Paralytic Shellfish Toxins in Southern Rocklobsters (*Jasus edwardsii*)

Interim Food Safety Exposure Assessment

Catherine McLeod, Ian Stewart and Andreas Kiermeier

South Australian Research and Development Institute

December 2012

SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

Government of South Australia

Executive Summary

In October 2012 a shipment of mussels (*Mytilus edulis planulatus*) derived from the east coast of Tasmania was rejected by Japanese import authorities due to the presence of unacceptable levels of Paralytic Shellfish Toxins (PSTs). Mussel samples were collected and tested from the implicated consignment and harvest area and it became apparent that the mussels had bioaccumulated PSTs through feeding on a bloom of the dinoflagellate algae *Alexandrium tamarense*.

Following the initial discovery of *A. tamarense*, additional seawater and bivalve sampling of sites spanning the majority of the east coast of Tasmania revealed the presence of *A. tamarense* cells and PSTs in shellfish (oysters and mussels) at several sites between Eddystone Point and Marion Bay. By early November it was revealed that scallops (*Pecten fumatus*) and rocklobsters (*Jasus edwardsii*) were also impacted by PSTs. While lobster hepatopancreas samples from the affected area have been found to be above the maximum level for PSTs in bivalves (0.8 mg kg^{-1}), lobster muscle tissue tested to date has not shown the presence of PSTs. Questions have been raised as to whether the presence of PSTs in lobster hepatopancreas from the affected region poses a significant human health risk. To assist risk managers to evaluate the risk to public health an interim exposure assessment has been undertaken.

The exposure assessment has resulted in a series of scenarios being developed which provides estimates of the number of lobster hepatopancreas that may be consumed without exceeding the Designated Dose for PSTs (as calculated from the current PST maximum limit for bivalves in Australia of 0.8 mg kg^{-1}). For example:

- A 70 kg adult, eating a maximum-sized raw lobster hepatopancreas at the maximum PST level detected in the Tasmanian 2012 event to date could consume up to 0.9 hepatopancreas without exceeding the Designated Dose.
- A 15 kg child, eating an average-sized raw lobster hepatopancreas at the maximum PST level detected in the Tasmanian 2012 event could consume up to 0.4 hepatopancreas without exceeding the Designated Dose.

These results are broadly consistent with similar data from the US and Canada, where public health regulators have also issued advisories about the consumption of lobster hepatopancreas as part of their risk management.

A significant number of data gaps have been identified which has prevented a full characterisation of the risk (e.g. determining the probability of illness). Consequently, there is considerable uncertainty in the calculation of the Designated Dose and subsequent number of lobster hepatopancreas that can be consumed. The most pressing data needs relate to: (1) obtaining robust data on consumption patterns regarding lobster hepatopancreas; and (2) information on the kinetics of uptake and elimination of PSTs from southern rocklobsters. Such information could support the future development of management strategies such as the potential for harvesting PST contaminated lobsters and depuration prior to live sale.

Table of Contents

Background

In October 2012 Japan import authorities detected paralytic shellfish toxins (PSTs) in a consignment of mussels (*Mytilus edulis planulatus*) harvested from Spring Bay on the east coast of Tasmania. Mussel samples were collected and tested from the implicated consignment and harvest area and it became apparent that the mussels had bioaccumulated PSTs through feeding on a bloom of the dinoflagellate algae *Alexandrium tamarense*.

Mainly non-toxic strains of *A. tamarense* have been detected in Tasmanian waters. While a few isolates of PST producing *A. tamarense* are described in the literature (sampled from cyst beds) [62], they have not been associated with the detection of PSTs in bivalve shellfish in Tasmania previously. From this perspective, the discovery of PSTs in mussels was unexpected.

Following the initial discovery of *A. tamarense*, additional seawater and bivalve sampling of sites spanning the majority of the east coast revealed the presence of *A. tamarense* cells and PSTs in shellfish at several sites between Eddystone Point and Marion Bay (Figure 1). By early November it was revealed that scallops (*Pecten fumatus*) from the east coast fishery were also impacted by PSTs and concerns were raised that other fishery products may also be impacted.

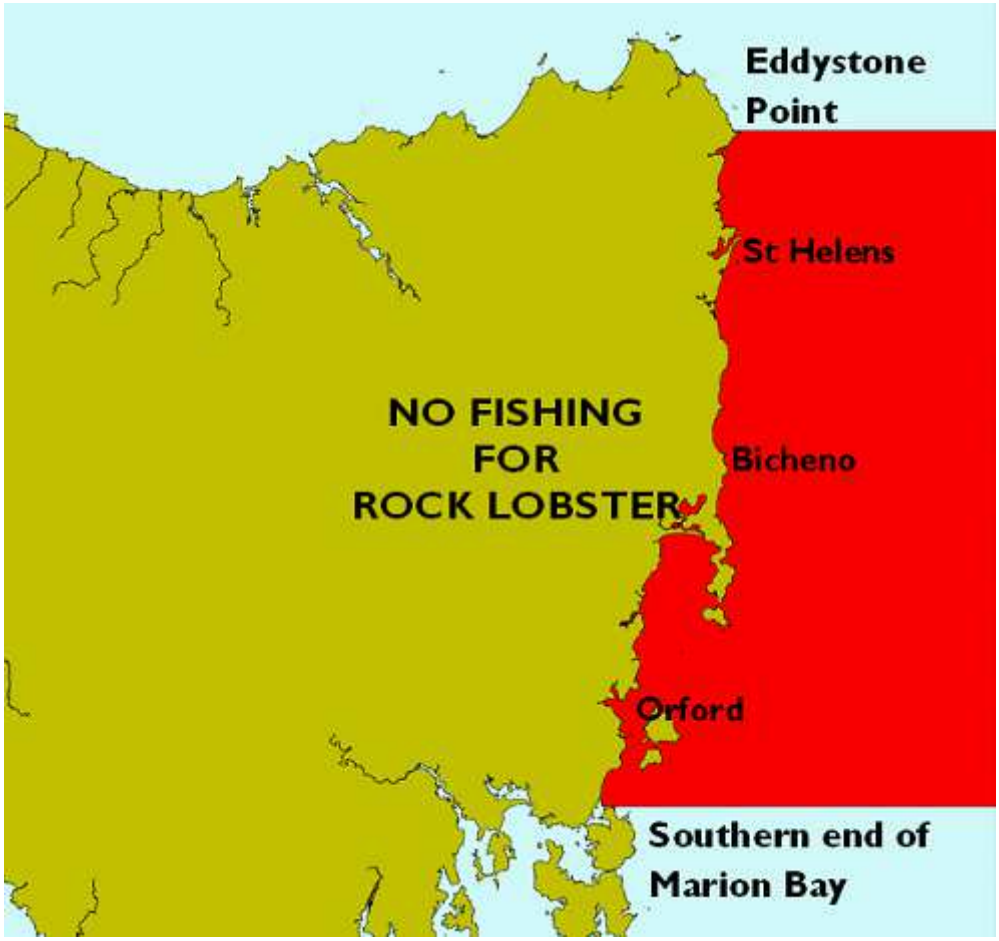
To investigate these concerns public health authorities facilitated collection of 10 lobsters (*Jasus edwardsii*) (along with other important fishery species) from various sites within the Spring Bay region; hepatopancreas and flesh (muscle) tissues of each lobster were analysed for PSTs. These tests revealed that 7 of the 10 lobster hepatopancreas samples had PST levels above the maximum level for bivalves[1] of 0.8 mg kg^{-1}. Lobster muscle tissue tested to date has not shown the presence of PSTs. Additional sampling rounds have subsequently been undertaken and confirmed that lobsters as far north as Eddystone are impacted by PSTs, however lobsters south of Marion Bay were found to be compliant with the maximum level of 0.8 mg kg^{-1} (Figure 1). With the support of the Tasmanian Rocklobster Fishermen's Association, this portion of the east coast (Figure 1) was not opened to lobster fishing on 15 November 2012 and remains closed to commercial rocklobster fishing and public health advisories not to consume the gut of lobsters are in place.

Questions have been raised as to whether the presence of PSTs in lobster hepatopancreas from the affected region poses a significant human health risk. To assist risk managers to evaluate the risk to public health an interim exposure assessment has been undertaken[2].

[1] A maximum permissible level has not been specifically derived for lobsters to our knowledge, however some countries utilise the bivalve PST level of 0.8 mg kg^{-1} as a basis for issuing public health warnings and restricting trade of potentially contaminated lobster hepatopancreas.

[2] Basic information from a risk assessment on PSTs in Australian abalone has been utilised to inform the hazard identification and hazard characterisation of this interim exposure assessment [1].

Figure 1: Region affected by the *Alexandrium tamarense* bloom and 'no fishing' zone for lobsters.



Statement of Purpose

This project was commissioned by the Tasmanian Department of Primary Industries, Parks, Water and Environment to provide an interim assessment of the risks of PSTs to consumers of lobster hepatopancreas. The specific project objectives were:

1. To provide an interim assessment of the paralytic shellfish toxin (PSTs) content of rocklobster hepatopancreas (sourced from Tasmania) in relation to the reference dose.

2. To provide preliminary information to support consumption advice for Rocklobster hepatopancreas.

Approach

It is acknowledged that a comprehensive risk assessment needs to be undertaken to fully evaluate the public health impact related to PSTs in rocklobsters harvested from the north east of Tasmania. A full risk assessment would take into account a wide range of factors and questions, including:

- The size of the human population consuming lobster viscera and meat (tail and leg muscle tissue) (Tasmania, Australia, key overseas markets).
- Average and 95th percentile meal sizes for lobster viscera and meat.
- Frequency of consumption.
- Format in which products are consumed (e.g. cooked, raw, paté etc).
- Amount of lobster harvested in Tasmania available for human consumption (recreational and commercial catch).
- Occurrence of PSTs in rocklobsters in Tasmania.
- Considerations on contaminated lobsters reaching the market.
- Influence of processing on PST levels, including cooking and freezing.
- Adsorption, distribution (viscera vs. flesh), uptake and elimination of PSTs in lobsters.
- Toxin profiles in lobster viscera and meat (cooked and raw).
- Acute and oral toxicity of PSTs.
- Observations in humans.

This information would inform the characterisation of risk to humans from the consumption of a variety of rocklobster products (including lightly cooked muscle, viscera based patés, hepatopancreas based mustard, etc). This would also include explicit identification of major data gaps and uncertainties. A full risk assessment accounting for the above factors is likely to take a significant period of time to complete (e.g. 6 – 12 months) due to the need to gather basic information and data on consumption patterns and toxin uptake, tissue distribution and elimination which are not readily available at this time.

As an interim measure due to the need for a rapid assessment to provide early guidance, preliminary risk assessment work has been undertaken, including an initial hazard identification, hazard characterisation and exposure assessment. This has involved an evaluation of the following factors:

- A review of PSTs detected in lobsters and other crustaceans globally and public health advisories issued by overseas regulatory agencies.
- An evaluation of past PST illnesses associated with the consumption of crustaceans.
- The mean and maximum weight of lobster hepatopancreas of lobsters harvested from Tasmania (lobsters implicated in 2012 PST event in the north east of Tasmania).
- The average and maximum toxin levels detected in lobster hepatopancreas during this event.
- The toxin profiles of lobster hepatopancreas tested during the 2012 event.
- A designated dose for PSTs (derived from the maximum permissible level for PSTs in bivalves) has been compared to levels recorded in raw lobster hepatopancreas and cooked lobster viscera.
- Potential loss of toxin through a standard cooking process e.g. boiling.

The following report provides a summary of the above information.

Risk Analysis Framework and Definitions

Risk analysis is a systematic approach to assess risks associated with food and to develop, implement and communicate risk management decisions. The risk analysis framework sets out to provide answers to fundamental questions regarding food safety, such as:

- What are the potential problems?
- What is the probability of the problem occurring?
- What would the consequences be if it went wrong?
- What can be done to reduce the likelihood and/or seriousness of it going wrong?

Risk analysis is made up of three separate components: Risk Assessment, Risk Management and Risk Communication. The following provides definitions of these terms, along with others that are commonly used in the risk analysis process. The definitions used are primarily based on the Codex Alimentarius Commission Principles and Guidelines for the Conduct of Microbiological Risk Assessment [2] and supplemented with definitions found in other key texts.

Acute reference dose (ARfD) - An estimate of the amount of substance in food, normally expressed on a body-weight basis (mg kg^{-1} or mg kg^{-1} of body weight), that can be ingested in a period of 24 hours or less without appreciable health risk to the consumer on the basis of all known facts at the time of assessment.

Exposure Assessment - The qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food, as well as exposures from other sources if relevant.

Hazard - A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect.

Hazard Characterisation - The qualitative and/or quantitative assessment of the nature of the adverse health effects associated with the hazard.

Hazard Identification - The identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods.

LD50 - The dose of a substance required to cause death in half the members of a tested population after a specified test duration.

Lowest Observed Adverse Effect Level (LOAEL) - The lowest concentration or amount of a substance found by experiment or observation which causes an adverse alteration of morphology, function, capacity, growth, development or life span of a target organism distinguished from normal organisms of the same species under defined conditions of exposure.

No Observable Adverse Effect Level (NOAEL) - The level of exposure of an organism found by experiment or observation, at which there is no biologically or statistically significant increase in the frequency or severity of any adverse effects in the exposed population when compared to its appropriate control.

Paralytic Shellfish Toxins (PSTs) – A group of about 30 hydrophilic toxins produced primarily by dinoflagellates.

Paralytic Shellfish Poisoning (PSP) – Human illness induced by the consumption of significant levels of PSTs.

Risk - A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food.

Risk Analysis - A process consisting of three components: risk assessment, risk management and risk communication.

Risk Assessment - A scientifically based process consisting of the following steps:

(i) hazard identification;

(ii) hazard characterisation;

(iii) exposure assessment; and

(iv) risk characterisation.

Risk Characterization - The process of determining the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterisation and exposure assessment.

Risk Communication - The interactive exchange of information and opinions concerning risk and risk management among risk assessors, risk managers, consumers and other interested parties.

Risk Estimate - Output of Risk Characterisation.

Risk Management - The process of weighing policy alternatives in the light of the results of risk assessment and, if required, selecting and implementing appropriate control options, including regulatory measures.

Hazard Identification

Paralytic Shellfish Toxins (PSTs)

The PSTs are a group of hydrophilic toxins comprised of about 30 related congeners that have been identified in toxic algae (predominantly dinoflagellates) and various shellfish products [3-6].

There are four main groups of PST congeners:

(i) The carbamate toxins (e.g. STX, GTX 1-4 and neoSTX);
(ii) The decarbamoyl toxins (e.g. dcSTX, dcGTX1-4);
(iii) The N-sulfocarbamoyl toxins (e.g. B1 [GTX5], B2 [GTX6] and C1–4); and
(iv) The deoxydecarbamoyl toxins.

The potencies of the congeners differ, with the carbamate toxins being the most potent group [3, 7-9]. The toxicity (toxin profile and concentration) of the dinoflagellates which produce PSTs varies and appears to be influenced by a wide range of factors, including: geographical location, nutrient availability, temperature and other environmental factors [10-13].

The terms “paralytic shellfish toxin” and “paralytic shellfish poisoning” could be considered misnomers, as saxitoxins produced by dinoflagellate blooms can become widely distributed through marine food webs. These toxins can bioaccumulate not only in bivalve filter-feeding molluscs but in carnivorous molluscs, cephalopod molluscs, fish – particularly planktivores, marine mammals (pinnipeds and cetaceans) and birds, and many other invertebrates, including decapod crustaceans [7, 14, 15]. Regarding crustaceans, PSTs have been found in crabs, lobster, freshwater crays, and prawns. By far the largest body of literature on PSTs in decapods is devoted to crabs.

The potential for dietary exposure to PSTs through consumption of lobster was recognized in 1967; specimens of Cape lobster (*Jasus lalandii*) were sampled and tested during a bloom of *Gonyaulax catenella* (now *Alexandrium catenella*) off the west coast of South Africa. Tail meat and “digestive gland” were not toxic, but stomach contents of lobsters collected from locations where toxic mussels were harvested were also toxic. Mussels were known as an important food item for the Cape lobster [16].

Studies on PST profiles show differences between the causative dinoflagellates when compared with contaminated seafood products. This is likely due to inter-conversion of the congeners and this is probably catalysed by enzymes and bacteria contained within the seafood [6, 17, 18].

Consistent with this, STX has been found to be the main congener in abalone muscle (foot) tissue compared with GTX and C toxins detected in the causative dinoflagellate algae [17, 19-22]. Additionally, in abalone from the D’entrecasteux Channel (Tasmania) the major congener found is deoxydecarbamoylsaxitoxin (doSTX), which is only found in small quantities in the causative dinoflagellates (*Gymnodinium catenatum*) and in mussels harvested at the same time and location. This indicates a high degree of toxin transformation in abalone.

With regard to rocklobster hepatopancreas, Haya *et al* [50] noted that GTX-1, GTX-2 and neosaxitoxin were found in higher proportions in hepatopancreas than in the toxic scallops fed to lobsters. Saxitoxin, neosaxitoxin and decarbamoylsaxitoxin concentrations increased and GTX 1-4 decreased during the later stages of depuration, with the implication either that gonyautoxins were biotransformed to saxitoxins or that lobster hepatopancreas has a greater affinity for more basic toxins. Lawrence *et al* [51] found that saxitoxin was the predominant congener in samples from Québec, whereas GTX-2,3 predominated in lobsters captured from Nova Scotia. No C-toxins were seen in any samples. The gonyautoxins were markedly reduced in concentration after cooking; saxitoxin concentrations less so. Cembella and Desbiens [52] found GTX-2,3, saxitoxin, decarbamoyl-STX and c-toxins were measured in both muscle and hepatopancreas, but with different profiles seen in these tissues.

Symptoms

Paralytic Shellfish Poisoning (PSP) intoxification in humans results in a variety of symptoms ranging from mild to severe (Table 1) [3, 23, 24]. In fatal cases, death is caused by respiratory paralysis.

Table 1. Symptoms of Paralytic Shellfish Poisoning in Humans

Mild	Moderate	Severe
Prickly sensation in fingers and toes	Extremity numbness and tingling	Muscular/limb paralysis
Tingling sensation or numbness around lips	Incoherent speech	Pronounced respiratory difficulty
Headache	Stiffness and non coordination of limbs	Choking sensation
Dizziness	General weakness and feeling of lightness (floating sensation)	
Nausea	Slight respiratory difficulty/ shortness of breath and rapid pulse plus backache	
Vomiting		
Dry mouth		
Diarrhoea		

Paralytic Shellfish Poisoning (Illness Reports)

Accounts of PSP relating to the consumption of bivalves are well documented in the literature; these relate to shellfish grown and consumed throughout northern and southern America, Europe, Asia and the Pacific [3, 4, 24]. One report in the literature suggests there are ~2000 human cases of PSP globally per annum through fish or shellfish consumption with a 15% mortality rate [25].

In contrast, there have been few reported cases of PSP intoxication resulting from consumption of bivalve shellfish in Australia. Hallegraeff [26] reported only five mild cases of human poisoning from eating wild shellfish in Tasmania. In 2011, a probable case was reported to the Tasmanian Department of Health and Human Services following

consumption of mussels recreationally harvested during a bloom of Gymnodinium catenatum in Tasmania. None of the cases to date have been clinically confirmed by public health authorities. There are no confirmed reports of PSP illness resulting from the consumption of lobsters in Australia to our knowledge.

The majority of PSP cases globally involve the consumption of bivalves, however a few cases have resulted from the consumption of crustaceans, fish and gastropods. Numerous crab species are known to be capable of bioaccumulating PSTs, and several reports of "paralytic shellfish poisonings" including fatal human intoxications, attributable to consumption of crabs can be found in the literature. The following provides a review of the literature regarding potential PST illnesses related to the consumption of crustaceans.

Case reports date from 1928, but there are suspicions of earlier, similar incidents [27, 28]. Humans and domestic animals fed suspect crab died within hours of exposure; clinical descriptions of motor paralysis, dyspnoea and sensory anaesthesia are strongly indicative of PST intoxication. Specifically, in relation to crustaceans, early accounts of illness include the following:

1. Some early case descriptions describe acute signs and symptoms of neurointoxication, for example a report from South Africa in 1882 of a mass poisoning involving some 100 individuals that had eaten "crayfish." As well as gastroenteritis, sensory anaesthesia of the lips and feet were described, muscular weakness and "... a feeling of constriction of the throat." [29].

2. Case reports from the early 20th Century began to provide more details that paralysing intoxications were involved. In 1928, five members of a Japanese family were sickened after eating a meal of soup made from two different crabs and three lobsters. Two of the individuals that ate "only the abdomen of the lobster without soup" suffered "slight numbness and aphasia" and subsequently recovered. However, another two family members died within hours of eating the soup; domestic animals that ate remains of the meal and vomitus died rapidly. Clinical descriptions of motor paralysis, dyspnoea and sensory anaesthesia are strongly indicative of an acute paralysing neurointoxication [27, 28]. Many early reports involved consumption of fresh, cooked crabs and lobsters, and incubation times were very short – minutes to hours – thus supporting suspicions of acute intoxication rather than spoilage-related food poisoning.

3. Public health workers from California, who began the first systematic investigations into so-called PSP in the 1920s, found that the Pacific sand crab – a suspension feeder – was toxic, with the toxin concentrated "in the intestinal organs, probably the liver..." The toxic component was also noted to be water-soluble and heat stable in acidic solution [30]; these features were subsequently shown to be chemical properties of the PSTs.

4. Unconfirmed reports of illness [31] in the Quebec area of Canada following consumption of lobsters initiated a series of observational and experimental investigations into PST accumulation and depuration in lobster by Canadian researchers.

It should be noted that the early case descriptions of neurointoxication following consumption of crab or lobster tissues were not unequivocal findings of saxitoxin poisoning. Likewise for investigations of crab toxicity that were conducted solely by bioassay (usually employing laboratory mice). Such reports and investigations could not reliably discriminate between the presence of saxitoxin and other rapidly-acting neurotoxins, particularly tetrodotoxin or palytoxin, both of which have been found in crabs; tetrodotoxin and saxitoxin-group toxins can co-accumulate in crabs [32-34].

A more recent report of illness occurred in 2000. A young man died in East Timor after eating a xanthid crab. Significant levels of PSTs were detected by functional assays and High Pressure Liquid Chromatography (HPLC) in forensic samples and the remains of the crab [35].

Consistent with this illness report, xanthids and other crab families are known to be capable of bioaccumulating PSTs [7, 14, 15]; crabs sampled from Australian waters are among those found by confirmatory analytical procedures to contain saxitoxin analogues [36].

Hazard Characterisation

Following the consumption of toxic shellfish, PSP symptoms can occur within minutes or up to 12 hours after the meal [3, 23, 24, 37]. PSTs bind to voltage dependent sodium channels and effectively plug the channel and stop sodium from flowing through. This prevents nerve transmission impulses and leads to the paralytic effects of PSTs in humans e.g. muscular paralysis, respiratory distress etc.

Acute Toxicity

The intra-peritoneal toxicity of 14 major PST congeners to mice has been evaluated (Table 2) [9]. These values are used as the basis for Toxicity Equivalence Factors (TEFs) which are applied by laboratories that use chemical based methods of analysis to account for differences in toxicity between congeners [3].

Table 2. Specific toxicities (established via intra-peritoneal route in mice) of the Paralytic Shellfish Toxins and the 'Toxicity Equivalence Factors' proposed by the European Food Safety Authority (EFSA).

Toxin	Specific Toxicity (MU μmol^{-1})	EFSA Proposed TEFs
STX	2483	1.0
GTX1	2468	1.0
NeoSTX	2295	1.0
GTX4	1803	0.7
GTX3	1584	0.6
dcSTX	1274	1.0
dcGTX3	935*	0.4
GTX2	892	0.4
dcGTX2	382*	0.2
C2	239	0.1
B1 (GTX5)	160	0.1
C4	143	0.1
C3	33	-
C1	15	-

*dcGTX2 and dcGTX3 were originally reported as 1617 and 1872 MU μmol^{-1} respectively [9]. These values were corrected in a later publication.

It should be noted, that while the mouse intra-peritoneal potency of the major congeners has been determined, information on the oral potency of these congeners is scant.

The oral toxicity of PSTs to mice was assessed by Health Canada in the late 1950s. The toxicity study was undertaken using purified STX prepared from toxic shellfish. The LD_{50} of STX was found to be 263 µg/kg by oral administration (mode of oral administration not noted), compared with 10 µg/kg by intra-peritoneal inoculation [38]. Further oral dosing studies are required for the other major PST congeners found in shellfish to enable more

relevant TEFs to be established. A significant international effort led by the National Research Council (Canada) and the Cawthron Institute (Nelson, NZ) is underway and aims to provide information on the mouse oral toxicity of major PST congeners in the next 2-3 years.

Toxicity in Humans

In 2004 and 2009 the World Health Organisation/Intergovernmental Oceanographic Commission/Food and Agricultural Organisation (WHO/IOC/FAO) and the European Food Safety Authority (EFSA) respectively, reviewed data related to human poisonings from PSTs. This involved reviewing data from approximately 20 illness outbreaks in Canada [39] and around 500 reported cases of illness [3]. The illness cases reviewed were utilised to establish a lowest-observed-adverse-effect-level (LOAEL). The WHO/IOC/FAO Expert Consultation and EFSA both utilised a safety factor of 3.0 to arrive at a no-observed-adverse-effect level (NOAEL) and derive an acute reference dose (ARfD)[3]. Table 3 shows the LOAEL's and ARfD's estimated by the EFSA Panel and the WHO/IOC/FAO Expert Consultation.

Table 3. Lowest-Observed-Effect Level and Acute Reference Dose for PSTs

	EFSA Panel	WHO/IOC/FAO Expert Consultation
Lowest-Observed-Effect Level (µg kg^{-1} bw)	1.5	2.0
Acute Reference Dose (µg STX equivalents kg^{-1} b.w.)	0.5	0.7

bw = body weight

Maximum Permissible Level

Following the derivation of the acute reference dose, both the FAO/WHO/IOC and EFSA made recommendations on appropriate maximum permissible (guidance) levels in bivalve shellfish. These were based on various bivalve meal sizes. FAO/WHO/IOC suggested appropriate guidance levels of 0.11, 0.17 and 0.42 mg kg^{-1} shellfish (based on consumption of 380, 250 and 100g of shellfish respectively); whereas, EFSA suggested an appropriate guidance level of 0.075 mg kg^{-1} shellfish (based on a meal size of 400g). This compares with an existing maximum level for PSTs in shellfish of 0.8 mg kg^{-1}. The maximum level of 0.8 mg kg^{-1} has been in place since 1958 in northern America and is now the level utilised by the vast majority of shellfish producing countries [40]. The Australia New Zealand Food Standard Code (standard 1.4.1) also lists a maximum level of 0.8 mg kg^{-1} for PSTs in bivalve shellfish. It is widely accepted as providing adequate public health protection and is recommended in the draft Codex standard for bivalves. A maximum permissible level has not been specifically derived for lobsters to our knowledge, however it appears that some countries utilise the

[3] Direct Quotation from the EFSA Scientific Opinion on Marine Biotoxins in Shellfish – Saxitoxin Group: *"From the available reports on intoxications in humans, comprising more than 500 individuals, a lowest-observed-adverse-effect-level (LOAEL) in the region of 1.5 µg STX equivalents/kg b.w. could be established. Because many individuals did not suffer adverse reactions at higher intakes it is expected that this LOAEL is close to the threshold for effects in sensitive individuals. Therefore the CONTAM Panel concluded that a factor of 3 was sufficient to move from this LOAEL to an estimated no-observed-adverse-effect level (NOAEL) of 0.5 µg STX equivalents/kg b.w."*

bivalve PST level of 0.8 mg kg^{-1} as a basis for issuing public health warnings and restricting trade of potentially contaminated lobster hepatopancreas.

Risk Management Advisories

A brief review of the literature shows that various countries issue public health advisories for lobsters and crabs during algal blooms, examples of such advisories include:

1. Sabah, Malaysia: Public health circulars during toxic blooms advise that "All fish, shrimp, crabs and lobsters are safe to be eaten on condition that their gills and guts are removed..." [41]

2. Philippines: Public health announcements during red tide events advise that "fish and other invertebrates caught even from red tide areas are safe for human consumption, provided that they are fresh, eviscerated and washed thoroughly before cooking." [42]

3. USA: Advisories against eating lobster hepatopancreas have been "in effect for years in Maine, neighboring states, and by U.S. Food and Drug Administration (FDA), mostly due to the presence of PCB toxins, and more recently due to PSP". http://www.maine.gov/dmr/rm/public_health/shellfishadvisory.htm

4. USA: A conservative action level of 0.7 mg kg^{-1} viscera for Dungeness crab (*Cancer magister*) has been established by the State of Alaska Food Safety and Sanitation Program. When levels exceed 0.7 mg kg^{-1} viscera product cannot be marketed live or whole cooked but must be eviscerated at a processing facility where it is landed. http://www.dec.alaska.gov/eh/fss/seafood/Docs/2012CrabPSPMonitoringPlan_5-21-12.pdf

5. New Zealand: Advisories against eating abalone, crayfish and crab gut are issued during significant PSP bloom events. Several blooms are currently active in New Zealand and the following public warning has been issued: "Shellfish in the affected area should not be taken or consumed. Paua, crayfish and crabs can still be taken but as always, the gut should be removed before cooking". http://www.foodsmart.govt.nz/food-safety/hunting-collecting-fishing/seafood-gatherers/

Preliminary Exposure Assessment

Potential Mode of PST Uptake in Rocklobsters

Research results suggest that southern rocklobsters consume a range of foods, including ascidians, gastropods, urchins, macroalgae and bivalves [43]. There is also some suggestion that larger size lobsters (>80 mm) are more likely to consume bivalves and fish than smaller specimens [44]. Given the ability of bivalves (and gastropods to a lesser extent) to bioaccumulate significant levels of PSTs, it seems likely that PSTs concentrate in rocklobster hepatopancreas via the act of feeding on contaminated bivalves and gastropods. In the Australian context, no research has been undertaken on the kinetics of PST contamination of lobsters in the wild, so mode of uptake needs to be explored through monitoring of PST uptake and elimination in lobsters and their sources of food.

Global Occurrence of Paralytic Shellfish Toxins in Rocklobsters

A review of the literature on PSTs in lobster has been conducted. The bibliographic databases PubMed and Web of Science were accessed using the search string "lobster AND (paralytic OR saxitoxin)." Journal titles and abstracts were perused to identify publications discussing the presence of PSTs in lobster from the perspectives of food safety, public health, toxinology, toxicology, analytical chemistry, phycology or oceanography. Bibliographies of papers thus identified were also reviewed to find additional reports that met these criteria. Google Scholar was also searched with the string "lobster AND saxitoxin (OR paralytic) AND illness AND hepatopancreas".

The above search identified 18 publications which describe the detection of PSTs in lobsters from a range of different countries, including South Africa, USA, Canada, Japan, China and Australia. It is acknowledged that PSTs may also occur in lobsters from other countries which were not revealed in this literature review (e.g. New Zealand), presumably because these findings have not been published in the peer reviewed scientific literature. Table 4 presents the results of the literature review, key highlights include:

- There is one report in the literature of PSTs being detected at approximately 2x the guidance level for shellfish in the 'gut tissue' of lobsters harvested from Victoria;
- Generally it appears that the hepatopancreas is the main affected organ;
- Four publications suggest that tail meat can contain low levels of PSTs;
- Cooking (via boiling or steaming) may reduce PST levels in the hepatopancreas;
- Several studies have investigated PST elimination time frames (in tanks) for Canadian lobsters, but no such work has been undertaken on southern rocklobsters.

Table 4. Paralytic Shellfish Toxins in Lobsters

Year	Region	n	Range PSTs (mg kg^{-1})	Other results	Action	Refs
1967	South Africa Cape rocklobster (*Jasus lalandii*)	NS	NS	Stomach contents toxic in lobsters sampled where toxic mussels found		[16]
NS	Maine Experimental uptake & depuration study	12	0.5 & 1.2 in "guts"	No toxicity in tail meat		[45]
NS	USA: New Hampshire Experimental feeding study	4	0.6 HP	0.03 mg kg^{-1} in tail muscle 0.21 mg kg^{-1} in appendage muscle		[46]
1976	Sabah, Malaysia Spiny lobster, *Panulirus versicolour & P. Longipes*	11	Tail meat: ND (*n*=5) Body only: ND (*n*=1) Whole lobster: 0.3 & 0.4 (*n*=2) Body & legs: 0.3 (*n*=1) Body only: 0.3 (*n*=1) Head & appendages: 0.3 (*n*=1)		As part of a survey of a wide range of seafood products following a shellfish-related PSP outbreak in 1976 in which 105 hospitalised & four fatalities	[47]
1990	Canada: Quebec & Nova Scotia (Gulf St Lawrence & Bay of Fundy)	30 raw HP 195 cooked HP Total 225	<0.4 – 6	Only HP toxic, no muscle toxicity	Advisories to consume no more than two HPs at any one sitting	[31]
1990	Canada: Novia Scotia & New Brunswick	20 (? *n*=10 per treatment or *n*=20 per Rx ?)	1.24 HP raw 0.64 HP cooked	Lobster cooked for 20 mins		[48]
NS	New Brunswick, Canada Experimental uptake & depuration study	NS	2.75 – 32 HP	Tail muscle non-toxic. Toxins "are readily excreted."		[49]
1991	Canada: Quebec, Newfoundland, Nova Scotia, New Brunswick, PEI	466 cooked HP	<0.4 – 7.2			[31]
NS	New Brunswick, Canada (?) Experimental uptake & depuration study	NS	21.5 after 4 weeks feeding of toxic scallop	After 8 weeks feeding with toxic scallops, feed switched to non-toxic feed. PSTs below 0.8 mg kg^{-1} by 70 days. Toxins never detected in muscle. Congener profile differed between scallop and lobster HP		[50]

Year	Region	n	Range PSTs (mg kg^{-1})	Other results	Action	Refs
NS	Quebec 7 Nova Scotia, Canada	?45 ?90	0.13 – 5.2 HP raw 0.04 – 2.65 HP steamed 0.02 – 1.5 HP boiled	Low concentrations of STX found in tail meat, although concentrations were higher after cooking (max 0.027mg kg^{-1})		[51]
NS	Gulf of Maine, USA Experimental feeding study	NS	4.3 raw HP 1.1 cooked HP			[15]
NS	Maine, USA	NS	16.5 raw HP 5.4 cooked HP	Muscle non-toxic		[15]
1990	Gaspé Bay, Quebec	139	0.45 – 15.5 HP; haemolymph non-toxic. All samples (meat & HP) contained measureable PSTs by HPLC: range 0.02 – 0.69mg kg^{-1} in muscle	High toxin variability despite homogeneous sampling of lobster. No significant depuration after 53 days in cold water. After cooking, 31% were more toxic & 69% were less toxic (but sample size and baseline toxicities not stated)		[52-54]
1992	Western Victoria, Australia	NS	Max 1.8 "gut tissues"			[55]
2008 2010	Maine & New Hampshire, USA	NS	NS	"...dangerous levels of [PSTs] in lobster tomalley."	Advice to not consume lobster hepatopancreas, regardless of origin (from Northeast Canada to South Carolina, inclusive)	[8, 56]
NS	China Experimental uptake and depuration studies Spiny lobster (*Panulirus stimpsoni*)	5	0.02 – 0.12 HP	x3 treatments: feeding with toxic scallops for 6 days; depuration 2 – 6 days Muscle and other tissues non-toxic		[57]
2003	New Brunswick, Canada	19	0.36 – 4.5 (means)	Up to 0.5 mg kg^{-1} in gonad; up to 0.16 mg kg^{-1} in tail meat		[58]
2007	New Brunswick, Canada	NS	0.54 HP			[59]

Lobster species were American lobster (*Homarus americanus*) except where indicated (Column: "Region")
PST concentrations reported in mouse units were converted to STX eq. 1MU = 0.18 µg STX [7]
PST concentration units: mg STX eq per kg lobster tissue
NS: Not stated

Occurrence of PSTs in Lobsters, North East Tasmania, 2012

Table 5 provides a summary of the PST levels (STX-equivalents) recorded in hepatopancreas samples of lobsters harvested (to date) during the 2012 *A. tamarense* bloom in north east (NE) Tasmania. All samples included in Table 5 represent individual lobster hepatopancreas samples (e.g. from single animals) which were sampled from locations within the area affected by the *A. tamarense* bloom (Figure 1). The average PST content of hepatopancreas samples tested across locations within the affected zone was 0.99 mg kg^{-1} compared with the maximum level of 0.8 mg kg^{-1} for bivalve shellfish. The maximum value to date was 2.82 mg kg^{-1}.

On several occasions replicate animals have been sampled from the same location at the same time, this has revealed large variation in PST levels in the hepatopancreas between animals harvested at the same site. For example, on 11/12/2012 five lobsters were harvested from the same location at Maria Island and the hepatopancreas of each animal was tested for PSTs. PST levels in the 5 hepatopancreas ranged from 'not detectable' to 1.9 mg kg^{-1} (2.4 times greater than the maximum regulatory level of 0.8 mg kg^{-1}).

Two lobster hepatopancreas samples (each sample comprised a pooled homogenate of three individual lobster hepatopancreas) from lobsters harvested outside (further south) the southern boundary (Marion Bay) of the affected zone were also tested, these lobsters only contained trace levels of dcGTX2,3 (0.073 and 0.056 mg kg^{-1}) and were compliant with the maximum permissible level for bivalves. This supports the contention that the elevated PST levels detected in hepatopancreas tissues of lobsters harvested within the affected zone are most likely related to the occurrence of the *A. tamarense* bloom.

Twelve samples of lobster muscle tissue (leg and tail meat) have also been tested from lobsters harvested within the affected region during the *A. tamarense* bloom event (muscle tissue from 10 individual lobsters harvested from 5 different locations were tested separately, and 2 samples of muscle tissue pooled from 3 different animals were tested). To date, no PSTs have been detected in any of the lobster muscle samples.

Table 5. Paralytic Shellfish Toxin concentrations in lobster hepatopancreas, NE Tasmania, 2012

Sample date	Location*	STX-eq (mg kg^{-1})
11/11/12	Sarah Ann Bay, Schouten Is	0.94
11/11/12	W Sarah Ann Bay	1.68
11/11/12	W Sarah Ann Bay	2.82
11/11/12	Buttons Bay Nth	ND (=0)
11/11/12	Haunted Bay, Sth Maria Is	0.27
11/11/12	Haunted Bay, Sth Maria Is	0.92
11/11/12	Hellfire Bluff	0.46
11/11/12	Painted Cliffs, Maria Is	1.96
11/11/12	Painted Cliffs, Maria Is	1
11/11/12	Painted Cliffs, Maria Is	2.71
29/11/12	Block 30C	ND (=0)
29/11/12	Block 30C	1.1
29/11/12	Block 30C	0.3
28/11/12	Maria Is 24C	1.1
28/11/12	Maria Is 24C	0.79
28/11/12	Maria Is 24C	0.85
7/12/2012	Bicheno	0.92
7/12/2012	Bicheno	0.98
7/12/2012	Bicheno	2.7
7/12/2012	Bicheno	2.1
7/12/2012	Bicheno	1.2
11/12/2012	Sarah Ann Bay	0.18
11/12/2012	Sarah Ann Bay	0.074
11/12/2012	Sarah Ann Bay	0.11
11/12/2012	Sarah Ann Bay	ND (=0)
11/12/2012	Sarah Ann Bay	0.35
11/12/2012	Painted Cliffs, Maria Is	1.9
11/12/2012	Painted Cliffs, Maria Is	ND (=0)
11/12/2012	Painted Cliffs, Maria Is	1.6
11/12/2012	Painted Cliffs, Maria Is	0.89
11/12/2012	Painted Cliffs, Maria Is	0.8
	Mean	**0.99**
	SD	**0.84**
	Max	**2.82**

*Note. Only samples collected from within the boundaries affected by the *A. tamarense* bloom (Figure 1) have been included.

Toxin Profile

Analysis of the PST monitoring data has been undertaken to investigate the major PST congeners present in various seafood species collected during the 2012 *A. tamarense* event. In relation to the samples included in this analysis, the following should be noted:

1. Only samples from within the affected region depicted in Figure 1 have been included;
2. Only samples on which HPLC confirmation tests were undertaken were included (screen results were excluded);
3. Samples containing <0.1 mg kg^{-1} STX-eq were excluded;
4. Other than samples meeting criteria 2 and 3 above, no other samples tested to date have been excluded to our knowledge.

The following provides an overview of the toxin profile (on a toxicity basis) for lobster hepatopancreas, mussel, oyster and scallop roe samples.

The toxin profile of lobster hepatopancreas is dominated by GTX-2,3 and STX, these are mid-range and high toxicity congeners respectively (as determined by the intraperitoneal route). On average (n=19) 45% of the toxins present in the samples was attributable to GTX-2,3 and 29% to STX when reported on a toxicity basis (Figure 2). Also present were C1,2 (11%), dcGTX-2,3 (7%) and dcSTX (5%). Of note, GTX-1,4 and C3,4 were not detected in any of the hepatopancreas samples. The toxin profile for southern rocklobster appears similar to that described previously for other species (see Hazard Identification section).

Similarly, mussels (n=9) tested during the same algal bloom event were also dominated by GTX-2,3 (53%) and STX (18%). C1,2 (17%), dcGTX-2,3 (7%) and GTX-1,4 (3%) were also present in the mussel samples (Figure 3).

In contrast to lobster hepatopancreas and mussels, oysters (n=4) and scallops (n=5) sampled from within the affected region of the bloom had relatively simple toxin profiles with only 3 congeners detected in each species (Figures 4 and 5). STX and GTX-2,3 was detected in both scallops (contributing 72% and 24% to the total toxicity respectively) and oysters (contributing 30% and 27% to the total toxicity respectively). C1,2 also contributed approximately 43% of the total PST toxicity in oysters, and a small amount of dcSTX was detected in scallops.

It should be noted that only a small number of samples were used to generate the average percentage contribution values for oysters and scallops, further monitoring data are required to improve the confidence in these results. Further data analysis should also be undertaken to ascertain the toxicity profiles on a molar basis to supplement the information obtained from these interim results.

Figure 2: Plot shows the contribution of each congener to the total PSP level on a toxicity basis in Tasmanian lobster hepatopancreas samples analysed using the PSP confirmation test (n=19). Samples with <0.1 mg kg^{-1} STX-eq were excluded from the analysis.

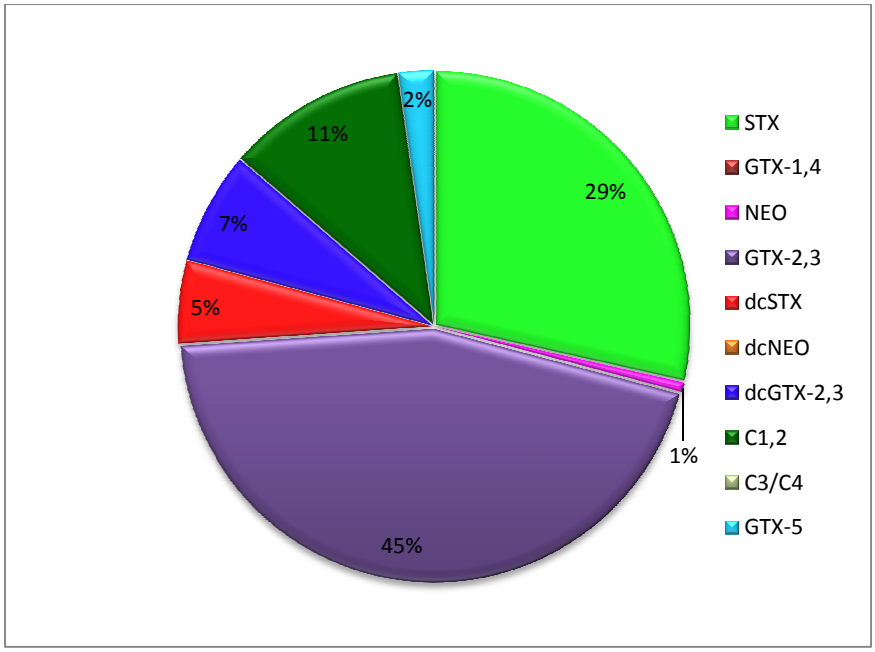


Figure 3 Plot shows the contribution of each congener to the total PSP level on a toxicity basis in Tasmanian mussel samples analysed using the PSP confirmation test (n=9). Samples with <0.1 mg kg^{-1} STX-eq were excluded from the analysis.

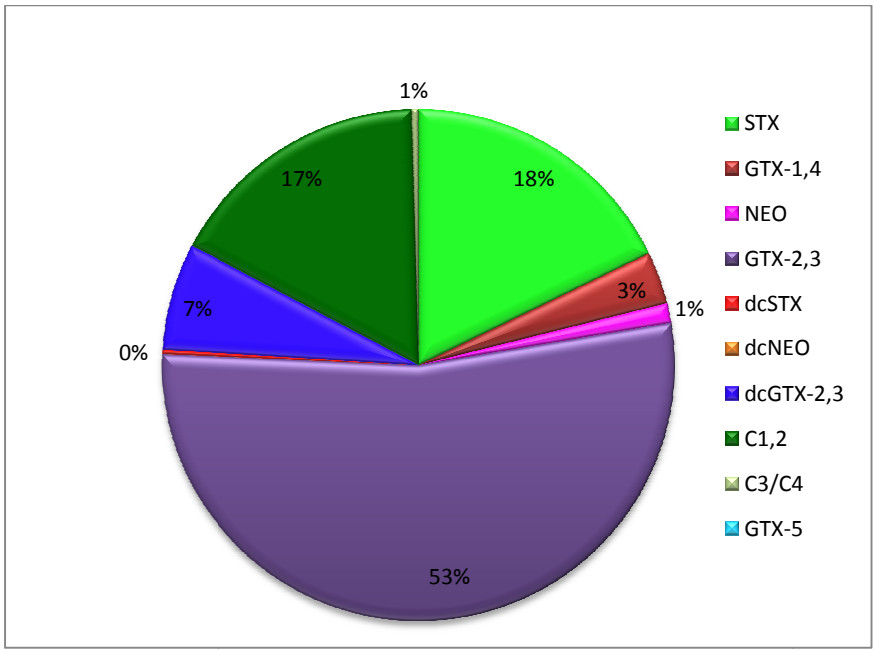


Figure 4: Plot shows the contribution of each congener to the total PSP level on a toxicity basis in Tasmanian oyster samples analysed using the PSP confirmation test (n=4). Samples with <0.1 mg kg^{-1} STX-eq were excluded from the analysis.

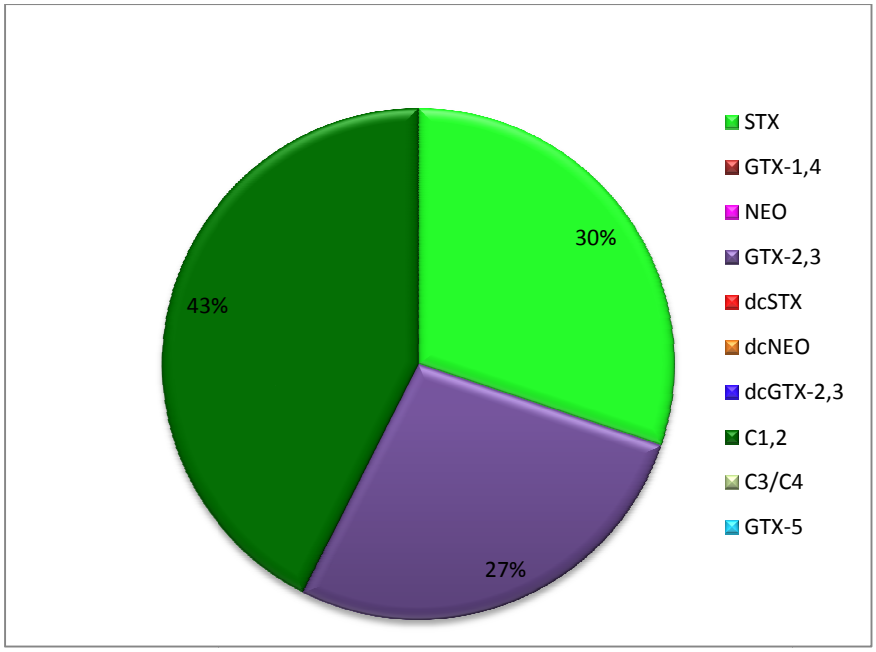
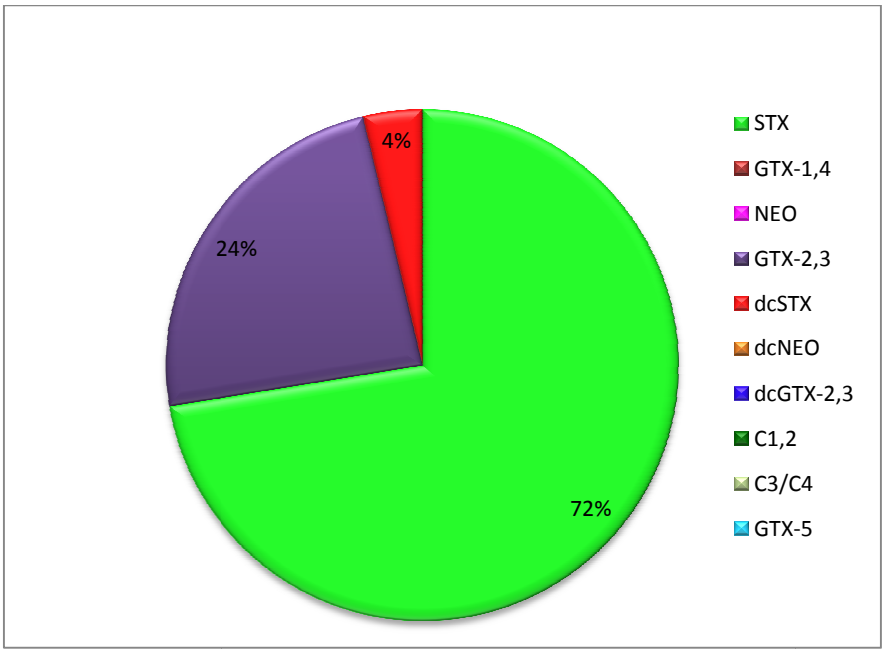


Figure 5: Plot shows the contribution of each congener to the total PSP level on a toxicity basis in Tasmanian scallop roe samples analysed using the PSP confirmation test (n=5). Samples with <0.1 mg kg^{-1} STX-eq were excluded from the analysis.



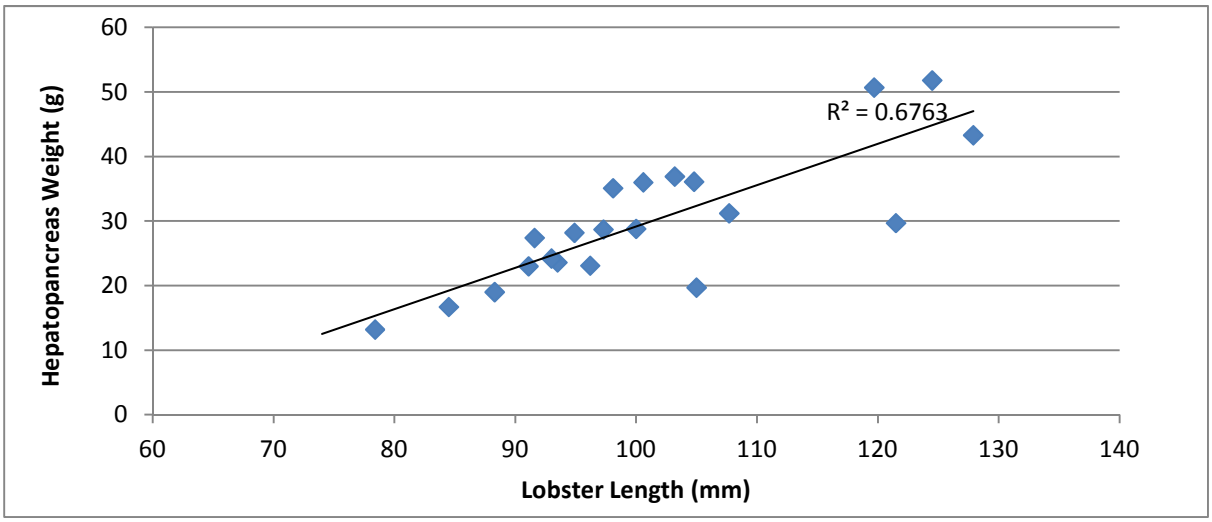
Lobster Length, Weight and Sex

As noted previously, it is likely that lobsters become contaminated through feeding off bivalves and gastropods which contain PSTs and there may be some differences in the feeding habits of larger vs. smaller size lobsters, with larger lobsters potentially more likely to consume bivalves than smaller lobsters [44]. Given the foregoing, we undertook a brief analysis of data to ascertain whether there was any relationship between lobster size, lobster hepatopancreas weight and PST levels in the hepatopancreas.

Briefly, for lobsters collected from the affected area during this algal bloom event for which length, weight, sex and toxin data are available (n=23):

- There was a reasonable linear relationship between lobster length (mm) and hepatopancreas weight (g) (Figure 6).
- No relationship was observed between the length of the lobster and PST level in the hepatopancreas, or between the weight of the hepatopancreas and the PST level (data not shown).
- No relationship was observed between sex of the lobster and PST level in the hepatopancreas (data not shown).

Figure 6. Lobster length vs. hepatopancreas weight for animals collected from the north east region of Tasmania during the 2012 *Alexandrium tamarense* event.



Exposure to PSTs through Consumption of Lobster Hepatopancreas

As noted previously, an Acute Reference Dose (ARfD) for saxitoxin equivalents was determined by EFSA in 2009, this led to the recommended guidance level of 0.075 mg kg^{-1} shellfish (based on a meal size of 400g). However, the Australia New Zealand Food Standard Code (standard 1.4.1) lists a maximum level of 0.8 mg kg^{-1} for PSTs in bivalve shellfish (as do Codex) and this is widely accepted as providing adequate public health protection. The 0.8 mg kg^{-1} maximum level is the established health guideline value for saxitoxins in bivalve

shellfish used by Food Standards Australia New Zealand (FSANZ) and Shellfish Quality Assurance Programs around Australia and has therefore, been utilised for exposure considerations in this assessment[4].

As noted in Table 5, lobster hepatopancreas have been found to exceed the maximum level of 0.8 mg kg^{-1} during the 2012 *A. tamarense* bloom. A question has been posed as to whether the consumption of PSTs in lobster hepatopancreas from the affected region poses a risk to human health. To assist risk managers to evaluate the risk to public health a comparison between the amount of PSTs present in lobster hepatopancreas with a Designated Dose (DD) has been made. The DD of PST has been derived based on the existing maximum level of 0.8 mg kg^{-1} and using the following equation[5]:

$$\mathrm{DD} = (\mathrm{ML} \times \mathrm{Serve}) \div \mathrm{BW}$$

Where:

- **ML** denotes Maximum Level (0.8 mg kg^{-1})
- **BW** denotes Body Weight (74 kg)
- **DD** denotes Designated Dose (µg kg^{-1} body weight)
- **Serve** denotes serving size (178 g)

To derive the DD, we assumed a maximum meal size for mussels of 178 g (as this was the most relevant consumption data available), as applied to an average 74k g adult [60], and utilised the Food Standards Code maximum level of 0.8 mg kg^{-1} STX eq in shellfish (and assumed this is a safe level based on no historical human illness)[6]. Using this approach, the DD is 1.9 µg STXeq kg^{-1} body weight.

To account for varying body weight of humans, various exposure scenarios for children and adults weighing between 10 and 100 kg are presented, using the average and maximum lobster hepatopancreas PST concentrations detected in the *A. tamarense* event (as determined in Table 5), and the average and maximum hepatopancreas sizes. Table 6 gives estimates of the maximum number (or part thereof) of lobster hepatopancreas that may be consumed without exceeding the DD (as calculated above) in a 24-hour period.

This provisional exposure assessment has been conducted using a simple formula with body weight as the sole consideration of relative differences in exposure. We have extended the formula to incorporate paediatric body weights, but the data generated from this exercise needs to be viewed with caution. The developmental toxicity of saxitoxin is poorly understood; there are significant knowledge gaps regarding the impact of these sodium channel antagonists on developing neural systems and the related capacity of developing

[4]A maximum permissible level has not been specifically derived for lobsters to our knowledge, however it appears that some countries utilise the bivalve PST level of 0.8 mg kg^{-1} as a basis for issuing public health warnings and restricting trade of potentially contaminated lobster hepatopancreas. The use of the maximum level for bivalves in this assessment was recommended by FSANZ and Tasmanian DHHS as it is considered more relevant to the Australian situation than the ARfD that EFSA set in 2009.

[5]Based on an equivalent calculation utilised by EFSA to obtain the acute reference dose (ARfD).

[6]No data is available on average size of lobster hepatopancreas meals. Advice was received from FSANZ and Tasmanian DHHS that the most appropriate data available for use in Australia relates to that of mussels as published in Mulvenna *et al* [60]. The average adult weight (74kg) utilised in the Mulvenna publication was also used for these estimates.

organs of metabolism and elimination. The adage that "children are not just small adults" should be considered here [61].

Number of specified lobster tissues (hepatopancreas or muscle) consumed to reach the DD $= \frac{BW*DD}{Conc*TW}$

Where

- **BW** denotes body weight (kg)
- **DD** denotes Designated Dose of 1.9 μg kg^{-1} body weight as determined from the existing maximum level of 0.8 mg kg^{-1} (see above)
- **Conc** denotes measured concentration (mean or maximum) of STXeq in lobster tissue (μg kg^{-1} tissue weight)
- **TW** denotes the weight (mean or maximum) of lobster tissue consumed (kg).

Table 6. Number of lobster hepatopancreas that may be eaten during the 2012 NE Tasmania event without exceeding the Designated Dose of 1.9 μg kg^{-1} body weight.

Body weight (kg)	**STX consumed to reach Designated Dose (μg)**	**Number of lobster hepatopancreas that may be consumed without exceeding the Designated Dose for PSTs (rounded)**			
		[1]Average HP size – raw		**[1]Maximum HP size - raw**	
		[2]Mean STX	**[2]Max STX**	**[2]Mean STX**	**[2]Max STX**
10	19	0.8	0.3	0.4	0.1
15	29	1.1	0.4	0.6	0.2
40	77	3.0	1.1	1.5	0.5
60	115	4.5	1.6	2.3	0.8
70	135	5.3	1.9	2.6	0.9
85	164	6.4	2.3	3.2	1.1
100	192	7.6	2.7	3.8	1.3

[1]Average weight of raw lobster hepatopancreas sampled during the 2012 bloom = 25.7 g; maximum weight of raw lobster hepatopancreas = 51.8 g (n = 37; SD: 11.0).

[2]Average STXeq concentration in raw hepatopancreas sampled during the 2012 bloom: 0.99 mg kg^{-1}; maximum STXeq concentration in raw hepatopancreas: 2.82 mg kg^{-1} (n = 31; SD: 0.84).

In summary, Table 6 suggests:

- A 70 kg adult, eating a maximum-sized raw lobster hepatopancreas at the maximum PST level detected in the Tasmanian 2012 event to date could consume up to 0.9 hepatopancreas without exceeding the DD (as calculated above from the current PST maximum limit in Australia of 0.8 mg kg^{-1}).
- A 15 kg child, eating an average-sized raw lobster hepatopancreas at the maximum PST level detected in the Tasmanian 2012 event could consume up to 0.4 hepatopancreas without exceeding the DD (as calculated above from the current PST maximum limit in Australia of 0.8 mg kg^{-1}).

Mean and maximum STX levels utilised in Table 6 are for lobsters collected from zones in which other fisheries – mussels, oysters, scallops, etc – were impacted by the 2012 *A. tamarense* PST event, and subject to temporary closures as a result. Lobsters sampled from outside these impacted zones and analysed for PST concentrations were not included in the dataset used for determination of mean PST levels in hepatopancreas and muscle.

For comparative purposes, similar scenarios have been developed for humans weighing between 10 and 100 kg exposed to lobster hepatopancreas PST concentrations detected in selected US and Canadian algal bloom events (from the early to mid 1990s). Table 7 gives estimates of the maximum number (or part thereof) of lobster hepatopancreas that may be consumed in a 24-hour period from these historical US and Canadian events.

Table 7. Number of lobster hepatopancreas (and tail meat tissue) that may be eaten without exceeding the Designated Dose of 1.9 µg kg^{-1} body weight during selected Canadian and US PST events.

Body weight (kg)	**STX consumed to reach Designated Dose (µg)**	**Number of lobster hepatopancreas that may be consumed without exceeding the Designated Dose for PSTs (rounded)**						
		Reference: [51]		**Reference: [49]**	**Reference: [48]**		**Reference: [31]**	**Reference: [53]**
		[1]Raw HP: 5.2 mg kg^{-1} STXeq (maximum)	**[1]Boiled HP: 1.5 mg kg^{-1} STXeq (maximum)**	**[1]HP 32 mg kg^{-1} STXeq (maximum)**	**[1]Raw HP: 1.24 mg kg^{-1} STXeq (mean)**	**[1]Cooked HP: 0.64 mg kg^{-1} STXeq (mean)**	**[1]Cooked HP: 7.2 mg kg^{-1} STXeq (maximum)**	**[2]Raw tail meat 0.69 mg kg^{-1} STXeq (maximum)**
10	19	0.1	0.9	0.02	0.6	2.0	0.2	0.2 (28g)
15	29	0.2	1.3	0.04	0.9	3.0	0.3	0.3 (42g)
40	77	0.6	3.4	0.1	2.5	8.0	0.7	0.7 (112g)
60	115	0.9	5.1	0.1	3.7	12.0	1.1	1.0 (167g)
70	135	1.0	6.0	0.2	4.3	14.0	1.2	1.2 (195g)
85	164	1.3	7.3	0.2	5.3	17.0	1.5	1.5 (237g)
100	192	1.5	8.6	0.2	6.2	20.0	1.8	1.7 (279g)

[1]All calculations based on the assumptions of Shumway [15]: 25 g raw hepatopancreas weight and 15 g cooked HP weight from a 570 g lobster.

[2]For tail meat assumptions, we used an average recovery of muscle tissue as a proportion of whole lobster weight (=28.5 %; n = 42; SD: 5.05) from the Tasmanian dissection work, applied to the "typical" lobster weight of 570 g assumed by Shumway [15], i.e. 162 g.

In summary, Table 7 suggests:

- A 15 kg child eating lobster tail meat measured at 0.69 mg kg^{-1} could consume the flesh from 0.3 lobsters, i.e. 42 g, without exceeding the DD (as calculated above from the current PST maximum limit in Australia of 0.8 mg kg^{-1}).

- A 70 kg adult eating hepatopancreas measured at 32 mg kg^{-1} could consume 0.2 hepatopancreas without exceeding the DD (as calculated above from the current PST maximum limit in Australia of 0.8 mg kg^{-1}).

It should be recognised that no actual data are available on serving sizes of lobster hepatopancreas, the way in which lobster hepatopancreas is extracted and prepared prior to consumption, or whether it is combined with other foods prior to consumption. Lobster bisque, seafood chowder, lobster mustard (as a prepared dish), lobster paté, and hepatopancreas-infused choux pastry are just some examples by which exposure to lobster hepatopancreas can occur. Given the lack of actual data on consumption, the above estimates are based on the consumption of raw hepatopancreas when served alone without other food. Equimolar concentrations of PSTs in lobster hepatopancreas used to prepare such dishes will likely result in very different exposure scenarios, due to effects such as dilution factors, cooking techniques and portion sizes.

Cooking

Workers from North America have described effects of cooking on PST concentrations in lobster tissues. Watson-Wright *et al* [48] and Lawrence *et al* [51] reported significantly lower PST hepatopancreas levels in lobsters that were cooked or steamed, compared to control groups. Two unpublished reports from the USA cited by Shumway [15] also show considerably reduced levels of toxin in cooked lobster hepatopancreas. Desbiens and Cembella [54] acquired paired data on individual lobsters, sub-sampling tissues (including hepatopancreas) for analysis before and after cooking. The authors noted inconsistent changes across their sample, with 31% showing increased total PST toxicity, and 69% with decreased toxicity. Thermal transformation of PST congeners during cooking was posited as a likely explanation.

In order to assess the impact of cooking on southern rocklobster hepatopancreas, ten specimens were collected from a single location in Tasmania (Sarah Ann Bay) on 11th December 2012 and randomly allocated to two groups of *n*=5 (cooked *vs.* uncooked). Lobsters were cooked according to the protocol for boiling as described on the website of Southern Rocklobster Ltd:

http://www.southernrocklobster.com/cooktechnique/boilingblanching.aspx

Briefly, lobsters were chilled, then cooked in boiling salted water (25g NaCl in 2.5L) for between seven and nine minutes, depending on size, after which the cooking process was halted by placing the animals in an ice slurry for five minutes. Lobsters were then dissected and tissues removed, weighed and prepared for PST analysis as for uncooked lobsters. Table 8 shows results of toxin analysis in this homogeneous sample.

Table 8. PST levels in hepatopancreas of cooked and uncooked lobsters, Tasmania 2012

Specimen ID	Treatment	Total PST (SXT eq) mg kg^{-1}
LOB11-1	Uncooked	0.18
LOB11-2	Uncooked	0.074
LOB11-3	Uncooked	0.11
LOB11-4	Uncooked	ND (=0)
LOB11-5	Uncooked	0.35
LOB11-6	Cooked	0.21
LOB11-7	Cooked	0.45
LOB11-8	Cooked	0.044
LOB11-9	Cooked	ND (=0)
LOB11-10	Cooked	ND (=0)

An unpaired t-test was used to compare PST levels in cooked and uncooked lobster hepatopancreas. No significant difference was seen at the 5% level: t=0.02; df=8; p=0.99. We therefore did not consider for this exposure assessment the influence of cooking on PST concentrations in lobster hepatopancreas.

The dynamics of uptake, retention and bio-transformation of PSTs in various lobster tissues prepared for human consumption will require more detailed investigation to better assess the public health implications. As discussed below, the variety of ways in which lobster is marketed, prepared and consumed is at present poorly understood by risk managers. Integral to a contemporary understanding of product preparation will be knowledge of how various tissues, including hepatopancreas, are cooked.

Data Gaps

Several data gaps have been identified in this exposure assessment. A summary of these is provided below. These data gaps mean that it is not possible at this time to estimate the probability of occurrence and severity of adverse health effects in a given population related to the presence of PSTs in lobster hepatopancreas (e.g. risk characterisation).

Probability of Exposure

To assess the probability of human exposure to PSTs through consumption of lobster hepatopancreas, the following information would be required:

- Marketing and distribution of various product formats, including how lobster hepatopancreas is extracted, marketed, prepared, combined with other ingredients and consumed.
- Data on the proportion of the Australian and overseas (predominantly Chinese) populations that consume lobster hepatopancreas.
- Dietary practices in particular ethnic communities with high rates of seafood consumption.
- Data regarding serving sizes of lobster hepatopancreas (or flesh) in Australia or key overseas markets.
- Data on the frequency of lobster hepatopancreas consumption by Australian or overseas consumers (including production and export volumes).

Probability of Contamination

To assess the likelihood of lobsters becoming contaminated with PSTs, the following information is required:

- Confirmation of the mode of uptake of PSTs into lobster hepatopancreas is required (e.g. how do lobsters accumulate PSTs in the hepatopancreas? Are they concentrating the toxins through consumption of contaminated bivalves or are there are other potential sources?). The following sub-points would assist in answering these questions:
    - Continued monitoring of lobsters in the affected area over the ensuing months to track toxin levels, this may enable a relationship between the *A. tamarense* bloom, levels in other shellfish and PST levels in the lobster hepatopancreas to be established.
    - PST analysis of key food items being consumed by lobsters during this *A. tamarense* bloom event (to assist in confirming contamination pathway).
    - Data on the potential for lobsters to accumulate PSTs from different types of algal blooms. Currently, we have information in Australia to suggest that *A. tamarense* and *Gymnodinium catenatum* (limited data) can lead to PST accumulation in lobster hepatopancreas, further investigations need to be undertaken to ascertain if lobsters can also accumulate high levels of PSTs following blooms of other PST-producing algae.
    - Data on 'background' level of PSTs in lobsters when harmful algal blooms are not occurring. This may assist in ruling out 'spurious' sources of PST.

- Data on the variability of PST levels in lobster hepatopancreas in animals taken from the same sites and between sites. This information is critical to inform the development of management and sampling plans, and will also assist in future risk assessment work in terms of assessing probability of contamination.
- Tissue distribution of PSTs in lobsters.
- Elimination timeframes (in wild and in tank).
- Influence of post harvest processing (e.g. cooking) composition and concentration of PSTs.

Conclusion

The review of information and data from the 2012 *A. tamarense* event in Tasmania has resulted in a series of scenarios being developed which provide estimates of the number of lobster hepatopancreas that may be consumed without exceeding the DD for PSTs (as calculated from the current PST maximum limit for bivalves in Australia of 0.8 mg kg^{-1}). The 0.8 mg kg^{-1} maximum level is the established health guideline value for saxitoxins in bivalve shellfish used by Food Standards Australia New Zealand (FSANZ) and Shellfish Quality Assurance Programs around Australia and has therefore, been utilised for exposure considerations in this assessment[7].

This assessment of PST exposure suggests that:

- A 70 kg adult, eating a maximum-sized raw lobster hepatopancreas at the maximum PST level detected in the Tasmanian 2012 event to date could consume up to 0.9 hepatopancreas without exceeding the DD.
- A 15 kg child, eating an average-sized raw lobster hepatopancreas at the maximum PST level detected in the Tasmanian 2012 event could consume up to 0.4 hepatopancreas without exceeding the DD.

These results are broadly consistent with (i.e. fall within the range of) similar data from the US and Canada, where public health regulators have also issued consumption advisories about the consumption of lobster hepatopancreas as part of their risk management.

While there are no confirmed reports of PSP illness resulting from the consumption of lobsters in Australia to our knowledge, this review has found several recorded instances of potential PSP illness related to the consumption of crabs and lobsters in other countries. These illnesses have again resulted in issuing of public health consumption advisories not to consume the gut of lobsters from the PST affected region. In addition, the PST toxin profile in lobster hepatopancreas is comprised of predominantly high and mid-range toxicity congeners which are well known to be responsible for illness when present in bivalves at levels above the maximum limit.

A significant number of data gaps have been identified which has prevented a full characterisation of the risk (e.g. determining the probability of illness). Consequently, there is considerable uncertainty in the calculation of the DD and subsequent number of lobster hepatopancreas that can be consumed. The most pressing data needs relate to: (1) obtaining robust data on consumption patterns regarding lobster hepatopancreas; and (2) information on the kinetics of uptake and elimination of PSTs from southern rocklobsters. Such information could support the future development of management strategies such as the potential for harvesting PST contaminated lobsters and depuration prior to live sale.

[7]A maximum permissible level has not been specifically derived for lobsters to our knowledge, however it appears that some countries utilise the bivalve PST level of 0.8 mg kg^{-1} as a basis for issuing public health warnings and restricting trade of potentially contaminated lobster hepatopancreas. The use of the maximum level for bivalves in this assessment was recommended by FSANZ and Tasmanian DHHS as it is considered more relevant to the Australian situation than the ARfD that EFSA set in 2009.

Acknowledgements

Funds were gratefully received from the Department of Primary Industries, Parks, Water and Environment to support this work. Data and technical advice on aspects of the Exposure Assessment were received from: Alison Turnbull and Rosalind Harrison (Department of Health and Human Services Tasmania), Hilary Revill, Grant Pullen and Rob Gott (Department of Primary Industries, Parks, Water and Environment), Utz Mueller and Matthew O'Mullane (Food Standards Australia New Zealand), and Caleb Gardner and Rafael Leon (Institute for Marine and Antarctic Studies, University of Tasmania).

References

1. McLeod, C., et al., *Semi-quantitative risk assessment of paralytic shellfish toxins in canned Australian abalone*, 2010, South Australian Research & Development Institute: Adelaide. 56 pp.

2. World Health Organization – Codex Alimentarius Commission, *Principles and guidelines for the conduct of microbiological risk assessment, CAC/GL-30*, 1999, WHO: Geneva. 6 pp.

3. Panel on contaminants in the food chain, *Scientific opinion: marine biotoxins in shellfish - saxitoxin group.* Eur Food Saf Auth J, 2009. 7(4): 76 pp.

4. Van Egmond, H.P., M.E. Van Apeldoorn, and G.J.A. Speijers, *Marine biotoxins - FAO food and nutrition paper 80*, 2004, Food and Agriculture Organization of the United Nations: Rome. i-xii; 1-278.

5. Dell'Aversano, C., P. Hess, and M.A. Quilliam, *Hydrophilic interaction liquid chromatography--mass spectrometry for the analysis of paralytic shellfish poisoning (PSP) toxins.* J Chromatogr A, 2005. 1081(2):190-201.

6. Dell'Aversano, C., et al., *Isolation and structure elucidation of new and unusual saxitoxin analogues from mussels.* J Nat Prod, 2008. 71(9):1518-23.

7. Deeds, J.R., et al., *Non-traditional vectors for paralytic shellfish poisoning.* Mar Drugs, 2008. 6(2):308-48.

8. Etheridge, S.M., *Paralytic shellfish poisoning: seafood safety and human health perspectives.* Toxicon, 2010. 56(2):108-22.

9. Oshima, Y., *Postcolumn derivatization liquid-chromatographic method for paralytic shellfish toxins.* J AOAC Int, 1995. 78(2):528-532.

10. Anderson, D.M., et al., *Biogeography of toxic dinoflagellates in the genus* Alexandrium *from the northeastern United States and Canada.* Mar Biol, 1994. 120(3):467-478.

11. Anderson, D.M., et al., *Toxin composition variations in one isolate of the dinoflagellate* Alexandrium fundyense*.* Toxicon, 1990. 28(8):885-93.

12. Chang, F.H., et al., *Toxin production of* Alexandrium minutum *(Dinophyceae) from the Bay of Plenty, New Zealand.* Toxicon, 1997. 35(3):393-409.

13. Wang, D.Z., et al., *Paralytic shellfish toxin profiles and toxin variability of the genus* Alexandrium *(Dinophyceae) isolated from the Southeast China Sea.* Toxicon, 2006. 48(2):138-51.

14. Landsberg, J.H., *The effects of harmful algal blooms on aquatic organisms.* Rev Fish Sci, 2002. 10(2):113-390.

15. Shumway, S.E., *Phycotoxin-related shellfish poisoning: bivalve molluscs are not the only vectors.* Rev Fish Sci, 1995. 3(1):1-31.

16. Grindley, J.R. and N. Sapieka, *The cause of mussel poisoning in South Africa.* S Afr Med J, 1969. 43(10):275-9.

17. Jaime, E., G. Gerdts, and B. Luckas, *In vitro transformation of PSP toxins by different shellfish tissues.* Harmful Algae, 2007. 6(3):308-316.

18. Llewellyn, L., A. Negri, and A. Robertson, *Paralytic shellfish toxins in tropical oceans.* Toxin Rev, 2006. 25(2):159-196.

19. Homan, N., et al., *Uptake, distribution and depuration of paralytic shellfish toxins in Australian greenlip abalone,* Haliotis laevigata 2010, South Australian Research and Development Institute: Adelaide. 26 pp.

20. Kotaki, Y., et al., *Identification of a calcareous red alga as the primary source of paralytic shellfish toxins in coral reef crabs and gastropods.* Nippon Suisan Gakkai Shi, 1983. 49(2):283-286.

21. Martinez, A., et al. *PSP toxicity in* Haliotis tuberculata *from NW Spain*. in *5th International Conference on Toxic Marine Phytoplankton*. 1993. Newport, RI: Elsevier pp. 419-423.

22. Pitcher, G.C., et al., *Paralytic shellfish poisoning in the abalone* Haliotis midae *on the west coast of South Africa.* J Shellfish Res, 2001. 20(2):895-904.

23. Gessner, B.D. and J.P. Middaugh, *Paralytic shellfish poisoning in Alaska: a 20-year retrospective analysis.* Am J Epidemiol, 1995. 141(8):766-70.

24. Van Dolah, F.M., *Marine algal toxins: origins, health effects, and their increased occurrence.* Environ Health Perspect, 2000. 108 Suppl 1:133-41.

25. Hallegraeff, G.M., *A review of harmful algal blooms and their apparent global increase.* Phycologia, 1993. 32(2):79-99.

26. Hallegraeff, G.M., *Harmful algal blooms in the Australian region.* Mar Pollut Bull, 1992. 25(5-8):186-190.

27. Garth, J.S. and A.C. Alcala. *Poisonous crabs of Indo-West Pacific coral reefs, with special reference to the genus* Demania *Laurie*. in *3rd International Coral Reef Symposium*. 1977. Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL pp. 645-651.

28. Hashimoto, Y., et al., *Occurrence of toxic crabs in Ryukyu and Amami Islands.* Toxicon, 1967. 5(2):85-90.

29. Halstead, B., *Poisonous and venomous marine animals of the world. Volume 1 - invertebrates*. 1965, Washington DC: US Department of Defense. 994 pp.

30. Sommer, H., *The occurrence of the paralytic shell-fish poison in the common sand crab.* Science, 1932. 76(1981):574-5.

31. Todd, E.C.D., et al. *Recent illnesses from seafood toxins in Canada: paralytic, amnesic and diarrhetic shellfish poisoning*. in *5th International Conference on Toxic Marine Phytoplankton*. 1993. Newport, RI: Elsevier pp. 335-340.

32. Alcala, A.C., et al., *Human fatality due to ingestion of the crab* Demania reynaudii *that contained a palytoxin-like toxin.* Toxicon, 1988. 26(1):105-7.

33. Asakawa, M., et al., *Toxicity Assessment of the Xanthid Crab* Demania cultripes *from Cebu Island, Philippines.* J Toxicol, 2010. 2010:172367.

34. Tsai, Y.H., et al., *Toxicity and toxic components of two xanthid crabs,* Atergatis floridus *and* Demania reynaudi*, in Taiwan.* Toxicon, 1997. 35(8):1327-35.

35. Llewellyn, L.E., et al., *Post-mortem analysis of samples from a human victim of a fatal poisoning caused by the xanthid crab,* Zosimus aeneus*.* Toxicon, 2002. 40(10):1463-69.

36. Negri, A. and L. Llewellyn, *Comparative analyses by HPLC and the sodium channel and saxiphilin (3)H-saxitoxin receptor assays for paralytic shellfish toxins in crustaceans and molluscs from tropical North West Australia.* Toxicon, 1998. 36(2):283-298.

37. Chung, P.H., S.K. Chuang, and T. Tsang, *Consumption of viscera as the most important risk factor in the largest outbreak of shellfish poisoning in Hong Kong, 2005.* Southeast Asian J Trop Med Public Health, 2006. 37(1):120-5.

38. Wiberg, G.S. and N.R. Stephenson, *Toxicologic studies on paralytic shellfish poison.* Toxicol Appl Pharmacol, 1960. 2:607-15.

39. Anon, *Report of the joint FOA/IOC/WHO ad hoc expert consultation on biotoxins in bivalve molluscs*, 2004. 40 pp.

40. McFarren, E.F., et al., *Public health significance of paralytic shellfish poison.* Adv Food Res, 1960. 10:135-79.

41. Wong, J.T.S. and T.M. Ting. *Management of red tides in Sabah, Malaysia*. in *Management and Training Workshop*. 1989. Bandar Seri Begawan, Brunei Darussalam: Fisheries Department, Ministry of Development, Brunei Darussalam and International Center for Living Aquatic Resources Management, Manila, Philippines pp. 135-139.

42. Gonzales, C.L. *Management of toxic red tides in the Philippines*. in *Management and Training Workshop*. 1989. Bandar Seri Begawan, Brunei Darussalam: Fisheries Department, Ministry of Development, Brunei Darussalam and International Center for Living Aquatic Resources Management, Manila, Philippines pp. 141-147.

43. Guest, M.A., et al., *Trophic effects of fishing southern rock lobster* Jasus edwardsii *shown by combined fatty acid and stable isotope analyses.* Mar Ecol Prog Ser, 2009. 388:169-184.

44. Mayfield, S., et al., *Diet of the West Coast rock lobster* Jasus lalandii*: influence of lobster size, sex, capture depth, latitude and moult stage.* S Afr J Mar Sci, 2000. 22:57-69.

45. Yentsch, C.M. and W. Balch, *Lack of secondary intoxification by red tide poison in the American lobster* Homarus americanus*.* Environ Lett, 1975. 9(3):249-54.

46. Foxall, T.L., et al. *Secondary intoxication with PSP in* Cancer irroratus. in *2nd International Conference on Toxic Dinoflagellate Blooms*. 1979. Key Biscayne, FL: Elsevier/North-Holland pp. 413-418.

47. Sang, J.W.T. and T.T. Ming. *Red tide and paralytic shellfish poisoning in Sabah, Malaysia*. in *Proceedings of a consultative meeting held in Singapore*. 1984. Singapore: Southeast Asian Fisheries Development Center and International Development Research Centre pp. 35-42.

48. Watson-Wright, W., et al. *Monitoring of PSP in hepatopancreas of lobster from Atlantic Canada [abstract]*. in *2nd Canadian Workshop on Harmful Marine Algae*. 1991. Bedford Institute of Oceanography, Dartmouth, NS: Department of Supply and Services Canada pp. 27-28.

49. Haya, K., et al. *Uptake and excretion of paralytic shellfish toxins by lobster fed scallop digestive glands [abstract]*. in *3rd Canadian Workshop on Harmful Marine Algae*. 1992. Maurice Lamontagne Institute, Mont-Joli, Québec: Department of Supply and Services Canada p. 38.

50. Haya, K., Y. Oshima, and W.W. Young-Lai. *Profile of paralytic shellfish poisoning toxins in lobsters during uptake and depuration [abstract]*. in *4th Canadian Workshop on Harmful Marine Algae*. 1994. Institute of Ocean Sciences, Sidney, B. C.: Department of Public Works and Government Services Canada p. 17.

51. Lawrence, J.F., M. Maher, and W. Watson-Wright, *Effect of cooking on the concentration of toxins associated with paralytic shellfish poison in lobster hepatopancreas.* Toxicon, 1994. 32(1):57-64.

52. Cembella, A.D. and M. Desbiens, *Fate of paralytic shellfish toxins in the American lobster* Homarus americanus *[abstract].* J Shellfish Res, 1994. 13(1):302.

53. Desbiens, M. and A.D. Cembella. *Occurrence and elimination kinetics of PSP toxins in the American lobster (*Homarus americanus*)*. in *6th International Conference on Toxic Phytoplankton*. 1995. Nantes: Lavoisier pp. 433-438.

54. Desbiens, M. and A.D. Cembella, *Retention and possible transformation of paralytic shellfish toxins in lobster (*Homarus americanus*).* Bull Aquac Assoc Can, 1997. 97(2):75-7.

55. Arnott, G.H., *Toxic marine microalgae: a worldwide problem with major implications for seafood safety.* Adv Food Saf, 1998. 1(4):24-34.

56. State of Maine, Department of Marine Resources. *Press release July 8, 2010. Paralytic shellfish poisoning (red tide) reminder*. [webpage] 2010 8 July 2010 [cited 2012 19 December]

http://www.maine.gov/dmr/rm/public_health/shellfishadvisory.htm

57. Jiang, T.J., T. Niu, and Y.X. Xu, *Transfer and metabolism of paralytic shellfish poisoning from scallop (*Chlamys nobilis*) to spiny lobster (*Panulirus stimpsoni*).* Toxicon, 2006. 48(8):988-94.

58. Sephton, D.H., et al., *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, 2007. 6(5):745-58.

59. International Council for the Exploration of the Sea, *Report of the ICES-IOC Working Group on harmful Algal Bloom Dynamics (WGHABD), 10-13 March 2008, Galway, Ireland*, 2008, ICES: Copenhagen. p. 64 pp.

60. Mulvenna, V., et al., *Health risk assessment for cyanobacterial toxins in seafood.* Int J Environ Res Public Health, 2012. 9(3):807-20.

61. Faustman, E.M., et al., *Mechanisms underlying children's susceptibility to environmental toxicants.* Environ Health Perspect, 2000. 108 Suppl 1:13-21.

62. Bolch, C.J.S. and M.F. de Salas, *A review of the molecular evidencefor ballast water introduction of the toxic dinoflagellates* Gymnodinium catenatum *and the* Alexandrium "catenatum *complex" to Australasia.* Harmful Algae, 2007. 6(4):465-485.