PSPs using mouse bioassay (MBA). Sommer and Meyer (1937) defined a mouse unit as the amount of toxin that killed a 20 g mouse within 10–20 min (using an extract of 100 g sample tissue boiled in HCl). Based on extractions this was 200 MU 100 g^{-1} shellfish. Using a conversion factor of 0.2 μg STX eq equal to 1 MU, the limit of detection (LOD) was defined as 40 μg STX eq 100 g^{-1}. The limit of 80 was set as a compromise between the MBA LOD and the minimal toxicity of 200 μg STX eq 100 g^{-1} reported at that time for causing illness. To date this action level has been effective. Modern illnesses, at least in the US, result primarily from recreational harvest and subsistence fishing from closed or untested waters.

A recent summary, however, by the European Food Safety Authority (EFSA) states the opinion that, based on the established acute reference doses, the current EU regulatory limit for STX-group toxins was not sufficiently protective (EFSA, 2009). They suggest a level of 75 μg STX eq kg^{-1} (7.5 μg STX eq 100 g^{-1}) as opposed to the current 800 μg STX eq kg^{-1} (80 μg STX eq 100 g^{-1}). If the proposed revision to the EU regulatory limit for STXs takes effect, there will be a need for a detection method with a sensitivity that allows for the rapid determination of product exceeding that established toxin limit (the MBA LOD will not allow this method to be used). While the Lawrence HPLC method can detect such lower concentrations, it has not been fully validated at concentrations much lower than the existing EU regulatory limit. The EFSA

opinion further suggested a change from 100 g to 400 g for what is considered a realistic portion size of shellfish.

5. Toxin detection methods

Given the recent reviews on PSP toxin detection methods (Ben-Gigirey and Villar-González, 2008), this summary is not exhaustive. Rather, this review highlights PSP detection methods that currently are used (or demonstrate advanced promise for use) for seafood safety and human health purposes. There is a need for detection options that span the range from screening and field methods to validated laboratory confirmatory tests.

The mouse bioassay is considered the official regulatory method in most countries for determining PSP toxins in shellfish (AOAC, 2000), meaning that this is the method most often required for making determinations regarding opening and closing harvest areas for seafood. The MBA is an NSSP (National Shellfish Sanitation Program) approved method which allows for its implementation as a regulatory method in the US. Despite its widespread use and recognition as the gold standard, the many disadvantages of the MBA include: poor specificity with low sensitivity, low sample throughput, fairly high cost, labor-intensive, high variability, and animal usage. The observed variability is mostly due to salt effects which lead to an underestimation of toxicity as high salt content of samples is known to interfere with the MBA, suppressing toxic effects (Schantz et al., 1958). Over estimations of toxicity are plausible as well. For example, zinc accumulation in oysters has resulted in lethal effects in mice at insignificant toxin levels (Aune et al., 1998). Given the numerous limitations of the MBA and, in particular, the ethical concerns of using animals, there are great efforts to establish alternative methods for PSP toxin detection. The European Commission has gone as far as publishing directives to establish other methods for official PSP control. The European Animal Protection Legislation (Council Directive 86/609/EEC) was developed to encourage movement away from animal usage towards other validated methods.

There are numerous assays currently being used (or evaluated for use) for seafood safety and human health purposes. A single laboratory validation of the receptor binding assay (RBA) was successful (Van Dolah et al., in press), and an AOAC collaborative study is currently in progress. The RBA is highly specific, exploiting the interaction of the toxins with the native receptor site (i.e. voltage-gated sodium channels). This functional based assay allows toxins to bind to the receptors according to their affinity, yielding an integrated toxic potency. The RBA was implemented for detecting PSP toxins by Davio and Fontelo (1984) and Vieytes et al. (1993). Both of these studies demonstrated the possibility of using the RBA to detect PSP toxins in matrices of interest such as human plasma and mussel extracts, respectively. Later the assay was further improved by Doucette et al. (1997) who transformed it into a microtiter plate-based assay which increased sample throughput and efficiency. Results showed a close agreement between toxicity determined by RBA compared to the MBA. The method was further improved by Powell and Doucette (1999) by using microplate scintillation technology which resulted in decreasing the assay time to 4 h. This study also demonstrated the utility of the RBA for a range of sample matrices, including algal and zooplankton extracts as well as human fluids. A strong correlation was found in this study between RBA based toxicity and that determined from HPLC results for algal extracts and human urine and serum samples. Usup et al. (2004) characterized the detection of a range of toxin congeners using the RBA and found that the rat brain sodium channel preparations in the assay reliably represented total toxicity of the congeners present in a sample. They reported binding affinities in the order of STX > GTX1/4 > NEO > GTX2/3 > dcSTX > GTX5 which is similar to the order found based on mouse toxicity. All results to date demonstrate the ability of the RBA to provide accurate estimates of overall toxicity and agree well with other established methods. The existing AOAC collaborative study indicates the potential for this method to be fully validated in the near future, enhancing the likelihood of approval by regulatory authorities as an alternative regulatory method. It is noteworthy, however, that the RBA would need to be approved as an NSSP method before it could easily be implemented for regulatory purposes in the US. The main limitation of widespread implementation of the RBA is the need for radiolabeled material (^{3}H-STX). This is an impediment because the material is in short supply and it is also difficult for some laboratories to fulfill requirements for performing assays that involve radioactive materials.

Immunoassays, which depend on antibody-based detection, are also becoming more readily available for STX detection. Commercial ELISAs (enzyme-linked immunosorbent assays) include those produced by Abraxis, R-Biopharm, and Beacon Analytical Systems, Inc. Advantages of these kits include ease of use and high throughput. Yet a major challenge is that they suffer from cross-reactivity limitations, where antibody binding with the range of congeners does not necessarily reflect human oral potency. The ELISA method developed by Usleber et al. (1991) was used to produce the commercially available Ridascreen™ test kit (R-Biopharm Darmstadt, Germany), and these kits were used by CDC to evaluate clinical samples associated with puffer fish poisoning (M. Early, pers. comm.). They were also used by Etheridge et al. (2006) to test puffer fish extracts associated with a human illness. In these cases ELISA results compared well with other methods, although this was because the samples fortuitously possessed a congener profile that reacted with the antibody in a fashion that matched the potency. Good agreement between ELISAs and MBA was also reported in other studies (Chu et al., 1996; Usleber et al., 1997), but not in all (Kasuga et al., 1996). As stated earlier, toxin profiles can be quite complex, making the cross-reactivity challenge with antibodies an impediment to widespread use of the kits. To improve immuno-based assays for PSP toxins, an antibody or, more likely, a mixture of antibodies with a cross-reactivity to congeners reflecting toxicity is needed. To address this issue Beacon Analytical Systems, Inc. has introduced an additional ELISA for the detection of NEO. Antibodies to all toxin congeners are not readily available. Successful production of antibodies appears to remain on small scales (e.g. Chu et al., 1992), with the exception of what is produced by or in association with test kit developers for their own purposes. The lack of available antibodies is

mostly due to the lack of purified toxin congeners from which to produce them. None of these kits have undergone AOAC validation nor are they NSSP approved (with the exception of approval for the Abraxis ELISA to be used as part of a pilot study to assess the Onboard Screening Dockside Testing Protocol as a management strategy – described below).

Lateral flow immuno-chromatography test kits are also antibody-based assays that have potential for detecting PSP toxins for seafood safety and human health purposes. The only such commercially available kit is the Jellett Rapid Test (JRT) manufactured by Jellett Rapid Testing, Ltd. The JRT kit has not been AOAC validated; however, they have approval by the ISSC (Interstate Shellfish Sanitation Conference) as an NSSP approved method for regulatory use in the US under very specific circumstances. Specifically, the JRT can be used in the following ways: (1) as a method to determine when to perform an MBA in a previously closed area, (2) a negative result can be substituted for an MBA to maintain an area in the open status, and (3) a positive result shall be used for a precautionary closure. The JRTs are faced, however, with the same antibody cross-reactivity challenge as other immuno-based methods. Evaluations of the JRT have been conducted by several regulatory laboratories. For example, concerns were reported in the UK about potential false negatives when using the kits (CEFAS, 2007). In the US, however, studies have concluded that false positives are the main problem. California's public health laboratory conducted a parallel study using the MBA and the JRT in 2004 for a total of 910 samples (Oshiro et al., 2006). Throughout the year there were no false negatives found, meaning there were no cases when the JRT gave a negative result when the MBA tested positive. However, 28.5% of the samples resulted in false positives (positive by JRT and negative by MBA). Unfortunately there were no toxin profile data available to see if discrepancies were related to the toxin congeners present in the samples. The authors concluded that the JRT could be used as part of California's PSP surveillance program as a screening tool for determining when to conduct MBAs, thereby reducing animal usage. Similarly, Costa et al. (2009) found no false negatives when using the JRT to test shellfish collected from the Aleutian Islands, Alaska. The percentage of false positives was greater in this study, with 73% false positives reported for the JRT when compared to the RBA. Likewise at the US FDA, we conducted a study investigating shellfish toxicity tested by JRT compared to the RBA and no false negatives were reported. However, false positives were fairly frequent (Fig. 2). Even Jellett Rapid Testing Ltd. reports false positive rates ranging between 1.4% and 55%, depending on the toxin profile (Laycock et al., 2010). From a public health perspective false negatives are to be avoided entirely, whereas false positives err on the side of protection. A problem arises though when the rate of false positives is too high and the kits become ineffective tools. Unfortunately, there is no known toxin profile that exists ubiquitously that could be used for kit validation. Thus, false positives are inevitable.

While the assays stated above are more widely used and/or considered for seafood safety and human health uses, there are others that may serve as effective detection tools in the future. Surface plasmon resonance (SPR) biosensors represent an example. They have been applied mostly in biomedical research and industry settings to assess molecular interactions especially for drug analysis. Relatively recently this platform has been evaluated for detecting and investigating small molecular weight toxins (e.g. Yu et al., 2005; Taylor et al., 2008). An SPR biosensor assay was developed by Fonfría et al. (2007) to detect PSP toxins using an inhibition type antibody binding approach. They report being able to detect STX in the range of 15–400 μg 100 g^{-1}; however, the antibody challenges described earlier apply here as well. Another study describes an approach of using calyx[4]arene derivative monolayers in an SPR assay and found the crown ether self-assembled monolayer to bind STX well, even at low concentrations (Chen et al., 2007). Campbell et al. (2007) investigated the use of three different PSP protein binders in an SPR inhibition-based assay which included sodium channel receptors, a monoclonal antibody raised to GTX2/3 and a polyclonal antibody raised to STX. They reported the ability to bind STX using all three proteins with the rabbit polyclonal demonstrating cross-reactivity with the widest range of PSP toxins. The data, however, indicated stability issues with the sodium channel preparations that were used during the assay and that improvements are necessary before native receptors can serve as the molecular recognition element in this assay. The potential for coupling SPR with mass spectrometry for confirmatory analysis of PSP toxins is also being investigated (Marchesini et al., 2009). An assay for detecting PSP in shellfish using an SPR biosensor is currently the focus of a multi-laboratory collaborative study through BioCop Project, which seeks to supply regulators, consumers and industry with long-term solutions to the complex problems associated with chemical contaminant monitoring (http://www.biocop.org/index.html [accessed 9 November 2009]). Advantages of SPR biosensors include having real-time, label-free, high throughput, detection capabilities that use very little solvents or sample. It is foreseeable that this method could play a more prominent role for regulatory purposes in the future.

In addition to assays there are also analytical methods that allow for the separation of individual toxin congeners so that they can each be identified and quantified. Of those, high-performance liquid chromatography (HPLC) is most well-established for PSP toxins. HPLC methods for these toxins involve either a pre- or post-column oxidation step so that the derivatized form of the toxins can be detected by fluorescence. The post-column oxidation HPLC method reported by Sullivan and Wekell (1984) and Sullivan et al. (1985) was based on the original fluorometric method of Bates and Rapoport (1975) and it was found to compare well with the MBA. One challenge of the Sullivan (1988) post-column oxidation method is that it is challenging to differentiate STX from dcSTX (van Egmond et al., 1994). The most commonly used method for research application is the post-column oxidation HPLC method by Oshima (1995). The limitation is time consumption since three separate isocratic runs are needed in order to separate, identify and quantify the range of toxins present. This particular HPLC

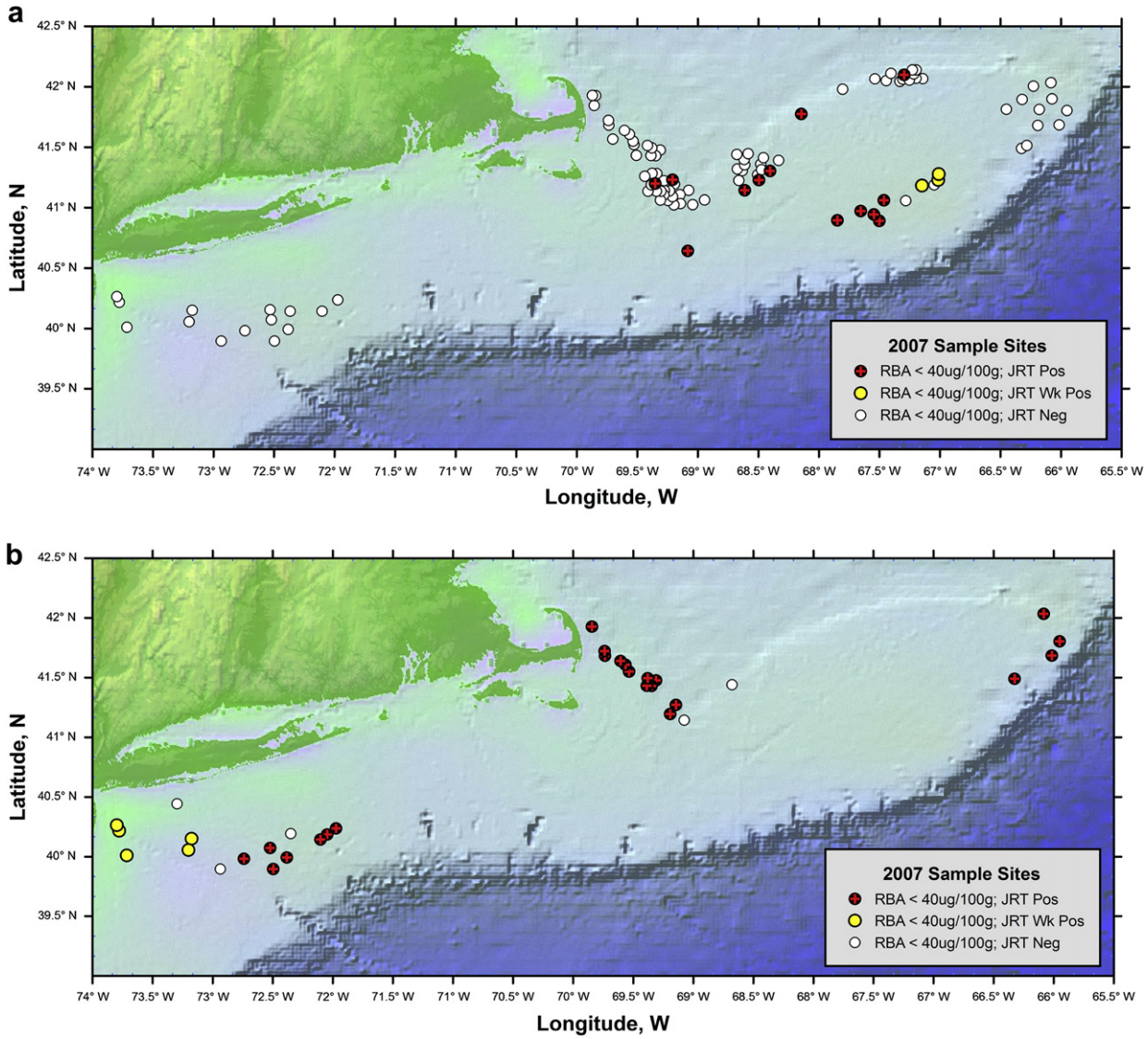

Fig. 2. Sea scallop (a) gonad and (b) viscera toxicity compared using the JRT and RBA. All scallop samples collected during the survey of waters off the northeast coast of the US in 2007 that tested less than 40 μg STX eq 100 g^{-1} by RBA were plotted showing the JRT results (green = negative; yellow = weakly positive; red = positive). The red symbols, thus, denote samples for which the JRT yielded a false positive. No false negatives were found during that survey. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

method has not been AOAC validated nor is it approved as an NSSP method for regulatory purposes.

The Oshima (1995) post-column oxidation method was modified by Thomas et al. (2006) and then further by Rourke et al. (2008). Originally, the method required three separate injections per sample under different chromatographic and post-column conditions in order to detect the full range of toxins. The modified version only requires two separate injections per sample. The major advantages of this modified method include higher throughput and faster turnaround time of positive samples. A successful AOAC single laboratory validation was performed with this method (Rourke et al., 2008), and it is currently the focus of an AOAC collaborative study. Additionally, in October 2009 this post-column HPLC approach was accepted as a Type IV NSSP method for the determination of paralytic shellfish toxins in shellfish, making it the most recently approved PSP method for regulatory purposes in the US.

The pre-column oxidation HPLC technique that is becoming more established, especially as a regulatory tool, is the Lawrence AOAC (2005) approved method. This pre-column oxidation HPLC method has been validated for STX, NEO, GTX2/3, GTX1/4, dcSTX, B1 (GTX5), C1/2 and C3/4 in mussels, clams, oysters and scallops. In the UK, this method has already been implemented as the primary regulatory tool (Food Standards Agency (FSA), 2007). In order for state monitoring laboratories in the US to implement this method, it would still need to be deemed an NSSP approved

method; however, even with that approval it is unlikely that many state laboratories would switch to this method due to time constraints, instrument expense and the need for a skilled scientist to analyze samples. The method has not been adopted for use in Canada even though this is where the method was developed. Guy and Griffin (2009) cited instrumentation costs and lack of certified reference materials as the major obstacles of implementation. Many of the PSP congeners are actually available through the National Research Council in Canada. The congeners that are fairly common that are still not available include B2 (GTX6) and C3/4. The lack of these additional standards would of course be the same limitation to any other analytical method. It is likely that the major drawback and leading reason for the Lawrence method not being implemented as a regulatory tool is due to the time it takes to complete a sample analysis (Ben-Gigirey and Villar-González, 2008). This is especially the case in Canada where they find a large number of positive samples that have to undergo the full Lawrence analysis, thereby reducing sample throughput and increasing costs.

To date, these method validation studies have focused on shellfish extracts and there is no validated method for detecting PSP toxins in clinical matrices. The focus of a current study (Etheridge et al., unpublished) is the extension of the Lawrence HPLC method for use with human urine and serum. Given the limited number of clinical samples typically encountered, the time constraints of this method would be less of a concern compared to using it for shellfish monitoring purposes. Having a validated method for detecting individual toxins in clinical samples will enhance our ability to obtain as much information as possible about toxins in humans for those rare samples that are collected in time, stored adequately, and sent to an appropriate laboratory that can perform the analyses.

In general another challenge of HPLC and fluorescence detection is the possibility that naturally occurring fluorescent compounds in samples may interfere with toxin identification, as was demonstrated by the GTX4 "imposters" by Baker et al. (2003). Etheridge et al. (2006) discussed this challenge and steps that can be taken in addition to simple chromatographic runs to confirm the presence of PSP toxins. In brief, it is necessary to, at a minimum, run subsets of samples without the post-column oxidation to assist with distinguishing toxins from naturally occurring fluorescent compounds in the matrix. Other limitations of HPLC-based toxin determination include lack of availability of certain toxin standards as described above and converting toxin concentration back to toxicity, which is the most relevant measure with respect to human illness. Both of these limitations of course apply to other analytical methods as well.

The other analytical separation method becoming more established for PSP toxins is liquid chromatography with mass spectrometry detection (LC–MS). Dell'Aversano et al. (2005) improved LC–MS detection capabilities by establishing a method based on hydrophilic interaction liquid chromatography (HILIC) which was more sensitive than previous methods. Since this method does not use mobile phases with ion-pairing agents, it does not suffer from reduced ionization efficiency as did previous methods. Application of a zwitterionic hydrophilic interaction liquid chromatography (HILIC) column by Diener et al. (2007) separated underivatized PSP toxins using an LC–MS/MS method to separate all 3 groups of PSP toxins in a single run. This area of research is expanding rapidly and promises to be an effective detection tool for these toxins (Deeds et al., 2008b). Challenges that currently remain are improving limits of detection and obtaining labeled internal standards to be used for concentration determination. The greatest advantage of LC–MS methods is that it is confirmatory.

Regardless of the detection method used, sample extraction plays a significant role. The most common is the boiling hydrochloric acid extraction used within the AOAC MBA (AOAC 2000). This process has the potential to convert some of the less potent toxins into congeners of higher toxicity (e.g. C toxins into their respective GTX counterparts). Since there is also the possibility that similar conditions may occur during human digestion, the extraction is considered acceptable as it errs on the side of caution. However, if one is interested in the inherent toxin profile and toxicity of a sample less harsh procedures (e.g. acetic acid) are needed. Vale et al. (2008) recently published a study on the effect of pH during extraction on PSP toxin potency and showed that a variation in pH by 2 units during extraction can lead to large discrepancies in sample toxin profiles and toxicity. The critical issue of toxin extraction must be considered when selecting a particular detection method to ensure that the measurement provided answers the regulatory need.

6. Monitoring and management strategies

The STXs are heat and acid stable, thus cooking the seafood does not denature the toxins. The combination of acid and heat can result in the hydrolysis of the carbamyl-N-sulfo congeners to their corresponding carbamate form (Boyer et al., 1986), making seafood potentially more toxic. While the cooking process does not destroy toxins per se, it is possible that certain procedures could reduce toxin levels. For example, Lawrence et al. (1994) demonstrated that boiling or steaming lobster tomalley could reduce toxicity by approximately 65% compared to raw toxicity. This observed decrease is the result of toxins migrating out of the lobster and into the water used for boiling/steaming. Therefore, when seafood is boiled or steamed, toxin extraction can make cooking liquids very toxic (Mons et al., 1998).

Operations of an industrial canning process were also evaluated for decreasing toxins, and found that boiling and sterilization reduced toxins (Berenguer et al., 1993). Takata et al. (1994) studied the effect of boiling and retorting on toxicity in oysters and reported that boiling and retorting for 60 min was more effective than boiling and then retorting for 5 min. While adjusting cooking processes is not solely a strategy for managing this public health risk, there are some benefits to certain cooking methods that extract the toxin into a cooking liquid that can then be discarded.

A harvesting management strategy is to only harvest parts of the organisms known to be safe and to discard the parts of the organism that may pose a threat. The most noteworthy example of such a practice is the harvesting of sea scallop adductor muscle only. In certain waters off the coast of the US, shellfish beds are only open for scallop adductor muscle harvesting and are closed to harvesting of all other molluscan bivalves, due to the threat of PSP. This practice is allowed because scallop adductor muscle is typically considered free of PSP toxins. Reports by Murakami and Noguchi (2003) found small toxin levels in the adductor muscle from the scallop *Patinopecten yessoensis* that were frozen as a whole body and then separated after thawing. Therefore, scallop adductors are considered safe when prepared from live, fresh scallops with careful removal of the remaining organs and tissues which is the basis for the required separation at sea upon harvest before freezing. Another example of management strategies dealing with the harvest and processing of certain body parts is the abalone in South Africa. It was suggested by Pitcher et al. (2001) that PSP toxin levels could be reduced by separating the abalone foot and epipodial fringe and scrubbing them.

A more recent study investigating the potential for decreasing toxin content in bivalves by industrial processes can be found by Reboreda et al. (2010). In addition to presenting a brief review of previous reports on procedures for detoxifying bivalves, the authors evaluated four methods for decreasing marine toxin levels in mussels, scallops, clams, and cockles: freezing, evisceration, ozonation, and thermal processing. Specifically for PSP toxins, they assessed the effects of thermal processing and freezing on mussels, clams, and cockles. Notably, they found that PSP toxicity decreased after thermal processing and the effect of freezing was highly dependent on the matrix. The bivalves used for this study, however, were not highly toxic. In order to fully assess the degree of successful detoxification these studies should be repeated starting with material with a higher toxin burden.

Since the seafood harvesting, processing and cooking methods have limited impact in most cases on reducing toxin loads and making seafood products safe from this hazard, there are other major monitoring and management strategies in place to protect public health. As already stated, the action level must not be exceeded for seafood to be harvested; therefore monitoring tools are critical for making decisions to open/close seafood resources as quickly and efficiently as possible. Many countries have established successful programs and many states within the US have very successful monitoring strategies for seafood collected from waters in their purview. One major challenge facing the FDA is the extensive offshore shellfish beds that are under federal jurisdiction, making them FDA's responsibility for ensuring their safety. FDA, however, does not have statutory authority to close a fishery, thus a request is made in these cases to the National Marine Fisheries Service to close the beds under their authority given by the Magnuson–Stevens Act. Federal waters represent areas that are nominally 3 miles or greater offshore and cover an enormous area, making it a serious challenge for traditional sample monitoring such as that typically done by state laboratories. Thus, innovative strategies are being investigated for monitoring and managing these offshore resources. Currently, the focus is on an Onboard Screening Dockside Testing pilot study (http://www.nero.noaa.gov/sfd/clams/ [accessed 9 January 2009]). This strategy involves giving a fishing vessel a permit to fish in closed waters with restrictions in place to ensure public health is protected. The protocol calls for industry to use available test kits in the field to test the seafood product upon harvest. If all samples from a minimal number of required sampling locations per area are negative using the field test, the product may be harvested and landed. However, once the product is landed it must be tested using a regulatory method (i.e. MBA) to confirm the product is safe for consumption before it can be released from the processing plant. Pilot studies are currently underway and involve the use of two onboard screening methods: the Jellett Rapid Test (JRT) and the ELISA by Abraxis.

Another management tool for seafood safety is through the Hazard Analysis Critical Control Point (HACCP) program. The FDA implemented HACCP in the mid-1990s for the seafood industry to (1) analyze hazards, (2) identify and monitor critical control points, (3) establish corrective actions to be taken when monitoring shows that a critical limit has not been met, and (4) verify that the system is working and documented properly. The FDA published and periodically updates the Fish and Fishery Products Hazards and Controls Guide to assist processors in HACCP plan development. The safety features of FDA's seafood HACCP regulations have also been incorporated into NOAA's National Seafood Inspection Program.

In the US, the ISSC and the NSSP serve to promote seafood safety. The ISSC (http://www.issc.org [accessed 9 January 2009]) was formed in 1982 *to foster and promote shellfish sanitation through the cooperation of state and federal control agencies, the shellfish industry, and the academic community.* The ISSC has its own Biotoxin Committee and a Laboratory Methods Review Committee for approving methods of detection for toxins in seafood for regulatory purposes. The NSSP is the *federal/state cooperative program recognized by the FDA and ISSC for the sanitary control of shellfish produced and sold for human consumption*. The NSSP provides the Guide for the Control of Molluscan Shellfish (http://www.fda.gov/Food/FoodSafety/Product-SpecificInformation/Seafood/FederalStatePrograms/NationalShellfishSanitationProgram/ucm046353.htm [accessed 9 January 2009]). Both entities play major roles in guiding policy/regulation in the US with respect to seafood safety.

On a more global scale the Task Force on Marine and Freshwater Toxins was developed under the auspices of AOAC (http://www.aoac.org [accessed on 9 January 2009]) *to work on prioritizing, funding, and accelerating validation studies of methods for marine and freshwater toxins. This is formed by an international group of experts on these toxins and stakeholders who have a strong and practical interest in the development and validation of methods for detection of these toxins.* Also on a global scale, the Codex Alimentarius (http://www.codexalimentarius.net [accessed 9 January 2009]) *was created in 1963 by FAO and WHO to develop food*

standards, guidelines and related texts such as codes of practice under the Joint FAO/WHO Food Standards Programme.

7. Summary

Paralytic shellfish poisoning is a serious syndrome and the toxins responsible for this illness pose a great risk to seafood safety and public health. Our understanding of the sources, vectors, viable toxin testing methods and monitoring/management practices has reduced illnesses and fatalities over the years. However, our knowledge base continues to expand even as new sources/vectors emerge. With efforts directed at establishing more appropriate regulatory methods and seeking alternatives to the MBA, our ability to monitor for toxins in a range of matrices of interest will improve. Innovative management strategies, such as the onboard screening dockside testing study, should be considered to further enhance our capacity to ensure that safe seafood products reach the market. Through these enhancements it is anticipated that we not only further decrease the number of PSP illnesses and fatalities, but that we make closures as efficiently as possible so that safe resources are available and are not unnecessarily closed – a balance that respects the economy and livelihood of the seafood industry and protecting the public.

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Conflict of interest

None.

References

Abbott, J.P., Flewelling, L.J., Landsberg, J.H., 2009. Saxitoxin monitoring in three species of Florida puffer fish. Harmful Algae 8, 343–348.

Anderson, D.M., Kulis, D.M., Sullivan, J.J., Hall, S., 1990. Toxin composition in one isolate of the dinoflagellate *Alexandrium fundyense*. Toxicon 28, 885–893.

Anderson, D.M., Kulis, D.M., Doucette, G.J., Gallagher, J.C., Balech, E., 1994. Biogeography of toxic dinoflagellates in the genus *Alexandrium* from the northeastern United States and Canada. Mar. Biol. 120, 467–478.

ANZFA, November 2001. Shellfish toxins in food. A toxicological review and risk assessment. In: Technical Report Series No. 14. Australia New Zealand Food Authority (ANZFA). Available at: www.nicnas.gov.au/australia/fsanz.htm.

AOAC Official Method, 2000. In: Horwitz, W. (Ed.), Official Methods of Analysis of AOAC International, 17th ed. AOAC International, Gaithersburg, MD.

AOAC Official Method 2005.06, A.M, 2005. In: Official Methods of Analysis.

Aune, T., Stabell, O.B., Nordstoga, K., Tjotta, K., 1998. Oral toxicity in mice of algal toxins from the diarrheic shellfish toxin (DST) complex and associated toxins. J. Nat. Toxins 7 (2), 141–158.

Baker, T.R., Doucette, G.J., Powell, C.L., Boyer, G.L., Plumley, F.G., 2003. GTX4 imposters: characterization of fluorescent compounds synthesized by *Pseudomonas stutzeri* SF/PS and *Pseudomonas*/*Alteromonas* PTB-1, symbionts of saxitoxin-producing *Alexandrium* spp. Toxicon 41, 339–347.

Bates, H.A., Rapoport, H., 1975. A chemical assay for saxitoxin, the paralytic shellfish poison. J. Agric. Food Chem. 23, 237.

Ben-Gigirey, B., Villar-González, A., 2008. Chemical analysis. In: Botana, L. M. (Ed.), Seafood and Freshwater Toxins: Pharmacology, Physiology, and Detection, second ed. CRC Press, Taylor and Francis Group LLC, pp. 177–196.

Berenguer, J.A., Gonzalez, L., Jimez, I., et al., 1993. The effect of commercial processing on the paralytic shellfish poison (PSP) content of naturally contaminated *Acanthocardia tuberculatum* L. Food Addit. Contam. 10 (2), 217–230.

Berry, J.P., Lind, O., 2010. First evidence of "paralytic shellfish toxins" and cylindrospermopsin in a Mexican freshwater system, Lago Catemaco, and apparent bioaccumulation of the toxins in "tegogolo" snails (*Pomacea patula catemacensis*. Toxicon 55 (5), 930–938.

Bodager, D., 2002. Outbreak of saxitoxin illness following consumption of Florida pufferfish. Fla. J. Environ. Health 179, 9–13.

Boyer, G.L., Sullivan, J.J., Andersen, R.J., Taylor, F.J.R., Harrison, P.J., Cembella, A.D., 1986. Use of high-performance liquid chromatography to investigate the production of paralytic shellfish toxins by *Protogonyaulax* spp. Mar. Biol. 93, 361–369.

Bricelj, V.M., Lee, J.H., Cembella, A.D., 1991. Influence of dinoflagellate cell toxicity on uptake and loss of paralytic shellfish toxins in the northern quahog *Mercenaria mercenaria*. Mar. Ecol. Prog. Ser. 74, 33–46.

Bricelj, V.M., Connell, L., Konoki, K., Macquarrie, S.P., Scheuer, T., Catterall, W. A., Trainer, V.L., 2005. Sodium channel mutation leading to saxitoxin resistance in clams increases risk of PSP. Nature 434, 763–767.

Bricelj, V.M., Shumway, S.E., 1998. Paralytic shellfish toxins in bivalve mollusks: occurrence, transfer kinetics, and biotransformation. Rev. Fish. Sci. 6, 315–383.

Campbell, K., Stewart, L.D., Doucette, G.J., Fodey, T.L., Haughey, S.A., Vilariño, N., Kawatsu, K., Elliott, C.T., 2007. Assessment of specific binding proteins suitable for the detection of paralytic shellfish poisons using optical biosensor technology. Anal. Chem. 79, 5906–5914.

Carmichael, W.W., Falconer, I.R., 1993. Diseases related to freshwater blue-green algal toxins, and control measures. In: Falconer, I.R. (Ed.), Algal Toxins in Seafood and Drinking Water. Academic Press, pp. 187–209.

Castonguay, M., Levasseur, M., Beaulieu, J.-L., Grégoire, F., Michaud, S., Bonneau, E., Bates, S.S., 1997. Accumulation of PSP toxins in Atlantic mackerel: seasonal and ontogenetic variations. J. Fish Biol. 50, 1203–1213.

Catteral, W.A., 1989. Molecular properties of voltage-sensitive sodium and calcium channels. ICSU Short Rep. 9, 14–15.

Catterall, W.A., 1984. The molecular basis of neuronal excitability. Science 223, 653–661.

Catterall, W.A., 1986. Molecular properties of voltage-sensitive sodium channels. Annu. Rev. Biochem. 55, 953–985.

Catterall, W.A., 2000. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. Neuron 26, 13–25.

Catterall, W.A., Cestèle, S., Yarov-Yarovoy, V., Yu, F.H., Konoki, K., Scheuer, T., 2007. Voltage-gated ion channels and gating modifier toxins. Toxicon 49, 124–141.

CEFAS (Centre for Environment, Fisheries and Aquaculture Science), 2007. The Use of the Jellett Rapid Testing Kit for PSP Toxins Detection in the UK Statutory Monitoring Programme for Marine Toxins. Biotoxin Team Report, 34 pp..

Cembella, A.D., 1998. Ecophysiology and metabolism of paralytic shellfish toxins in marine microalgae. In: Anderson, D.M., Cembella, A.D., Hallegraeff, G.M. (Eds.), Physiological Ecology of Harmful Algal Blooms. NATO ASI Series, vol. G41, pp. 381–403.

Cembella, A.D., Desbiens, M., 1994. Fate of paralytic shellfish toxins in the American lobster *Homarus americanus*. J. Shellfish Res. 13, 302.

Cembella, A.D., Therriault, J.-C., Beland, P., 1988. Toxicity of cultured isolates and natural populations of *Protogonyaulax tamarensis* from the St. Lawrence Estuary. J. Shellfish Res. 7, 611–621.

Cembella, A.D., Shumway, S.E., Lewis, N.I., 1993. Anatomical distribution and spatio-temporal variation in paralytic shellfish toxin composition in two bivalve species from the Gulf of Maine. J. Shellfish Res. 12, 389–403.

Chen, H., Kim, Y.S., Keum, S.R., Kim, S.H., Choi, H.J., Lee, J., An, W.G., Koh, K., 2007. Surface plasmon spectroscopic detection of saxitoxin. Sensors 7, 1216–1223.

Chu, F.S., Huang, X., Hall, S., 1992. Production and characterization of antibodies against neosaxitoxin. J. AOAC Int. 75 (2), 341–345.

Chu, F.S., Hsu, K.H., Huang, X., Barrett, R., Allison, C., 1996. Screening of paralytic shellfish poisoning toxins in naturally occurring samples with three different direct competitive enzyme-linked immunosorbent assays. J. Agric. Food Chem. 44 (12), 4043–4047.

Clemente, Z., Busato, R.H., Oliveira Ribeiro, C.A., Cestari, M.M., Ramsdorf, W.A., Magalhães, V.F., Wosiack, A.C., Silva de Assis, H.C., 2009. Analyses of paralytic shellfish toxins biomarkers a southern Brazilian Reservoir.

Costa, P.R., Baugh, K.A., Wright, B., RaLonde, R., Nance, S.L., Tatarenkova, N., Etheridge, S.M., Lefebvre, K.A., 2009. Comparative determination of paralytic shellfish toxins (PSTs) using five different toxin detection methods in shellfish species collected in the Aleutian Islands, Alaska. Toxicon 54, 313–320.

Davio, S.R., Fontelo, P.A., 1984. A competitive displacement assay to detect saxitoxin and tetrodotoxin. Anal. Biochem. 141, 199–204.

Deeds, J.R., Landsberg, J.H., Etheridge, S.M., Pitcher, G.C., Longan, S.W., 2008a. Non-traditional vectors for paralytic shellfish poisoning. Mar. Drugs 6, 308–348.

Deeds, J.R., White, K.D., Etheridge, S.M., Landsberg, J.H., 2008b. Concentrations of saxitoxin and tetrodotoxin in three species of puffers from the Indian River Lagoon, Florida, the location of multiple cases of saxitoxin puffer poisoning from 2002–2004. Trans. Am. Fish. Soc. 137 (5), 1317–1326.

Dell'Aversano, C., Hess, P., Quilliam, M.A., 2005. Hydrophilic interaction liquid chromatography–mass spectrometry analysis of paralytic shellfish poisoning (PSP) toxins. J. Chromatogr. A 1081, 190.

Desbiens, M., Cembella, A.D., 1997. Retention and possible transformation of paralytic shellfish toxins in lobster (*Homarus americanus*). Bull. Aquacul. Assoc. Can. 97 (2), 75–77.

Diener, M., Erler, K., Christian, B., Luckas, B., 2007. Application of a new zwitterionic hydrophilic interaction chromatography column for determination of paralytic shellfish poisoning toxins. J. Sep. Sci. 30, 1821–1826.

Doucette, G.J., Logan, M.M., Ramsdell, J.S., Van Dolah, F.M., 1997. Development and preliminary validation of a microtiter plate-based receptor binding assay for paralytic shellfish poisoning toxins. Toxicon 35 (5), 625–636.

Doucette, G.J., Kodama, M., Franca, S., Gallacher, S., 1998. Bacterial interactions with harmful algal bloom species: bloom ecology, toxigenesis, and cytology. In: Anderson, D.M., Cembella, A.D., Hallegraeff, G.M. (Eds.), Physiological Ecology of Harmful Algal Blooms. Springer-Verlag, Heidelberg, pp. 619–647.

Doucette, G.J., Turner, J.T., Powell, C.L., Keafer, B.A., Anderson, D.M., 2005. Trophic accumulation of PSP toxins in zooplankton during *Alexandrium fundyense* blooms in Casco Bay, Gulf of Maine, April–June 1998. 1. Toxin levels in *A. fundyense* and zooplankton size fractions. Deep-Sea Res. 52, 2764–2783.

EFSA (European Food Safety Authority), 2009. Scientific opinion of the panel on contaminants in the food chain on a request from the European Commission on marine biotoxins in shellfish – summary on regulated marine biotoxins. EFSA J. 1306, 1–23.

van Egmond, H.P., Speyers, G.J.A., Habermehl, G., 1992. Current situation on worldwide regulations for marine phycotoxins. Nat. Toxins 1, 67–85.

van Egmond, H.P., Van den Top, H.J., Paulsch, W.E., Goenaga, X., Vieytes, M. R., 1994. Paralytic shellfish poison reference materials: an intercomparison of methods for the determination of saxitoxin. Food Addit. Contam. 11 (1), 39–56.

Etheridge, S.M., Deeds, J., Hall, S., White, K., Flewelling, L., Abbott, J., Landsberg, J., Conrad, S., Bodager, D., Jackow, G., 2006. Detection methods and their limitations: PSP toxins in the southern puffer fish *Sphoeroides nephelus* responsible for human poisoning events in Florida in 2004. Afr. J. Mar. Sci. 28, 383.

Etheridge, S.M., Roesler, C.S., 2005. Effects of temperature, irradiance, and salinity on photosynthesis, growth rates, total toxicity, and toxin composition for *Alexandrium fundyense* isolates from the Gulf of Maine and Bay of Fundy. Deep-Sea Res. 52, 2491–2500.

Falconer, I.R., 1993. Measurement of toxins from blue-green algae in water and foodstuffs. In: Falconer, I.R. (Ed.), Algal Toxins in Seafood and Drinking Water. Academic Press, pp. 165–175.

FAO (Food and Agriculture Organization of the United Nations), 2004. Marine Biotoxins. Paper 80. FAO Food and Nutrition, Rome, pp. 5–52.

Fonfría, E.S., Vilariño, N., Campbell, K., Elliot, C., Haughey, S.A., Ben-Gigirey, B., Vieites, J.M., Kawatsu, K., Botana, L.M., 2007. Paralytic shellfish poisoning detection by surface plasmon resonance-based biosensors in shellfish matrixes. Anal. Chem. 79 (16), 6303–6311.

FSA (Food Standards Agency), 2007. Introduction of a New Chemical Screen Test to the UK Statutory Marine Biotoxin Monitoring Programme Available from: http://www.food.gov.uk/multimedia/pdfs/biotoxinmonitoringprogramme.pdf.

Galvão, J.A., Oetterer, M., Bittencourt-Oliveira, M., Gouvêa-Barros, S., Hiller, S., Erler, K., Luckas, B., Pinto, E., Kujbida, P., 2009. Saxitoxins accumulated by freshwater tilapia (*Oreochromis niloticus*) for human consumption. Toxicon 54, 891–894.

García, C., Bravo, M., Lagos, M., Lagos, N., 2004. Paralytic shellfish poisoning: post-mortem analysis of tissue and body fluid samples from human victims in the Patagonia fjords. Toxicon 43, 149–158.

García, C., Rodriguez-Navarro, A., Diaz, J.C., Torres, R., Lagos, N., 2009. Evidence of in vitro glucuronidation and enzymatic transformation of paralytic shellfish toxins by healthy human liver microsomes fraction. Toxicon 53, 206–213.

Geraci, J.R., Anderson, D.M., Timperi, R.J., St-Aubin, D.J., Early, G.A., Prescott, J.H., Mayo, C.A., 1989. Humpback whales (*Megaptera novaeangliae*) fatally poisoned by dinoflagellate toxin. Can. J. Fish. Aquat. Sci. 46, 1895–1898.

Gessner, B.D., Bell, P., Doucette, G.J., Moczydlowski, E., Poli, M.A., Van Dolah, F., Hall, S., 1997. Hypertension and identification of toxin in human urine and serum following a cluster of mussel-associated paralytic shellfish poisoning outbreaks. Toxicon 35 (5), 711–722.

Gessner, B.D., Middaugh, J.P., 1995. Paralytic shellfish poisoning in Alaska: a 20-year retrospective analysis. Am. J. Epidemiol. 141, 766–770.

Grabowski, J.H., Gaudette, J., 2008. The role of food limitation in lobster dynamics in coastal Maine, United States and New Brunswick, Canada. N.Z. J. Mar. Freshw. Res. 42.

Guy, A., Griffin, G., 2009. Adopting alternatives for the regulatory monitoring of shellfish for paralytic shellfish poisoning in Canada: interface between federal regulators, science and ethics. Regul. Toxicol. Pharmacol. 54, 256–263.

Hall, S., Strichartz, G., Moczydlowski, E., Ravindran, A., Reichardt, P.B., 1990. The saxitoxins: sources, chemistry, and pharmacology. In: Hall, S., Strichartz, G. (Eds.), Marine Toxins: Origin, Structure, and Molecular Pharmacology. American Chemical Society.

Hallegraeff, G.M., 1993. A review of harmful algal blooms and their apparent global increase. Phycologia 32, 79–99.

Halsetead, B.W., 1978. Poisonous and Venomous Marine Animals of the World. Darwin, Princeton.

Hille, B., 1968. Pharmacological modifications of the sodium channels of frog nerve. J. Gen. Physiol. 51, 199–219.

Hille, B., 1975. The receptor site for tetrodotoxin and saxitoxin. A structural hypothesis. Biophys. J. 15, 615–619.

Hille, B., 1992. Ionic Channels of Excitable Membranes. Sinauer Associates, Inc., Sunderland, MA.

Hoeger, S.J., Shaw, G., Hitzfeld, B.C., Dietrich, D.R., 2004. Occurrence and elimination of cyanobacterial toxins in two Australian drinking water treatment plants. Toxicon 43, 639–649.

IPCS (International Programme on Chemical Safety), 1984. Aquatic (Marine and Freshwater) Biotoxins. In: Environmental Health Criteria 37. World Health Organization, ISBN 92 4 154097 4.

Jiang, T.-J., Niu, T., Xu, Y.-X., 2006. Transfer and metabolism of paralytic shellfish poisoning from scallop (*Chlamys nobilis*) to spiny lobster (*Panulirus stimpsoni*). Toxicon 48, 988–994.

Kao, C.Y., 1986. Structure activity relations of tetrodotoxin, saxitoxin, and analogues. Ann. N.Y. Acad. Sci. 479, 52–67.

Kao, C.Y., 1993. Paralytic shellfish poisoning. In: Falconer, I.R. (Ed.), Algal Toxins in Seafood and Drinking Water. Academic Press, pp. 75–86.

Kasuga, F., Harakudo, Y., Machii, K., 1996. Evaluation of enzyme-linked immunosorbent assay (ELISA) kit for paralytic shellfish poisoning toxins. J. Food Hyg. Soc. Jpn. 37 (6), 407–410.

Kodama, M., Ogata, T., Kawamukai, K., Oshima, Y., Yasumoto, T., 1983. Occurrence of saxitoxin and other toxins in the liver of pufferfish *Takifugu pardalis*. Toxicon 21, 897–900.

Kodama, M., Ogata, T., Sakamoto, S., Sato, S., Honda, T., Miwatani, T., 1990a. Production of paralytic shellfish toxins by a bacterium *Moraxella* sp. isolated from *Protogonyaulax tamarensis*. Toxicon 28, 707–714.

Kodama, M., Ogata, T., Sato, S., Sakamoto, S., 1990b. Possible association of marine bacteria with paralytic shellfish toxicity in bivalves. Mar. Ecol. Prog. Ser. 61, 203–206.

Kodama, M., Doucette, G.J., Green, D.H., 2006. Relationships between bacteria and harmful algae. In: Ecology of Harmful Algae. Springer-Verlag, Berlin, pp. 243–255.

Kodama, M., Sato, S., 2008. Metabolism of paralytic shellfish toxins incorporated into bivalves. In: Botana, L.M. (Ed.), Seafood and Freshwater Toxins: Pharmacology, Physiology, and Detection, second ed. CRC Press, Taylor and Francis Group LLC, pp. 165–175.

Kotaki, Y., Tajiri, Y., Oshima, Y., Yasumoto, T., 1983. Identification of a calcareous red alga as the primary source of paralytic shellfish toxins in coral reef crabs and gastropods. Bull. Jpn. Soc. Sci. Fish 49, 283–286.

Lagos, N., 1998. Microalgal blooms: a global issue with negative impact in Chile. Biol. Res. 31, 375–386.
Landsberg, J.H., 2002. The effects of harmful algal blooms on aquatic organisms. Rev. Fish. Sci. 10 (2), 113–390.
Landsberg, J.H., Hall, S., Johannessen, J.N., White, K.D., Conrad, S.M., Abbott, J.P., Flewelling, L.J., Richardson, R.W., Dickey, R.W., Jester, E.L.E., Etheridge, S.M., Deeds, J.R., Van Dolah, F.M., Leighfield, T.A., Zou, Y., Beaudry, C.G., Benner, R.A., Rogers, P.L., Scott, P.S., Kawabata, K., Wolny, J.L., Steidinger, K.A., 2006. Saxitoxin puffer fish poisoning in the United States, with the first report of *Pyrodinium bahamense* as the putative toxin source. Environ. Health Perspect. 114 (10), 1502–1507.
Lawrence, J.F., Maher, M., Watson-Wright, W., 1994. Effect of cooking on the concentration of toxins associated with paralytic shellfish poison in lobster hepatopancreas. Toxicon 32 (1), 57–64.
Laycock, M.V., Donovan, M.A., Easy, D.J., 2010. Sensitivity of lateral flow tests to mixtures of saxitoxins and applications to shellfish and phytoplankton monitoring. Toxicon 55, 597–605.
Linares, J., Ochoa, J., Martínez, A.G., 2009. Retention and tissue damage of PSP and NSP toxins in shrimp: is cultured shrimp a potential vector of toxins to human population? Toxicon 53, 185–195.
Liu, Y., Chen, W., Li, D., Shen, Y., Li, G., Liu, Y., 2006. First report of aphantoxins in China – waterblooms of toxigenic *Aphanizomenon flos-aquae* in Lake Dianchi. Ecotoxicol. Environ. Saf. 65 (1), 84.
Llewellyn, L., Dodd, M.J., Robertson, A., Ericson, G., de Koning, C., Negri, A. P., 2002. Post-mortem analysis of samples from a human victim of a fatal poisoning caused by the xanthid crab, *Zosimus aeneus*. Toxicon 40, 1463–1469.
Llewellyn, L., Negri, A., Quilliam, M., 2004. High affinity for the rat brain sodium channel of newly discovered hydroxybenzoate saxitoxin analogues from the dinoflagellate *Gymnodinium catenatum*. Toxicon 43, 101–104.
Marchesini, G.R., Hooijerink, H., Haasnoot, W., Buijs, J., Campbell, K., Elliott, C.T., Nielen, M.W.F., 2009. Towards surface plasmon resonance biosensing combined with bioaffinity-assisted nano HILIC liquid chromatography/time-of-flight mass spectrometry identification of paralytic shellfish poisons. Trends Anal. Chem. 28 (6), 792–803.
Molica, R.J.R., Oliveira, E.J.A., Carvalho, P.V.V.C., Costa, A.N.S.F., Cunha, M.C. C., Melo, G.L., Azevedo, S.M.F.O., 2005. Occurrence of saxitoxins and an anatoxin-a(s)-like anticholinesterase in a Brazilian drinking water supply. Harmful Algae 4, 743–753.
Mons, M.N., Van Egmond, H.P., Speijers, G.J.A., June 1998. Paralytic Shellfish Poisoning: a Review. RIVM Report 388802 005.
Murakami, R., Noguchi, T., 2003. Toxicity of Adductor Muscle of Markedly PSP-infested Scallop *Patinopecten yessoensis* which Was Discussed in Codex Plan by FAO/WHO. Available at: http://pop3.wfd.ntou.edu.tw/symposium/Toxins/Abstracts.htm.
Nakamura, M., Oshima, Y., Yasumoto, T., 1984. Occurrence of saxitoxin in puffer fish. Toxicon 22, 381–385.
Nakashima, K., Arakawa, O., Taniyama, S., Nonaka, M., Takatani, T., Yamamori, K., Fuchi, Y., Noguchi, T., 2004. Occurrence of saxitoxins as a major toxin in the ovary of a marine puffer *Arothron firmamentum*. Toxicon 43, 207–212.
Narahashi, T., Moore, J.W., Poston, R.N., 1967. Tetrodotoxin derivatives: chemical structure and blockage of nerve membrane conductance. Science 156, 976–979.
Negri, A.P., Jones, G.J., Hindmarsh, M., 1995. Sheep mortality associated with paralytic shellfish poisons from the cyanobacterium *Anabaena circinalis*. Toxicon 33 (10), 1321–1329.
Negri, A., Stirling, D., Quilliam, M., Blackburn, S., Bolch, C., Burton, I., Eaglesham, G., Thomas, K., Walter, J., Willis, R., 2003. Three novel hydroxybenzoate saxitoxin analogues isolated from the dinoflagellate *Gymnodinium catenatum*. Chem. Res. Toxicol. 16, 1029–1033.
Negri, A.P., Bolch, C.J.S., Geier, S., Green, D.H., Park, T.-G., Blackburn, S.I., 2007. Widespread presence of hydrophobic paralytic shellfish toxins in *Gymnodinium catenatum*. Harmful Algae 6, 774–780.
Negri, A.P., Jones, G.J., 1995. Bioaccumulation of paralytic shellfish poisoning (PSP) toxins from the cyanobacterium *Anabaena circinalis* by the freshwater mussel *Alathyria condola*. Toxicon 33 (5), 667–678.
Negri, A., Llewellyn, L., 1998. Comparative analyses by HPLC and the sodium channel and saxiphilin 3H-saxitoxin receptor assays for paralytic shellfish toxins in crustaceans and mollusks from tropical North West Australia. Toxicon 36, 283–298.
Noda, M., Suzuki, H., Numa, S., Stuhmer, W., 1989. A single point mutation confers tetrodotoxin and saxitoxin insensitivity on the sodium channel II. FEBS Lett. 259, 213–216.
Oikawa, H., Fujita, T., Saito, K., Watabe, S., Satomi, M., Yano, Y., 2004. Comparison of paralytic shellfish poisoning toxin between carnivorous crabs (*Telmessus acutidens* and *Charybdis japonica*) and their prey mussel (*Mytilus galloprovincialis*) in an inshore food chain. Toxicon 43, 713–719.
Okumura, M., Yamada, S., Oshima, Y., Ishikawa, N., 1994. Characteristics of paralytic shellfish poisoning toxins derived from short-necked clams (*Tapes japonica*) in Mikawa Bay. Nat. Toxins 2, 141–143.
Orellana-Cepeda, E., Martínez-Romero, E., Muñoz-Cabrera, L., López-Ramírez, P., Cabrera Mancilla, E., Ramírezcamarena, C., 1998. Toxicity associated with blooms of *Pyrodinium bahamense* var. *compressum* in southwestern Mexico. In: Reguera, B., Blanco, J., Fernandez, M., Wyatt, T. (Eds.), Harmful Algae, Proceedings of the VIII International Conference on Harmful Algae, (June 1999, Vigo, Spain). Xunta de Galicia and IOC of UNESCO, 60 pp.
Oshima, Y., 1995. Post-column derivatization HPLC methods for paralytic shellfish poisons. In: Hallegraeff, G.M., Anderson, D.M., Cembella, A.D. (Eds.), Manual on Harmful Marine Microalgae. IOC of UNESCO, pp. 81–94.
Oshima, Y., Blackburn, S.I., Hallegraeff, G.M., 1993. Comparative study on paralytic shellfish toxin profiles of the dinoflagellate *Gymnodinium catenatum* from three different countries. Mar. Biol. 116, 471–476.
Oshiro, M., Pham, L., Csuti, D., Dodd, M., Inami, G.B., Brenden, R.A., 2006. Paralytic shellfish poisoning surveillance in California using the Jellett Rapid PSP test. Harmful Algae 5, 69–73.
Pereira, P., Dias, E., Franca, S., Pereira, E., Carolino, M., Vasconcelos, V., 2004. Accumulation and depuration of cyanobacterial paralytic shellfish toxins by the freshwater mussel *Anodonta cygnea*. Aquat. Toxicol. 68, 339–350.
Pitcher, G.C., Franco, J.M., Doucette, G.J., Powell, C.L., Mouton, A., 2001. Paralytic shellfish poisoning in the abalone *Haliotis midae* on the west coast of South Africa. J. Shellfish Res. 20 (2), 895–904.
Powell, C.L., Doucette, G.J., 1999. A receptor binding assay for paralytic shellfish poisoning toxins: recent advances and applications. Nat. Toxins 7, 393–400.
Quayle, D.B., 1969. Paralytic shellfish poisoning in British Columbia. Bull. Fish. Res. Bd. Can. 168, 1–68.
Quilliam, M., Wechsler, D., Marcus, S., Ruck, B., Wekell, M., Hawryluk, T., 2004. Detection and identification of paralytic shellfish poisoning toxins in Florida pufferfish responsible for incidents of neurologic illness. In: Steidinger, K.A., Landsberg, J.H., Tomas, C.R., Vargo, G.A. (Eds.), Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission of UNESCO, St. Petersburg, FL, pp. 116–118.
Reboreda, A., Lago, J., Chapela, M.J., Vieites, J.M., Botana, L.M., Alfonso, A., Cabado, A.G., 2010. Decrease of marine toxin content in bivalves by industrial processes. Toxicon 55, 235–243.
Ritchie, J.M., Rogart, R.B., 1977. The binding of saxitoxin and tetrodotoxin to excitable tissue. Rev. Physiol. Biochem. Pharmacol. 79, 42–50.
Rodrigue, D.C., Etzel, R.A., Hall, S., De porras, E., Velasquez, O.H., Tauxe, R.V., Kilbourne, E.M., Blake, P.A., 1990. Lethal paralytic shellfish poisoning in guatemala. Am. J. Trop. Med. Hyg. 42, 267–271.
Rourke, W.A., Murphy, C.J., Pitcher, G., van de Riet, J.M., Burns, B.G., 2008. Rapid postcolumn methodology for determination of paralytic shellfish toxins in shellfish tissue. J. AOAC Int. 91 (3), 589–597.
Schantz, E.J., 1960. Biochemical studies of paralytic shellfish poisons. Ann. N.Y. Acad. Sci. 89, 843–855.
Schantz, E.J., 1969. Studies on shellfish poisons. J. Agric. Food Chem. 17 (3), 413–416.
Schantz, E.J., Mold, J.D., Stranger, D.W., Shavel, J., Riel, F., Bowden, J.P., Lynch, J.M., Wyler, R.S., Riegel, B., Sommer, H., 1957. Paralytic shellfish poison. VI. A procedure for the isolation and purification of the poison from toxic clam and mussel tissues. J. Am. Chem. Soc. 79, 5230–5235.
Schantz, E.J., McFarren, E.F., Schafer, M.L., Lewis, K.H., 1958. Purified shellfish poison for bioassay standardization. J. Assoc. Off. Anal. Chem. 41, 160–168.
Schick, D.F., Feindel, S.C., 2005. Maine Scallop Fishery: Monitoring and Enhancement. Final Report to the Northeast Consortium, 73 pp.
Sephton, D.H., Haya, K., Martin, J.L., LeGresley, M.M., Page, F.H., 2007. Paralytic shellfish toxins in zooplankton, mussels, lobsters, and caged Atlantic salmon, *Salmo salar*, during a bloom of *Alexandrium fundyense* off Grand Manan Island, in the Bay of Fundy. Harmful Algae 6, 745–758.
Shumway, S.E., 1990. A review of the effects of algal blooms on shellfish and aquaculture. J. World Aquacul. Soc. 21, 65–104.
Shumway, S.E., 1995. Phycotoxin-related shellfish poisoning: bivalve mollusks are not the only vectors. Rev. Fish. Sci. 3 (1), 1–31.
Shumway, S.E., Cembella, A.D., 1993. The impact of toxic algae on scallop culture and fisheries. Rev. Fish. Sci. 1, 121–150.
Shumway, S.E., Barter, J., Sherman-Caswell, S., 1990. Auditing the impact of toxic algal blooms on oysters. Environ. Audit 2, 41–56.
Silva, E.S., 1990. Intracellular bacteria: the origin of dinoflagellate toxicity. J. Environ. Pathol. Toxicol. Oncol. 10, 124–128.

Smith, P.T., 2008. Cyanobacterial toxins in aquaculture. In: Botana, L.M. (Ed.), Seafood and Freshwater Toxins: Pharmacology, Physiology, and Detection, second ed. CRC Press, Taylor and Francis Group LLC, pp. 787–806.
Sommer, H., Whedon, W.F., Kofoid, C.A., Stohler, R., 1937. Arch. Pathol. 24, 537.
Sommer, H., Meyer, K.F., 1937. Paralytic shellfish poisoning. Arch. Pathol. 24, 560–598.
Steneck, R.S., Wilson, C.J., 2001. Large scale and long-term, spatial and temporal patterns in demography and landings of the American lobster, *Homarus americanus*, in Maine. Mar. Freshw. Res. 52, 1303–1319.
Sullivan, J.J., 1988. Methods of analysis for DSP and PSP toxins in shellfish: a review. J. Shellfish Res. 7, 587–595.
Sullivan, J.J., Iwaoka, W.T., Liston, J., 1983. Enzymatic transformation of PSP toxins in the Little Neck clam (*Protothaca staminea*). Biochem. Biophys. Res. Commun. 114, 465–472.
Sullivan, J.J., Wekell, M.M., Kentala, L.L., 1985. Application of HPLC for the determination of PSP toxins in shellfish. J. Food Sci. 50, 26.
Sullivan, J.J., Wekell, M.M., 1984. Determination of paralytic shellfish poisoning toxins by high pressure liquid chromatography. In: Ragelis, E.P. (Ed.), Seafood Toxins. ACS, Washington, DC, p. 197.
Takata, K., Mizuta, M., Monden, T., 1994. Reduction in toxicity of PSP-infected oysters by heat treatment. J. Food Hyg. Soc. Jpn. 35, 624–630.
Taylor, A., Ladd, J., Etheridge, S., Deeds, J., Hall, S., Jiang, S., 2008. Quantitative detection of tetrodotoxin (TTX) by a surface plasmon resonance (SPR) sensor. Sens. Actuators B 130, 120–128.
Terlau, H., Heinemann, S.H., Stuhmer, W., Pusch, M., Conti, F., Imoto, K., Numa, S., 1991. Mapping the site of block by tetrodotoxin and saxitoxin of sodium channel II. FEBS Lett. 293, 93–96.
Thomas, K., Chung, S., Ku, J., Reeves, K., Quilliam, M.A., 2006. Analysis of PSP toxins by liquid chromatography with postcolumn oxidation and fluorescence detection. In: Henshilwood, K., Deegan, B., McMahon, T., Cusack, C., Keaveney, S., Silke, J., O'Cinneide, M., Lyons, D., Hess, P. (Eds.), Molluscan Shellfish Safety. The Marine Institute Galway, Ireland, pp. 132–138.
Usleber, E., Schnieder, E., Terplan, G., 1991. Direct enzyme immunoassay in microtitration plate and test strip format for the detection of saxitoxin in shellfish. Lett. Appl. Microbiol. 13, 275.
Usleber, E., Donald, M., Straka, M., et al., 1997. Comparison of enzyme immunoassay and mouse bioassay for determining paralytic shellfish poisoning toxins in shellfish. Food Addit. Contam. 14 (2), 193–198.
Usup, G., Leaw, C.P., Cheah, M.Y., Ahmad, A., Ng, B.K., 2004. Analysis of paralytic shellfish poisoning toxin congeners by a sodium channel receptor binding assay. Toxicon 44, 37–43.
Vale, C., Alfonso, A., Vieytes, M.R., Romaris, X.M., Arevalo, F., Botana, A.M., Botana, L.M., 2008. *In vitro* and in vivo evaluation of paralytic shellfish poisoning toxin potency and the influence of the pH of extraction. Anal. Chem. 80, 1770–1776.
Van Dolah, F.M., Leighfield, T.A., Doucette, G.J., Bean, L., Niedzwiadek, B. , Rawn, D.F.K. Single lab validation of the microplate receptor binding assay for paralytic shellfish toxins in shellfish. J. AOAC Int., in press.
Vieytes, M.R., Cabado, A.G., Alfonso, A., Louzao, M.C., Botana, A.M., Botana, L.M., 1993. Solid-phase radioreceptor assay for paralytic shellfish toxins. Anal. Biochem. 211, 87–93.
Wang, D.Z., 2008. Neurotoxins from marine dinoflagellates: a brief review. Mar. Drugs 6, 349–371.
Watson-Wright, W., Gillis, M., Smyth, C., Trueman, S., McGuire, A., Moore, W., McLachlan, D., Sims, G., 1991. Monitoring of PSP in hepatopancreas of lobster from Atlantic Canada. In: Gordon, D.C. (Ed.), Proceedings of the Second Canadian Workshop on Harmful Marine Algae. Can. Tech. Rep. Fish. Aquat. Sci. 1799, 27–28.
Wekell, J.C., Hurst, J., Lefebvre, K.A., 2004. The origin of the regulatory limits for PSP and ASP toxins in shellfish. J. Shellfish Res. 23 (3), 927–930.
Westrick, J.A., 2008. Cyanobacterial toxin removal in drinking water treatment processes and recreational waters. In: Hudnell, H.K. (Ed.), Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs. Adv. Exp. Med. Biol. 619, 275–290.
White, A.W., 1981. Sensitivity of marine fishes to toxins from the red-tide dinoflagellate *Gonyaulax excavata* and implications for fish kills. Mar. Biol. 65 (3), 255–260.
WHO (World Health Organization), 1999. Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management. In: Chorus, I., Bartram, J. (Eds.). E&FN Spon.
Yu, Q., Chen, S., Taylor, A., Homola, J., Hock, B., Jiang, S., 2005. Detection of low-molecular weight domoic acid using surface plasmon resonance sensor. Sens. Actuators B 107, 193–201.
Yuan, M., Carmichael, W.W., Hilborn, E.D., 2006. Microcystin analysis in human sera and liver from human fatalities in Caruaru, Brazil 1996. Toxicon 48, 627–640.

Stacey Etheridge received her Ph.D. in Oceanography from the University of Connecticut in 2002. She is currently a Biologist in the Office of Regulatory Science at the US Food and Drug Administration, Center for Food Safety and Applied Nutrition. Her research interests are related to food safety and food defense: detection methods development for seafood-borne toxins; traditional and emerging sources and vectors of seafood-borne toxins; and the dynamics of toxic algae and the transfer of toxins to seafood.