

Risk profile for paralytic shellfish toxins in Tasmanian sea urchins

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# **Executive summary**

This risk profile aims to determine if there is a human health risk associated with paralytic shellfish toxin accumulation in Tasmanian sea urchin roe that requires management.

The urchin industry in Tasmania has been processing and marketing roe on a small scale for decades, based mainly on the native species *Heliocidaris erythrogramma* (Shortspined Sea Urchin). The industry has expanded in recent years, as a result of the incursion of the introduced pest urchin species *Centrostephanus rodgersii* (Longspined Sea Urchin), which causes large-scale urchin barrens on the east coast of Tasmania, and concomitant impact on valuable fisheries and marine biodiversity [1].

An impediment to the growth of this industry is the risk of biotoxin accumulation during the recurrent blooms of paralytic shellfish toxin (PST) producing microalgae *Gymnodinium* catenatum in south-east Tasmania and Alexandrium catenella, which occurs generally between July and November along the east coast of Tasmania [2].

Little is known about PST accumulation by sea urchins, and a conservative management approach has been taken thus far to protect both public health and market access. In such scenarios, risk managers will commonly outsource a preliminary risk assessment (known as a risk profile).

Risk profiles provide a summary of all information pertinent to food safety associated with the specific hazard/food combination. The purpose of a risk profile is to assist initial risk management activities, such as identifying future actions required (if any), and the options for food safety management programmes. They also inform the level of resourcing required to control the hazard/food pairing.

The consequence of human exposure to PST through consumption of seafood varies with the concentration of toxin in the seafood, the amount of seafood consumed, and the body weight (bw) of the consumer. Illnesses from paralytic shellfish poisoning range from mild to severe, with fatalities a rare end point.

A survey of 228 Tasmanian urchin roe samples consisting of at least 353 individual urchins (71 of these sampled when adjacent bivalve molluscs exceeded the regulatory level and a further 30 when PST were detected in bivalves below the regulatory level), found only one confirmed detection of PST above the laboratory level of reporting (0.1 mg STX equiv. /kg) in a pooled sample of *H. erythrogramma* roe taken during a *Gymnodinium catenatum* bloom (0.12 mg STX equiv. /kg). Trace levels of PST below the laboratory level of reporting were found during confirmatory analysis of an additional two urchin samples (<0.03 mg STX equiv. /kg). A further 14 urchin samples returned low level PST screen results and confirmation of PST levels did not occur. Thus all samples were well below the regulatory level for bivalves of 0.8 mg STX equiv. /kg).

There is some evidence from overseas that some urchin species can accumulate PST. The maximum PST level reported is 8.34 mg STX equiv. /kg in all viscera (internal organs including roe) of a non-commercial, Chilean sea urchin *Pseudichinus magellanicus*.

A review of serving sizes determined a range of 6 – 170 g of roe per meal.

A small adult consuming a large portion of roe at the maximum PST concentration reported in the present risk profile will consume 0.34  $\mu$ g STX equiv. /kg bw. This exposure level is less than both the European Food Safety Authority [3] and Food and Agricultural Organisation/World Health Organisation [4] acute reference doses (ARfD) of 0.5 and 0.7  $\mu$ g STX equiv. /kg bw respectively, and considerably lower than the ARfD estimated by Finch et al. [5] of 7.3  $\mu$ g STX.2HCL equiv. /kg bw.

Tasmanian sea urchins are exposed to PST on a regular basis as they are harvested from coastal areas that support regular blooms of toxic algae. There is considerable evidence that Tasmanian urchins do not accumulate PST to levels of concern in the roe (the consumed tissue for urchins) during *A. catenella* blooms. Whilst this may also be the case during *G. catenatum* blooms, we cannot rule out PST accumulation in this circumstance due to a lack of sampling effort during these blooms.

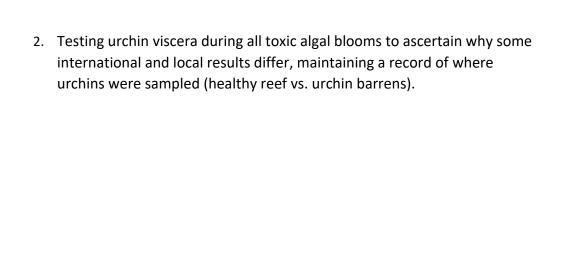
On the basis of the results presented in this risk profile, the probability of Tasmanian urchin roe accumulating concerning levels of PST during *Alexandrium catenella* blooms is low. Risk during *G. catenatum* blooms is currently unknown due to limited sampling during these blooms. The current control measures are highly conservative. There is no evidence that controls are needed to mitigate PST risk during low to moderate *A. catenella* blooms, although monitoring during more extensive blooms may be appropriate, as few urchin samples (n=5) have been collected during *A. catenella* blooms when PST in bivalves exceeded 10 mg STX equiv./kg. This is based on extensive sampling (101 sea urchins) during risk periods, where PST in bivalve shellfish exceeded 0.1 mg STX equiv./kg at the time and location of urchin sampling. Among the urchin samples collected during these periods, 70% were collected when bivalve PST levels had exceeded the ML (i.e. 71 urchins, including 45 *H. erythrogramma*, 7 *C. rodgersii* and 19 urchins where species was not recorded). These animals were collected on 15 different sampling occasions and analysed for PST as 42 individual and 4 pooled samples.

We recommend a review of the current risk controls based on the information presented in this risk profile. In particular:

- Consideration of when risk controls are necessary;
- 2. De-linking urchin testing from PST results in abalone on east coast;
- 3. Using risk monitoring results from other seafood biotoxin monitoring in Tasmania to indicate potential PST risk associated with *G. catenatum*, considering both where and when harvest activity is occurring.

We also recommend consideration of the following activities to address the current knowledge gaps:

1. Testing of urchins for PST during elevated PST activity associated with *G. catenatum* and during high *A. catenella blooms* when PST in bivalves exceed 10 mg STX.equiv. /kg, with consideration given to more frequent (e.g. weekly monitoring) during and after these blooms.



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#### Introduction

The urchin industry in Tasmania has been processing and marketing roe on a small scale for decades, based mainly on the native species *Heliocidaris erythrogramma*. The industry has expanded in recent years, as a result of the incursion of the introduced pest urchin species *Centrostephanus rodgersii*, which causes large-scale urchin barrens on the east coast of Tasmania, and concomitant impact on valuable fisheries and marine biodiversity [1]. As a method to control *C. rodgersii*, the Tasmanian government has encouraged harvesting of this species through administration of a bounty for animals captured [6]. A viable fishery is developing based on the harvest of these species for both the export and domestic market. The fishery is operating year-round, with *C. rodgersii* harvest greatest from January to July, and *H. erythrogramma* harvest greatest from July to February [6].

An impediment to the growth of this industry is the risk of biotoxin accumulation during the recurrent blooms of paralytic shellfish toxin (PST) producing microalgae *Gymnodinium catenatum* (south-east Tasmania) and *Alexandrium catenella*, which occurs generally between July and November along the east coast of Tasmania, [2]. Since 2012, when *A. catenella* bloom activity was first reported, PST concentrations exceeding the bivalve regulatory level (0.8 mg STX equiv. /kg) have been detected in Southern Rock Lobster (*Jasus edwardsii*) hepatopancreas, in both foot and viscera of Blacklip Abalone (*Haliotis rubra rubra*), Blue Mussels (*Mytilus galloprovincialis*), Pacific Oysters (*Magallana gigas*) and scallops (*Pecten fumatus*) [7, 8]. Separate marine biotoxin management plans are used to manage the risk of PST accumulation in Tasmanian Southern Rock Lobster [9] and Blacklip Abalone [10], while bivalve shellfish are managed under the Shellfish Market Access Program (ShellMAP) [11]. Abalone in particular appear to hold on to PST for prolonged periods (i.e. multiple years) following east coast bloom events.

Little is known about PST accumulation by sea urchins, and a conservative management approach has been taken thus far to protect both public health and market access. At the moment, sea urchins as grazers are loosely grouped with abalone and periwinkles for risk management. Blocks closed to abalone due to prolonged retention of high toxin levels therefore require regular PST testing in periwinkles and urchins, even in the absence of bloom activity.

In scenarios where specific risks are poorly understood, risk managers will commonly outsource a preliminary risk assessment (known as a risk profile). Risk profiles are an important tool for risk managers and industry. They provide a summary of all information pertinent to food safety associated with the specific hazard/food combination. The purpose of a risk profile is to assist initial risk management activities, such as identifying future research needs, future actions required (if any), and the options for food safety management programmes. They also inform the level of resourcing required to control the hazard/food pairing.

This risk profile is supported by field monitoring of PST on the east coast during bloom periods, and a survey to identify target markets (both international and domestic), as well as understanding product types and approximate amounts of roe consumed at each sitting. The latter is necessary to understand whether the bivalve maximum regulatory limit for PST is appropriate to use in risk management for the sea urchin industry (should a risk be determined).

# Scope

This risk profile critically reviews the information available on the human health or market access risk associated with paralytic shellfish toxin accumulation in Tasmanian sea urchin roe to determine if there is a need for risk management activities.

This will be achieved by:

- 1. Collating all existing information regarding the risk of PST accumulation in commercially harvested Tasmanian sea urchins;
- 2. Providing an initial evaluation of the extent of any public health concerns associated with PST in the roe of commercially harvested Tasmanian sea urchin species;
- 3. Identifying any knowledge gaps and requirements for further action.

# Methodology

#### Literature review of PST in sea urchins

A systematic review of the available scientific literature was conducted to identify any reports of PST accumulation in sea urchins. The search followed the criteria outlined in a previous Tasmanian marine biotoxin risk ranking report by Turnbull et al. [7] to include all up to date information. The literature search employed the following search terms for the hazard: shellfish toxin, shellfish poison, biotoxin, saxitoxin, paralytic shellfish toxin. These terms were paired against the following search terms for the food: sea urchin, urchin, echinoderm, *Heliocidaris*, *Centrostephanus*, echinoid, Kina. All combinations of the above terms were searched for using the PubMed, Web of Science and Scopus search engines, searching all fields. For papers to be retained, they had to relate to a level of paralytic shellfish toxin being found in sea urchins; human illnesses related to PST in sea urchins; or publications relating to toxin transfer through the food web involving sea urchins. Where PST concentrations were provided in the literature as STX.2HCl, these were converted to the STX equiv. /kg by dividing by 1.24 to ensure consistent reporting in the units of measurement used in the Australian Food Standards Code [12].

#### Surveys on international markets

The European Union's Rapid Alert System for Food and Feed [13] was searched for reports of paralytic shellfish poisoning (PSP) or PST within the "biotoxins (other)" hazard category across all food commodities and countries. The 31 biotoxin trade detection occasions were individually inspected to determine whether sea urchins or echinoderms were identified as the commodity. Additionally, the US National Outbreak Reporting System [14] was searched for reports of "Paralytic Shellfish Poisoning" across all available data (2009-2020). The returned outbreak data (6 outbreaks) was inspected to determine whether sea urchins or echinoderms were identified as the food vehicle.

## Field sampling - Tasmania

Several data sets were used to inform this risk profile. As data was collected for various purposes, the number of sampling events and treatment of animals within each event differed. For this report we have defined a sampling occasion as the collection of one or more urchins that have been collected on the same date from the same abalone sub-block and their roe subsequently analysed for PST either individually or as a pooled roe sample across multiple animals.

#### The data sets used were:

- 1. *H. erythrogramma* and *C. rodgersii* collected from Mercury Passage/Triabunna region during the 2020/21 and the 2021/22 biotoxin season (75 samples in total) and analysed for PST by Analytical Services Tasmania.
- 2. *H. erythrogramma* and *C. rodgersii* collected during an acute biotoxin event in the White Beach area on the Tasman Peninsula (17<sup>th</sup> August to 24<sup>th</sup> October 2022).
- 3. Sea urchin PST testing data supplied by the small dive industry (2018-2022). s
- 4. Recent (2022) and historic data on PST in sea urchins and other species collected as part of ongoing Institute for Marine and Antarctic Studies (IMAS) research projects.

IMAS and ShellMAP data were reviewed to get an indication of heightened algal bloom/PST activity in the sampling areas at the times of collection. Dates where PST above the level of reporting were detected in either abalone (viscera and foot tissues), rock lobster (hepatopancreas), or bivalve shellfish (oysters or mussels) were considered to match urchin collections if they were collected from the identical abalone sub-block within two days of urchin sampling. In some of the historic sea urchin PST data, it was not apparent whether the entire viscera (stomach, intestine and roe) or only the roe were analysed for PST. Where this is the case (e.g. Table 8), these results have been marked with an asterisk. Unless otherwise indicated, all PST concentrations in this risk profile are expressed as STX equiv. /kg (not STX.2HCl equiv. /kg), using Food and Agricultural Organisation (FAO) toxin equivalency factors. PST data across all seafood species and analytical techniques (Lawrence screen, mouse bioassay and confirmatory analysis) were treated identically and analysed in greater detail once matched as described in results section.

#### Consumption data

A survey of Tasmanian sea urchin processors/wholesalers was conducted to identify how sea urchin roe is processed, packaged, sold, and consumed. Sea urchin processing, wholesale, and retail businesses were identified through the Tasmanian Seafood Industry Council, word of mouth, and online searches.

An online search for sea urchin products on sale in Australia was conducted and included the following search terms: "sea urchin product Aus", "sea urchin buy", "buy sea urchin Australia", "long spine sea urchin roe", "sea urchin wholesale". The product origin, species of urchin, type of preparation (fresh, frozen brined), packaging (tray, punnet), and weight of product were recorded.

A second online search was conducted to identify sea urchin recipes and associated serving sizes. The type of recipe (e.g. pasta, salad), type of product (e.g. fresh, frozen or brined roe), preparation technique (e.g. raw garnish, boil, steam) and quantity of urchin required for the recipe, as well as number of servings were recorded to determine the serving size per person. The search was conducted via the Google search engine, encompassing key words, such as "sea urchin recipe", "Tasmanian sea urchin recipe", "Australian sea urchin recipe." The search focused on Australian recipes/websites (.com.au domain) and was extended to international websites (.com domains) when no additional domestic results could be identified.

#### Fisheries data

Commercial dive zone specific catch data for Long- and Short-spined Sea Urchins for the years 2009-2020 was provided in an aggregated format by Dr John Keane (Institute for Marine and Antarctic Studies), originally sourced from divers' dockets supplied to the Natural Resources and Environment Tasmania (NRE; owner of data). Additional fishing data (total catch and catch per unit effort) for the 2017/18-2020/21 fishing season were supplied by the NRE Tasmania wild fisheries branch (Sharna Rainer).

### Hazard Identification

# The toxins - Paralytic shellfish toxins

PSTs are a group of non-proteinaceous toxins composed of 57 related analogues that are produced by various algae (predominantly dinoflagellates, [15-17]). Saxitoxin is the parent analogue, consisting of a 3,4-propinioperhydropurine tricyclic structure with the molecular formula  $C_{10}H_{17}N_7O_4$  (Figure 1). The saxitoxin analogues are classified structurally based on the presence of various side chains such as carbamate, sulphate, hydroxyl, hydroxybenzoate or acetate. The level of toxicity of each analogue varies depending on the configuration of side chains. Analogues with carbamate side chains (e.g. STX, NEO and GTX1-4) are considered the most important because they are of the highest toxicity in mammalian assays [16, 18-20]. The total toxicity of a sample is determined by quantifying each analogue then employing toxin equivalency factors (TEFs) that relate the toxicity of individual toxin analogues to that of the saxitoxin parent molecule [21]. Total PST concentrations are reported as the amount of saxitoxin equivalents contained within a specified weight of animal tissue. The Australian bivalve regulatory limit is currently set as 0.8 mg STX equivalents per kg of tissue [12].

In Tasmania, individual PST analogues are detected and quantified via chemical analytical techniques. Liquid chromatography fluorescence detection (LC-FLD, Lawrence PST method, AOAC 2005.06) was used prior to January 2020, and the Hydrophilic Interaction Chromatography coupled to tandem mass spectrometry (HILIC MS/MS, [22, 23]) has been used since. These methods separate the individual PST analogues via chromatography before analysing them. The Lawrence method uses two oxidation processes (periodate and peroxide) prior to separation. Analysis of the periodate oxidate only can result in determination of a screening result. A negative screen result is determinant for a non-detection of toxins, however a positive screen result generally provides an overestimation of total toxicity and is normally confirmed and refined using the peroxide oxidate. For this reason, screening results are considered separately from confirmed data in quantitative analyses. Historically mouse bioassays were often employed, however, these have been phased out due to ethical concerns, consistency of results, poor sensitivity, and lack of information on individual PST analogues.

The most common PSTs are hydrophilic (water soluble), but some analogues that have hydrophobic side chains have been described [16, 24]. PSTs are also often described as heat stable at acidic pH. However, the European Food Safety Authority (EFSA, 2009, [3]) note that when heated at pH 2-4 analogues with the N-sulfo-carbamoyl side (e.g. GTX5) chain could be converted to their more potent corresponding carbamate toxins (e.g. STX) through hydrolysis of the N-sulphated group [3].

Figure 1 Structure of saxitoxin and analogues. Source: Lawrence, Loreal [25].

#### The toxin producers – Paralytic shellfish toxins in Tasmania

PSTs are produced by certain species of marine dinoflagellates in the genera *Alexandrium*, *Gymnodinium*, and *Pyrodinium*. PST production has also been demonstrated in certain species of freshwater cyanobacteria belonging to the genera *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya* and *Planktothrix* [16, 26, 27]. The main known dinoflagellate sources of PSTs of concern to the marine seafood-producing sector in Australia include *Alexandrium minutum*, *Alexandrium catenella*, *Alexandrium tamarense* and *Gymnodinium catenatum* [28-30]. Of historic concern for the marine seafood-producing sector in Tasmanian in terms of PST have been the chain forming dinoflagellates *A. catenella* (previously designated as *A. tamarense* and belonging to the *A. tamarense* species complex) and *G. catenatum*. The composition of the PST toxins, referred to as the toxin profile, differs between both species. *G. catenatum* predominantly produces C-toxins (0.01-0.1 times the toxicity of saxitoxin), while *A. catenella* appears to produce more potent PST analogues in culture, such as gonyautoxin 1-4 and neosaxitoxin (0.4-2 times the toxicity of saxitoxin, *Table* 1).

PST exceeding the bivalve regulatory level have been detected in Blacklip Abalone, Southern Rock Lobster and bivalves during blooms of both species (see Table 1). Bivalves are good early indicators of biotoxin activity, as they tend to quickly (within days) accumulate PST from toxic phytoplankton suspended in the water column. As the predominant PST analogues produced by Tasmanian A. catenella and G. catenatum differ, their relative proportions (PST profile) in bivalves can be used to infer which species was/is blooming. Both G. catenatum and A. catenella produce resting stages (referred to as cysts) that can hibernate for prolonged periods and germinate to form blooms when environmental conditions become favourable again.

Table 1 Maximum PST concentrations reported in Tasmanian Southern Rock Lobster, Blacklip Abalone and bivalves during blooms of *Alexandrium tamarense* species complex and *Gymnodinium catenatum*. The predominant PST analogues (>5% of total toxin profile) produced by these algal blooms and their toxicity relative to saxitoxin (toxin equivalency factor) are presented.

Bloom	Southern Rock Lobster	Blacklip Abalone	Bivalves	Predominant PST analogues produced by microalgae (in culture)	Toxin equivalency factor (TEF) [21]	
Alexandrium	10.9 mg	1.3 mg STX	150 mg STX	GTX1	1.0	
tamarense	STX equiv.	equiv. /kg	equiv. /kg	GTX4	0.7	
species	/kg (Pirates	(Okehampto	(East coast	GTX2	0.4	
complex	Bay, 2017)	n Bay, 2017)	Tasmania)	GTX3	0.6	
			[2]	NEO	2.0	
				[31, 32]		
Gymnodiniu	1.1 mg STX	2.4 mg STX	340 mg STX	C1	0.01	
m catenatum	equiv. /kg	equiv. /kg	equiv. /kg	C2	0.10	
	(Garden	(Garden	(Desolation	C3	0.01	
	Island,	Island, 2011)	Bay, 1993)	C4	0.1	
	2013)	[33]	[7]	[34, 35]		

#### Alexandrium catenella

Since 2012, A. catenella has recurrently bloomed along the Tasmanian east coast. Related, non-toxic Alexandrium species bloom in the same area, but are not readily distinguishable from the PST producing A. catenella using light microscopy alone. Routine ShellMAP monitoring of bivalve shellfish production zones therefore reports Alexandrium species inclusively as the "A. tamarense complex".

A. catenella blooms appear to favour stratified water column conditions caused by either salinity and/or temperature gradients during late winter and spring (generally June-November, [2]). Blooms predominantly tend to occur in Mercury Passage (inside of Maria Island) and Great Oyster Bay, but significant concentrations of A. catenella and associated PST accumulation in bivalve shellfish have also been reported around St. Helens and as far South as Bruny Island (ShellMAP biotoxin monitoring program). These blooms are variable in that they do not occur every year and differ in their spatial extent, intensity and duration (blooms can last for 3 or more months [36]). Regular monitoring of PST in bivalve shellfish (oysters and mussels) along the Tasmanian east coast highlights the recurrent and variable nature of A. catenella blooms in recent years (Figure 2).

#### Gymnodinium catenatum

The first *G. catenatum* bloom was reported in the Derwent Estuary in 1980 and many bloom events have since then been reported in the Derwent and Huon Estuary regions, where extensive cysts beds are thought to seed localised blooms [37]. *G. catenatum* cells that are flushed out of these estuaries into oceanic waters appear moribund [35]. *G. catenatum* blooms in Tasmania pre-2012 have tended to occur when water temperatures range from 12-18 °C and salinities range from 28–34 [35]. Blooms decline when temperatures fall below 12 °C. Lower mortality rates in autumn blooms compared with summer blooms cause autumn—winter blooms to decline slower [35]. Similar to *A. catenella* blooms on the east coast of Tasmania, *G. catenatum* blooms do not occur every year and vary in size and duration (*G. catenatum* can bloom for up to 6 months [38]). Regular monitoring of PST in bivalves as part of the ShellMAP demonstrates the recurrent nature of blooms, with the extent of blooms varying between biotoxin seasons (Figure 3).

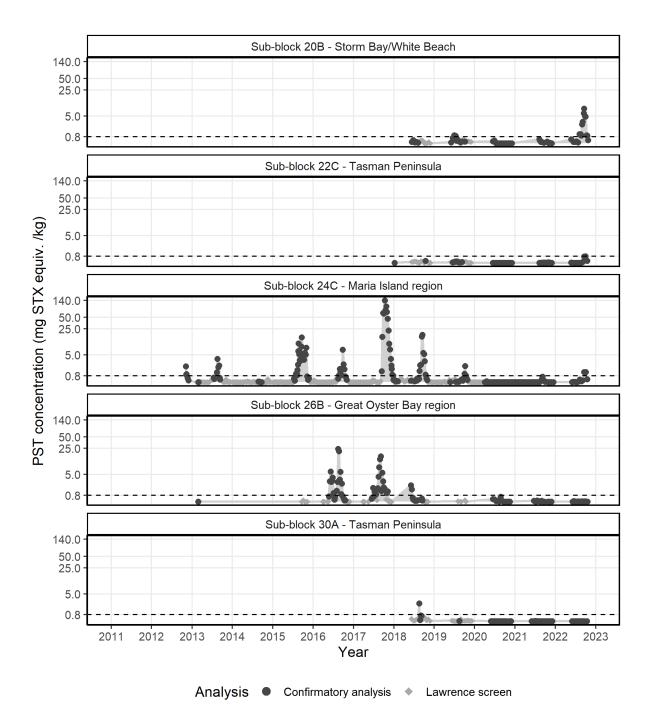


Figure 2 PST monitoring results for bivalve shellfish (mussels & oysters) along the Tasmanian east coast during *Alexandrium catenella* blooms. Monitoring of PST in sub-blocks 20B, 22C and 30A only commenced in 2018 when the Southern Rock Lobster sentinel monitoring program commenced. Note that Lawrence screen results can be up to 10 times higher than confirmatory analysis.

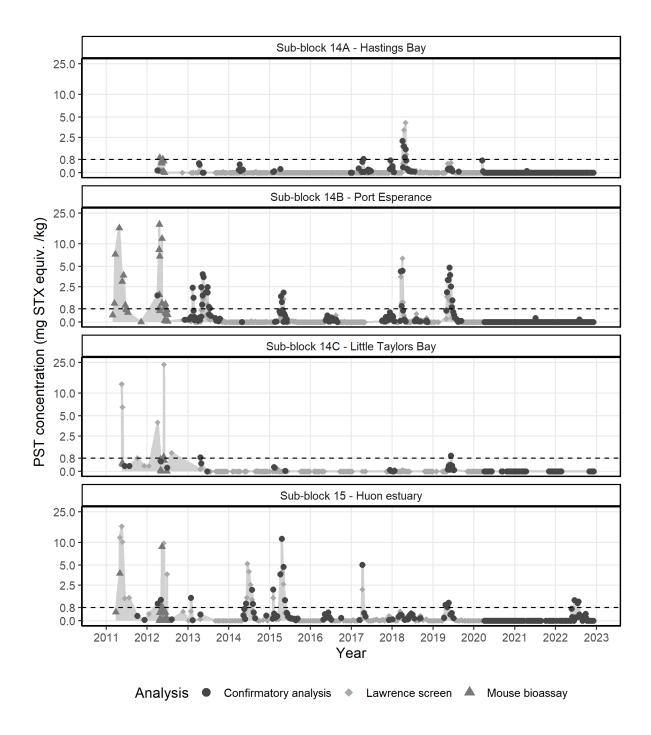


Figure 3 PST monitoring results for bivalve shellfish (mussels & oysters) in the Huon Estuary and D'Entrecasteaux Channel area during Gymnodinium catenatum blooms. Note that Lawrence screen results can be up to 10 times higher than confirmatory analysis.

#### The food – sea urchins

## Biology - Longspined Sea Urchin Centrostephanus rodgersii

The Longspined Sea Urchin, *Centrostephanus rodgersii*, or "Centro" is a large diadematid urchin found in south-eastern Australia, Norfolk Island, Lord Howe Island, The Kermadec Islands and Northern New Zealand [39]. The species is considered to have undergone a range expansion from mainland Australia and is now well established along the north and east coast of Tasmania, where it is reported as far south as Recherche Bay [40, 41]. *Centrostephanus rodgersii* have spines that are longer than half their test diameter (diameter of shell inside the spines) and are usually dark brown to black with a turquoise-like sheen on the spines and red down the centre (colours can vary) [6]. These urchins sexually mature at around 4 to 5 years old with a test diameter (diameter of shell inside the spines) of 40-60 mm, reaching up to ~130 mm at ~25-35 years of age [41]. Spawning generally occurs around August, when roe can make up in excess of 10% of the total body weight (including test, spines & coelomic fluid [40]).

Centrostephanus rodgersii are most often found around subtidal rocky reef structure at around 10-20 m depth [41]. They are light sensitive, spending the day in crevices and becoming more active after dusk to forage during the night before returning to their shelters. Individual urchins can move up to 10 m from their "home" crevices during their nightly feeding excursions [42] and exhibit strong site fidelity by returning to the same crevice at the end of the night [1]. Numerous animals often occupy the same crevice, leading to patchy aggregations. This localised feeding strongly contributes to the formation of urchin "barrens", where overgrazing removes all macroalgal cover [1].

Centrostephanus rodgersii is considered an omnivorous grazer, consuming a wide range of algal species, including drift algae, coralline algae, microalgae and sessile invertebrates, such as bryozoans and sponges (summarised in Byrne and Andrew [39] and Flukes et al. [1]). Food material is processed/removed from the substrate by a set of five individual teeth, called Aristotle's lantern. Macroalgae are considered to be the preferred food source, with other food sources playing a larger role on urchin barrens devoid of macroalgae [1].

#### Biology - Shortspined Sea Urchin Heliocidaris erythrogramma

The Shortspined Sea Urchin, Heliocidaris erythrogramma, "Helio" or purple sea urchin is endemic to Australia and Tasmania, commonly found from the intertidal zone down to a depth of ~35 m along the west, south and east Australian coasts [43]. It occurs in a range of habitats from algal-covered boulder fields to bare rock flats and sheltered sandy or seagrass areas [44]. In Tasmania, H. erythrogramma is predominantly found in sheltered to moderately exposed sites among boulders, rubble and ledges in less than 10 m of water, where it reaches up to 125 mm in test diameter [44]. It is found all around Tasmania, except for along the exposed south and south-west coast, where bull kelp (Durvilleae potatorum) is the dominant alga and the abalone H. rubra rubra a dominant herbivore (Dix 1977 cited in [43]). In Tasmanian waters, H. erythrogramma spawns in early summer to autumn at a test diameter of 40-50 mm (Dix 1977 cited in [43]). In the lead up to spawning, the roe can make up ~5-6% of the urchins body weight (including test, spines & coelomic fluid, [6]). Age at maturity is not well defined, but Sanderson et al. [45] suggested that maturity occurred at 5-10 years of age, with individuals >80 mm test diameter not abundant at most sites. The colour of the test and spines can differ significantly between individuals, including white, violet, green, dark red or occasionally pink [44].

Similar to *C. rodgersii*, the *H. erythrogramma* is predominantly nocturnal and also forms patchy aggregations (Wright et al. recorded up to 192 individuals per square meter in New South Wales waters [46]). Unlike *C. rodgersii*, *H. erythrogramma* does not have any fidelity to individual crevices and shelters (Andrew 1999 cited in [43]). *H. erythrogramma* feeds both by grazing or scraping on the substrate and capturing drift algae (summarised in [43]). In Tasmania, the preferred habitat and diet appears to be the kelp *Macrocystis pyrifera* [45]. Examinations of gut contents in Western Australia have shown that *H. erythrogramma* is primarily an algivorous herbivore, with macroalgae making up 98% of the gut contents (60% of which were brown algae and 35% red algae). Animal food (mostly sponge or ascidian material) and sand/rock made up the remainder at 1% each [47]). Similar studies have not been conducted on Tasmanian *H. erythrogramma*. Its diet is likely site specific, with Wright et al. documenting a shift in *H. erythrogramma* grazing to crustose algae in the absence of apparently preferred macroalgae [46].

# Fishery, production & markets *Fishery*

The Tasmanian sea urchin fishery targets both the endemic Shortspined Sea Urchin (H. erythrogramma) and the introduced Longspined Sea Urchin (C. rodgersii). The fishery forms part of the Tasmanian Commercial Dive Fishery, covering urchins & periwinkles and is divided into individual dive blocks within 5 management zones (see Figure 4). There are currently 53 commercial dive licences in Tasmania [6]. Centrostephanus rodgersii is considered invasive (urchin barren former) and does not have a size nor catch limit. Harvest of this species has been actively promoted since 2008 through government subsidies of up to \$1.5/kg (2022 fishing season, zone specific [6]). The native H. erythrogramma has a total allowable catch (TAC) of 175 tonnes and minimum size limit of 75 mm test diameter. The TAC for H. erythrogramma is divided across catch zones (44 t in the South-Eastern Zone, 45 t in the Central-East, 37 t in the North-East, 10 t in the Western, 39 t in the Northern Zone). Once the TAC for a zone is reached, the area is closed for the remainder of the licensing year [6]. Within the North-East, South-East and Central-East catch zones, further catch caps are placed on certain abalone sub-blocks located within this zone (Table 2). There are no size or possession limits for the recreational harvest of either of the two sea urchin species in Tasmania, although there is a recommendation that recreational fishers should apply a voluntary minimum size limit of 75 mm for H. erythrogramma urchins (identical to commercial size limit) and limit their catch to 50 individual *H. erythrogramma* urchins [48].

Table 2 *Heliocidaris erythrogramma* catch-caps for individual sub-blocks within Tasmanian Commercial Dive fishing zones during the 2022-23 fishing season [49].

Zone	Sub-bocks	Sub-bocks Area name			
North-East	30D	Georges Bay	7		
North-East	29D, 30A & 30B	St Helens	13		
North-East	24A, 24B & 24C	Mercury Passage	15		
Central-East	26B, 26C & 26D	Coles Bay	15*		
Central-East	23B	Dunalley	10		
South-East	18	Derwent River	6		
South-East	19B	Dodges, Sloping Island	15		

<sup>\*</sup>Each of the three sub-blocks in Coles Bay is managed to a 5 t catch-cap, for 15 t total.

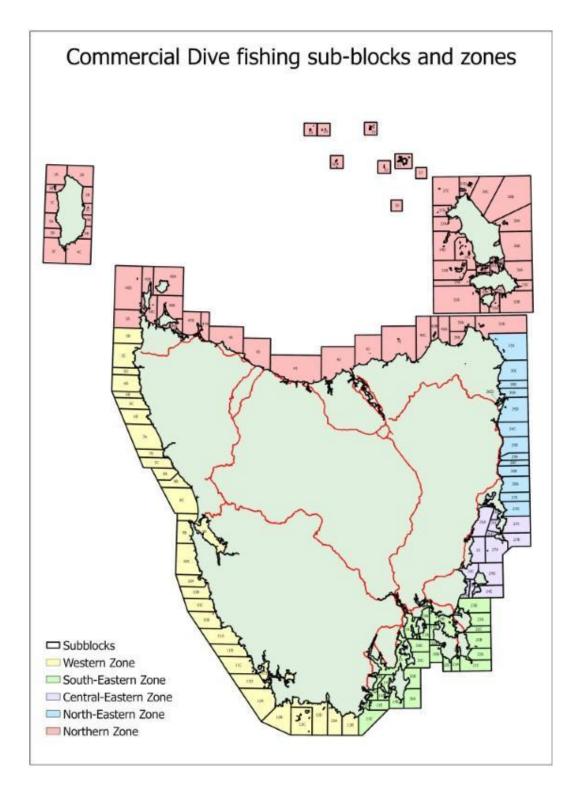


Figure 4 Tasmanian commercial dive fishery zones for the 2022/23 season. The fishery is split into individual blocks within 5 larger zones: Northern, Western, North-Eastern, Central-Eastern and South-Eastern Zone [6].

#### **Production & harvest**

Sea urchins are collected by divers by hand, operating out of small vessels (<10 m) and targeting individual urchins between 90-130 mm test diameter [50]. Catch weight, location and date is confirmed by a log recording from the processor who receives the catch. Divers are paid by total wet weight of catch (\$/kg) or by weight and quality of roe from the processor. For sea urchins, the term roe refers to both male and female gonads (in most other aquatic species the term roe applies specifically to eggs [51]). The quality of the urchin roe is seasonal and considered to be at its highest in the lead up to spawning with roe quality too low for harvest/market during and post-spawning. To ensure maximum roe quality, H. erythrogramma urchins are harvested from July until February, while C. rodgersii urchins are being targeted from January through to July. The majority of the total Tasmanian catch is made up by C. rodgersii and originates from the North-East and Central-East Zones (no TAC, total harvest of 497 t in 2021, Figure 5). Prior to 2019, the majority of the commercial catch originated from the North-East Zone (mainly around St. Helens area), but with the introduction of zone-specific government subsidies, catch effort has now increased (Figure 6) and spread across the Central-East Zone (\$0.75/kg for Central-East and 0\$ for North-East, subsidies as of 2019). The majority of the H. erythrogramma catch originates from the Central-East Zone, followed by the North-Eastern and South-Eastern Zones, as shown in Figure 5 [52]. Catch rates of *H. erythrogramma* have been increasing slightly since 2009 (Figure 7).

No information is available on the volume of the recreational or indigenous harvest, but the volumes are considered to be negligible relative to commercial harvesting [40].

# A. Heliocidaris erythrogramma harvest

# Catch Weight and CPUE by Block

# B. Centrostephanus rodgersii harvest

Catch Weight and CPUE by Block

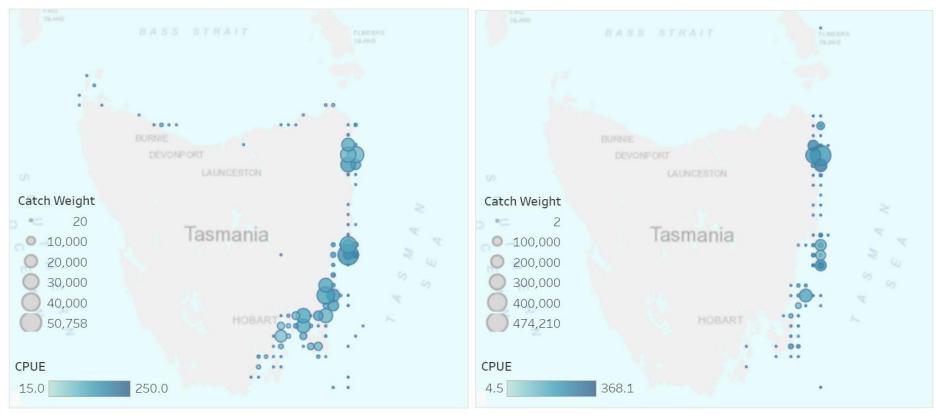


Figure 5 Commercial *Heliocidaris erythrogramma* (A) and *Centrostephanus rodgersii* (B) sea urchin harvest in Tasmanian waters during the 2017/18 to 2021/22 fishing seasons. The size of circles indicates the catch weight (in kg) and shading of circles the catch per unit effort (CPUE). Source: Sharna Rainer, NRE TAS.

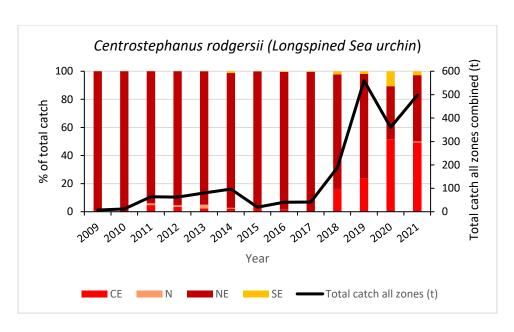


Figure 6 *Centrostephanus rodgersii* commercial dive fishery catch (2009-2021) per zone (coloured bars) and total catch across all zones (black line). Data supplied by John Keane (IMAS) and NRE (data owner).

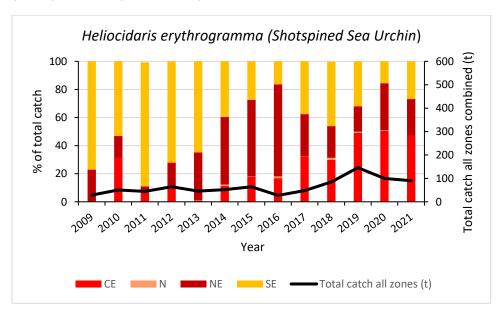


Figure 7 *Heliocidaris erythrogramma* commercial dive fishery catch (2009-2021) per zone (coloured bars) and total catch across all zones (black line). Data supplied by John Keane (IMAS) and NRE (data owner).

#### Processing & packaging

Live urchins are harvested by divers in the morning and arrive at the processor in the late afternoon to be held overnight at 10-12°C. The next morning, urchins are cracked by hand with specialised tools and the roe extracted (each urchin contains 5 lobes/tongues of roe). The remainder of the urchin is discarded. The urchin roe is placed on plastic trays and enters a series (3-4) of chilled saltwater baths, where teams of labourers manually pick off the membrane and clean the roe of any other urchin tissues. The roe then enters a final, chilled water bath with alum (potassium aluminium sulfate). Alum facilitates the drying process and maintains the fresh roe appearance. The roe is placed on paper towels and racked up in the blast chiller to dry. From here, urchins undergo a final manual clean and are graded into four grades based on physical appearance (freshness, colour, texture, size, shape of lobes) and taste. The highest grade, A, makes up approximately 40% of the extracted roe, followed by B (~30%), C (~25%) and the lowest grade, D (~5%). Roe quality and the relative percentages of products of a certain grade differ between harvest locations and seasons. The different grades of urchin roe are used for different products (see Table 3).

#### Market & trade

Australian and overseas markets for different types of urchin product were identified during the processor/wholesaler survey conducted as part of this risk profile. Tasmanian sea urchins and their product are sold both domestically and exported overseas. The sale of live sea urchins by processors or direct to mainland wholesalers by divers is limited and generally domestic market only, as long urchin spines necessitate larger packaging, thereby increasing transport costs per volume of product moved. The sale of extracted urchin roe is much more lucrative, as premium quality product (grade A and B) contained in trays sealed under normal atmosphere can be bulk packaged into boxes of 40-54 trays. Domestic sales include sales to Tasmanian retailers, restauranteurs and limited direct sales by processors. Product sold to mainland Australia generally goes to wholesalers in Victoria, New South Wales, and Queensland, who sell directly to the public, supply restauranteurs/fish markets or may export overseas. The amount of domestic vs. export sales varies between products, processors/wholesalers and fishing season, as sufficient quantity & quality of urchin roe needs to be available in order to offset bulk shipping costs. During the peak season, up to 80% of product may be exported. By far the biggest export markets are mainland China and Hong Kong, followed by Singapore and South Korea. New Zealand and the US present minor export markets, with the EU identified as an emerging market. The biotoxin regulations of trading partners (where relevant for PST in urchins/echinoderms) are identified below in Table 4. The European Union is the only regulatory body that specifically mentions echinoderms in their regulations, requiring that all live echinoderm product or products derived from echinoderms must meet the bivalve regulatory level for PST of 0.8 mg STX equiv. /kg [53]. Regulations in the United States and China/Hong Kong incorporate all aquatic species, requiring that all aquatic products need to meet the bivalve regulatory level. Maximum permissible PST levels in South Korea and Singapore could only be confirmed for bivalve shellfish. It is unknown whether these countries extend the application of this level to other wild harvested species, such as sea urchins.

Table 3 Tasmanian sea urchin roe product types, storage conditions, packaging and respective target markets as identified during sea urchin processor/wholesaler survey and online product searches.

Product type/grade	Product state	Storage matrix	Packaging	Package size (g of roe)	Storage temperature	Shelf life	Market
Live	Raw	None	Styrofoam box with ice packs	10-20 urchins/box, ~10 kg	Chilled to 2-3°C if air transport, held at 12-14 deg at processor	While alive	Limited domestic sales to Tasmania or wholesalers on mainland Australia. Very rarely export overseas.
A	Raw with alum	None	5 cavity trays	90 or 100 g	2-3°C	5-12 days	Both export & domestic
В	Raw with alum	None	5 cavity trays	90 or 100 g	2-3°C	5-13 days	Both export & domestic
A & B (A on top and B on bottom)	Raw with alum	None	5 cavity trays	90 or 100 g	2-3°C	5-13 days	Both export & domestic
С	Raw in brine	Brine	Pot	90 - 150 g	2-3°C or <8°C	12-14 days	Both domestic and export
D	Freeze dried	None	Tub	ТВА	<-18°C	ТВА	Currently being explored
	Frozen	None	Tub	500 g	<-18°C	6-12 months	Both domestic and export

Table 4 Biotoxin regulations (where available) for trading partners identified during the urchin processor/wholesaler survey and their respective biotoxin regulations as relevant to sea urchins. Note that not all countries specifically regulate for PST in sea urchins/echinoderms.

Trading partner	PST regulatory level	Seafood products specified in regulations
China & Hong Kong <sup>1</sup>	0.8 mg/kg, 4 MU	All aquatic products
United States <sup>2</sup>	0.8 mg STX equiv. /kg	All aquatic products
EU <sup>3</sup> (emerging market)	0.8 mg/kg	Bivalve molluscs, live echinoderms, tunicates and marine gastropods
South Korea <sup>4</sup>	0.8 mg/kg	Shellfish (oysters, mussels, cockles, clams, Spiny Topshell, whelks, abalone, pipis etc) and tunicates (sea squirts) only.
Singapore <sup>5</sup>	0.8 mg STX equiv. /kg	Bivalve shellfish only
New Zealand <sup>6</sup>	0.8 mg STX.2HCl equiv. /kg	Bivalve shellfish only. Sea urchins not regulated, but occasionally monitored
Australia <sup>7</sup>	0.8 mg STX equiv. /kg	Regulatory limit for bivalve shellfish only (FSANZ). Bivalve level employed as guidance by Tasmanian Wild Fisheries/Public Health Department and DAFF to lobster, abalone, sea urchins and periwinkles
CODEX <sup>8</sup>	0.8 mg STX.2HCl equiv. /kg	Bivalves & abalone only

Relevant international standards for PST

<sup>&</sup>lt;sup>1</sup>People's Republic of China Standard GB 2733-2015 [54].

<sup>&</sup>lt;sup>2</sup> Food and Drug Administration 2011. Fish and fishery products hazards and controls guidance.

<sup>&</sup>lt;sup>3</sup> Commission Regulation (EU). (EC No 853/2004). [53]

<sup>&</sup>lt;sup>4</sup> Korean Food Code 2019. [57]

<sup>&</sup>lt;sup>5</sup>Singapore Food Authority. Mycotoxins and marine toxins in food. Maximum limits for marine biotoxins [58]

<sup>&</sup>lt;sup>6</sup> New Zealand Animal Product Notice – regulated control scheme – bivalve shellfish. [59]

<sup>&</sup>lt;sup>7</sup>FSANZ: Schedule 19 Maximum levels of contaminants and natural toxicants. [12]

<sup>&</sup>lt;sup>8</sup>Standards for live and raw bivalve molluscs and abalone (CODEX STAN 292-2008 and CODEX STAN 312-2013).

## Food/hazard pairing

#### International literature search - PST in sea urchins

A comprehensive literature search identified six different studies reporting the detection of PST in nine different sea urchin species collected from the North Sea, South Pacific, North Atlantic and the Argentine Sea (summarised in Table 5). These reports largely consist of random surveys to identify potential non-traditional vectors for PST (i.e. sampling not triggered by algal bloom activity). As such, these studies are limited in sample size (all <10 animals, with the exception of 100 animals analysed for PST in Terrazas et al. [62]). Significant PST concentrations (>0.2 mg STX equiv. /kg were detected on four occasions, with one Argentinian sample of the non-commercial Little Pink Urchin (Pseudichinus magellanicus) collected after an Alexandrium bloom containing 8.34 mg STX equiv. /kg. Notably, a different urchin species, Arbacia dufresnii, collected at the same time only contained low concentrations of PST (0.096 mg STX equiv. /kg, pers. comm. Nora Montoya 2022). The above-mentioned studies analysed the entire urchin test contents (i.e. roe and all viscera). In case of the Chilean sea urchin Loxechinus albus, the highest PST concentrations were found in the roe (94% of total PST), followed by the viscera (max PST = 1.86 mg STX equiv./kg for all tissues combined [65]) during an ongoing bloom of A. catenella (PST in bivalves at the time of sampling exceeded 50 mg STX equiv./kg). This [65] is the only report of PST in urchin roe in the literature and it remains unknown whether PST uptake is urchin/algal species and/or environment specific. For example, where the urchin sample originated from in Chilean waters, intense algal blooms frequently occur, with total PST in bivalves often exceeding 100 mg STX equiv. /kg (pers. comm. Carlos Garcia). None of these studies reported any evidence of human illness related to the ingestion of sea urchins contaminated with PST. There is no information available in the scientific literature on uptake and depuration rates of PST in sea urchins.

#### Surveys in international markets

No records of any market detections of PST in sea urchins or sea urchin products were reported in the European Union Rapid Alert System for Food and Feed [13] nor the US National Outbreak Reporting System (2009-2020 [14]).

Table 5 Summary of scientific studies reporting the detection of PST in sea urchin tissues in different sea urchin species (includes both non-commercial and commercially fished species). Where reported, the number of samples and prevalence of PST positive samples is provided to indicate sampling effort.

Species	Region	Sampling related to algal bloom & tissue sampled	Number of animals tested	Frequency of PST detection <sup>a</sup>	Maximum PST detected (mg STX equiv. /kg)	Reference
Paracentrotus lividus (commercial species)	North Atlantic (Madeira, Portugal)	Random sampling, all viscera	1	0%	<loq<sup>c</loq<sup>	[63]
Arbacia lixula (non-commercial)	North Atlantic (Madeira, Portugal)	Random sampling, all viscera	1	0%	<loq<sup>c</loq<sup>	[63]
Echinus sp. (non-commercial)	North Sea	Random sampling, all viscera	6	100%	0.0218	[64]
Sphaerechinus granularis (commercial species)	North Atlantic (Azores, Portugal)	Random sampling, all viscera	5	40%	0.0350	[63]
Arbacia dufresnii (aquaculture species)	Argentine Sea (South America)	After Alexandrium bloom, collected from same sites as P. magellanicus, all viscera	Not reported	Not reported	0.0960 <sup>b</sup>	Pers. comm. Nora Montoya (2022)
Arbacia lixula (non-commercial)	North Atlantic (Azores, Portugal)	Random sampling, all viscera	2	50%	0.090	[63]

Species	Region	Sampling related to algal bloom & tissue sampled	Number of animals tested	Frequency of PST detection <sup>a</sup>	Maximum PST detected (mg STX equiv. /kg)	Reference
Loxechinus albus (commercial species)	Southern Pacific (Chile)	Bivalves at 50 mg STX equiv./kg at the time of sampling (intestine, stomach & roe)	100	15%	1.86 <sup>b</sup>	[65]
Psammechinus miliaris (non-commercial)	North Sea	Random sampling, all viscera	5	60%	0.207	[64]
Diadema africanum (non-commercial)	North Atlantic (Madeira, Portugal)	Random sampling, all viscera	2	100%	0.223	[63]
Paracentrotus lividus (commercial species)	North Atlantic (Portugal)	Random screening, all viscera	10	Not reported	0.323	[66]
Pseudichinus magellanicus (non-commercial)	Argentine Sea (South America)	After <i>Alexandrium</i> bloom, all viscera	Not reported	Not reported	8.34 <sup>b</sup>	[67]

 $<sup>^{\</sup>rm a}$  % samples greater than limit of detection (0.005 – 0.02 mg STX equiv. /kg)

<sup>&</sup>lt;sup>b</sup> Saxitoxin reporting units not specified

<sup>&</sup>lt;sup>c</sup> LOQ = limit of quantification

#### Field sampling - Tasmania

#### Sampling effort

Between 2012 and 2022, 228 Tasmanian pooled or individual sea urchin roe samples were analysed for PST. Pooled roe samples were made up of 2 or more urchins. For some of the older sampling dates (n=124), it could not be ascertained whether the PST result was representative of the roe of a single urchin or that of multiple pooled urchins. Assuming these all represent single animals only, the 228 PST data points available correspond to the roe of at least 353 individual animals. As multiple individual sea urchins from a single species were sometimes sampled on the same date, this represents 156 sampling occasions. A sampling occasion represents the collection of one or more urchins that have been collected on the same date from the same abalone sub-block and subsequently analysed for PST either individually or as a pooled roe sample across multiple animals. In total, the Tasmanian PST data set for all sea urchin species contains PST results for:

- 54 sampling occasions for *Heliocidaris* (equivalent to at least 166 individual animals)
- 94 sampling occasions for *Centrostephanus* (equivalent to at least 159 individual animals)
- 8 historic sampling occasions where the urchin species had not been recorded (equivalent to at least 28 individual animals).

Few sea urchin samples were tested for PST prior to 2020 (37 sampling occasions across all urchin species, see Table 6 below), with the majority of PST testing occurring as part of industry monitoring during harvest and IMAS research sampling in the last three years (119 sampling occasions in 2020-2022). The sampling effort has concentrated on the central-east coast (111 sampling occasions), followed by the north-east (31 sampling occasions). Considerably fewer samples have been collected along the lower east coast (n=3) and Storm Bay (n=10), with only a single sample collected in the D'Entrecasteaux Channel (see *Figure 8* across page).

Table 6 Sea urchin sampling effort as numbers of samples analysed for PST in Tasmanian waters grouped by year and urchin species. Where not recorded in the historic data, the urchin species is represented as "Unspecified". Sampling effort is quantified in terms of sampling occasions and the total number of animals analysed on those occasions. A sampling occasion represents the collection of one or more urchins that have been collected on the same date from the same abalone sub-block and subsequently analysed for PST either individually or as a pooled roe sample across multiple animals. Typically, at least 5 urchins from each species are collected on the same sampling occasion. Where the historic data did not specify whether a sample was pooled or individually analysed, the sample number is given in brackets. These samples were counted as one for the total number of animals tested (i.e. represents at least one urchin, but unknown exactly how many).

		H. erythro	ogramma			C. roa	lgersii			Unsp	ecified			To	tal	
Year	Number of sampling occasions	Number of pooled samples	Number of individual samples	Total of animals tested	Number of sampling occasions	Number of pooled samples	Number of individual samples	Number of animals tested	Number of sampling occasions	Number of pooled samples	Number of individual samples	Number of animals tested	Number of sampling occasions	Number of pooled samples	Number of individual samples	Number of animals tested
2012	1	(1)	0	1	1	(1)	0	1	0	0	0	0	2	(2)	0	2
2013	1	(1)	0	2	0	0	0	0	1	(1)	0	1	2	(2)	0	3
2014	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2015	0	0	0	0	0	0	0	0	2	(2)	0	2	2	(2)	0	2
2016	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2017	0	0	0	0	0	0	0	0	4	(1)	15	16	4	(1)	15	16
2018	5	4(1)	0	40	3	3	0	13	1	1	0	9	9	8(1)	0	62
2019	6	3(3)	0	18	12	3(15)	0	30	0	0	0	0	18	6(18)	0	48
2020	17	3(12)	10	37	34	3(32)	10	57	0	0	0	0	51	6(44)	20	94
2021	3	3	0	15	31	3(29)	0	44	0	0	0	0	34	6(29)	0	59
2022	21	(13)	40	53	13	(12)	2	14	0	0	0	0	34	(25)	42	67
Total	54	13(31)	50	166	94	12(89)	12	159	8	1(4)	15	28	156	26(124)	77	353

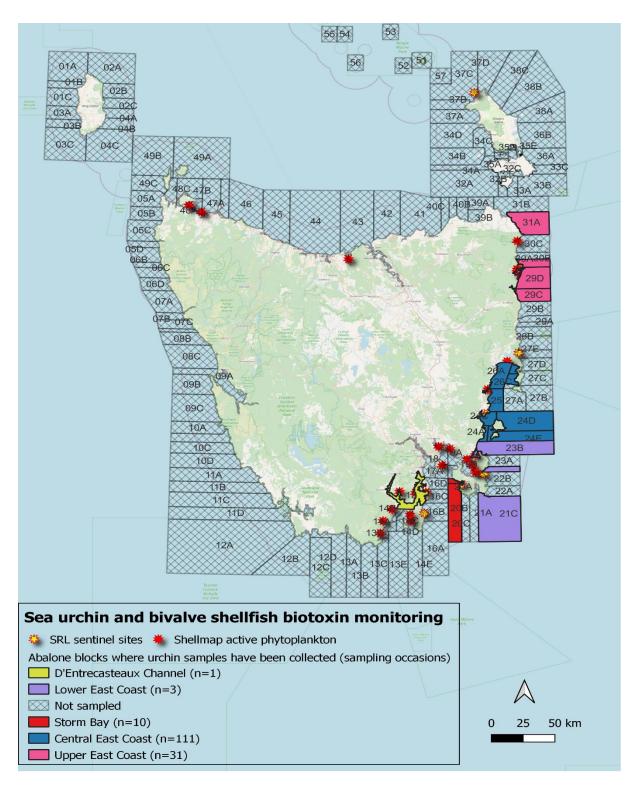


Figure 8 Tasmanian abalone fishery blocks and biotoxin monitoring sites. Coloured abalone sub-blocks indicate blocks from which sea urchin samples have been collected for PST analysis (2012-2022). Adjacent blocks are grouped into sampling zones. The hashed areas indicate abalone blocks for which no urchin PST data has been collected. Active ShellMAP phytoplankton sites and Rock Lobster sentinel sites are represented by red and yellow stars, respectively. Numbers in brackets indicate the number of occasions on which sea urchin samples were collected in each zone (multiple animals were collected and analysed for PST on each of these occasions).

#### PST monitoring results

#### PST detections in Tasmanian sea urchins

A total of 353 individual sea urchins have been tested for PST in Tasmania as either individual or pooled samples (equates to 228 PST analysis, consisting of 196 confirmed analysis and 32 unconfirmed Lawrence screen results). Reportable levels of PST were only detected in a single sample consisting of the pooled roe of 2 *H. erythrogramma* urchins collected during a *G. catenatum* bloom in the D'Entrecasteaux Channel in 2013 (0.12 mg STX equiv. /kg). On the east coast, trace amounts of PST (below the laboratory level of reporting) were only detected on two occasions in sea urchins collected from Georges Bay (St. Helens, 2017, 0.03 mg STX equiv. /kg, species unknown) and Okehampton Bay (Triabunna, 2018, 0.01 mg STX equiv. /kg, pooled sample containing 5 *H. erythrogramma*, 3 *C. rodgersii* and 1 egg urchin). The Okehampton Bay sample was collected at the start of a moderate bloom when bivalves were going up (2.57 mg STX equiv. /kg in bivalves at the time of urchin sampling), to peak at 17.5 mg STX equiv. /kg in bivalves 3 weeks later. No further urchin samples were collected during this bloom.

Lawrence screen results suggested the presence of low levels of PST in an additional 14 samples along the Tasmanian east coast between September 2018 and December 2019. The Lawrence screen results indicated maximum unconfirmed PST levels of 0.30 and 0.17 mg STX equiv. /kg in *C. rodgersii* and 0.11 mg STX equiv. /kg in *H. erythrogramma*, with PST levels in all other 11 screen results below 0.08 mg STX equiv./kg. It is important to note that these initial screen results from the Lawrence technique (now superseded by the Boundy method) can overestimate PST concentrations by up to a factor of 10. Since the screen results indicated the presence of only low levels of PST (<0.30 mg STX equiv. /kg), no confirmatory analysis of these samples was undertaken during industry sampling at the time. At the time that the three samples with screen results >0.08 mg STX equiv. /kg were collected, no bloom activity was recorded in either the Georges Rocks/Binalong Bay nor Great Oyster Bay/Little Swanport areas (max PST observed in bivalves at these locations was 0.23 mg STX equiv. /kg during a Lawrence screen test).

#### Sea urchin sampling during high-risk periods

There have been no confirmed detections of reportable levels of PST in sea urchin roe sampled during periods of biotoxin activity on the Tasmanian east coast, even during extreme conditions when PST levels in bivalves reached 75.5 mg STX equiv./kg (5 individual urchins tested on the same day). A period of biotoxin activity is here defined as a period where reportable levels of PST (>0.1 mg STX equiv./kg) were detected in bivalve molluscs within 2 days of urchins being tested. Bivalves are a widely accepted sentinel for biotoxin activity due to their filter feeding nature and rapid uptake of PST.

Of the 353 individual urchins collected for PST analysis in 2012-2023, 101 animals were collected during periods of biotoxin activity. These urchins were collected on 22 sample occasions and include PST analysis of 47 individual and 7 pooled samples, with a further 3 samples of unknown nature (i.e. either pooled or individually analysed, see Table 7). Among the urchin samples collected during these periods, 70% were collected during heightened biotoxin activity when bivalve PST levels had exceeded the ML (i.e. 71 urchins, including 45 H. erythrogramma, 7 C. rodgersii and 19 urchins of unknown species, see Table 7). These animals were collected on 15 different sampling occasions and analysed for PST as 42 individual and 4 pooled samples. High PST levels in bivalves within 1-2 days prior to urchin sampling provide a good indication that urchins were exposed to an active algal bloom at the time of sampling or immediately prior to sampling. However, due to the rapid uptake of PST by bivalves, if toxins were detected in bivalves 2 days after urchin sampling, the confidence that urchins were exposed 2 days prior may be reduced, particularly if only low PST levels were found in bivalves. During periods of heightened biotoxin activity, this latter scenario occurred only twice, but high PST levels in bivalves at the time (5.3 and 2.4 mg STX equiv. /kg) provide confidence that urchins had already been exposed to toxic algae during sampling 2 days prior. This is further supported by Southern Rock Lobster collected on the same date and location as urchins exceeding the bivalve ML (6.56 and 1.47 mg STX equiv. / kg in hepatopancreas). Table 8 below provides a detailed breakdown of PST levels across different seafood species and when bivalves were sampled relative to sea urchins. At all times when PST were detected in lobsters sampled within two days of sea urchins on the east coast, PST were also detected in bivalve molluscs.

With the exception of the single urchin sample where low levels of PST were detected in the D'Entrecasteaux Channel (abalone sub-block 15), the other matched sampling occasions originate from sampling during periods of *Alexandrium* bloom activity in the White Beach region (August-October 2022) or the central-east coast (2012-2021). The latter region is of particular interest, as considerable IMAS research sampling comparing PST levels between different species has occurred in this area from 2017 onward (Figure 9). Significantly elevated PST levels were detected in bivalve shellfish in this region during the 2017 (up to 139 mg STX equiv. /kg), 2018 and 2019 biotoxin seasons, but no PST reported in sea urchins sampled during and after these events. While only limited bloom activity was recorded in subsequent years (2020-2022), abalone appeared to contain significant concentrations of PST in between blooms years. Again, no reportable levels of PST were detected in sea urchins sampled during this period, while abalone harvest blocks remained closed due to the presence of elevated PST in abalone [68].

Table 7 Number of sampling occasions where urchins were collected during low or high-risk periods (indicated by PST concentration range in bivalve shellfish). On each sampling occasion, multiple urchins were collected and either analysed as a pooled sample, or individually. Typically, one pooled sample analysed for PST consisted of the combined roe of five individual urchins. Where the historic data did not specify whether a sample was pooled or individually analysed, the sample number is given in brackets. These samples were counted as one for the total number of animals tested (i.e. represents at least one urchin, but unknown exactly how many).

	PST in bivalves (mg STX equiv./kg)	0.1-0.5	0.5-0.8	0.8-1.6	1.6-10	>10	Total
	Number of sampling occasions	3	0	3	3	0	9
Heliocidaris	Number of pooled samples	2(1)	0	0	2	0	2
erythrogramma	Number of individual samples	5	0	15	20	0	40
	Total of animals tested	23	0	15	30	0	68
	Number of sampling occasions	4	0	0	6	0	10
Centrostephanus	Number of pooled samples	1(2)	0	0	1	0	1
rodgersii	Number of individual samples	0	0	0	2	0	2
	Number of animals tested	7	0	0	7	0	14
	Number of sampling occasions	0	0	0	2	1	3
Unspecified urchin	Number of pooled samples	0	0	0	1	0	1
species	Number of individual samples	0	0	0	5	5	10
	Number of animals tested	0	0	0	14	5	19
	Number of pooled samples	3(3)	0	0	4	0	7(3)
Total	Number of individual samples	5	0	15	27	0	47
iotai	Number of sampling occasions	7	0	3	11	1	22
	Number of animals tested	30	0	15	51	5	101
Maximum PST detected in urchin (mg STX equiv. /kg)		ND	ND	ND	ND	0.01	0.12

Table 8 Quantification of PST in different sea urchin species during periods of increased biotoxin risk (red, yellow and blue shading indicating very high, high and medium-risk respectively), as indicated by PST detections in either abalone, rock lobster or bivalve tissues sampled within two days of collection of urchin samples. Blank spaces indicate dates where no matching results were available for a particular seafood species and stars represent historic PST analysis where the tissue analysed is unknown (i.e. could be either roe or entire test content, including other viscera and faecal pellets). For pooled samples containing the roe of multiple individual urchins (P), the number in the brackets after the PST result indicates the number of urchins that were pooled on this sampling occasion. For sampling occasions where individual urchins where analysed (S), the number in the brackets provides the number of individual urchins that were analysed (typically 5). Where no records on the type of sample where available, these are denoted as unknown (U).

Date	Location		Sea urchins		Abal	one	Rock lobster	Bi	valves
Sampling sea urchins	Abalone sub-block	Centrostephanus rodgersii (n)	Heliocidaris erythrogramma (n)	Unspecified urchin species	Viscera	Foot	Hepatopancreas	Oysters & mussels	Bivalve sampling date relative to urchin sampling sampling date
30/10/2017	24C			ND (5S)	1.32	0.87		70.54	Same day
15/09/2022	20A	ND (1S)	ND (5S)			0.50		7.94	Same day
7/10/2019	24C	ND (5P)	ND *(5P)			0.38	6.56	2.27	1 day after
15/09/2022	20B		ND (5S)			0.31		6.04	Same day
4/10/2018	24C		ND *(5P)		0.24	0.62	1.47	5.32	2 days before
27/09/2022	20B		ND (5S)			0.29		5.05	Same day
27/09/2022	20A	ND (1S)				0.26		4.17	Same day
30/08/2022	20A		ND (5S)			0.14		2.76	Same day
4/09/2018	24C			0.01*(9P)	0.33	0.87	2.21	2.57	1 day before
13/12/2017	24C			ND (5S)				2.37	2 days before
22/10/2019	24C	ND (5P)	ND*(5P)				1.45	0.49	1 day before
17/08/2022	20B		ND (5S)			0.43		1.16	Same day
27/09/2021	24C	ND (5P)	ND (5P)			1.10			
4/04/2013	15		0.12(2P)				1.07		
12/10/2022	20B		ND (5S)			0.18		1.02	Same day
17/08/2022	20A		ND (5S)			0.75		0.90	Same day

Date	Location		Sea urchins		Abal	one	Rock lobster	Bi	valves
Sampling sea urchins	Abalone sub-block	Centrostephanus rodgersii (n)	Heliocidaris erythrogramma (n)	Unspecified urchin species	Viscera	Foot	Hepatopancreas	Oysters & mussels	Bivalve sampling date relative to urchin sampling sampling date
31/10/2018	24C		ND *(12P)		0.13	0.80	0.59	0.25	2 days before
9/10/2018	31A	ND *(3P)				0.69			
18/11/2020	26A		ND (5P)			0.57			
8/11/2018	22C	ND *(5P)				0.53	0.28		
28/09/2020	24D	ND (5S)	ND (5S)			0.52			
11/06/2019	24C	ND (5P)	ND *(5P)			0.50	0.18		
18/11/2020	26B		ND (5P)			0.43			
16/11/2020	24B	ND (5P)				0.38			
24/10/2022	20B		ND (5S)			0.24		0.38	Same day
28/09/2020	24C	ND (5S)	ND (5S)			0.34			
27/09/2021	24B	ND (5P)	ND (5P)			0.30			
30/06/2020	26B	ND (1U)						0.28	1 day before
16/11/2020	24A	ND (5P)	ND (5P)			0.23			
18/11/2020	26C		ND (5P)			0.22			
28/11/2012	24C	ND *(1U)	ND *(1U)					0.21	2 days after
8/10/2018	30A	ND *(5P)				0.18			
27/09/2021	24A	ND (5P)	ND (5P)			0.16			
16/10/2017	30A			0.03					

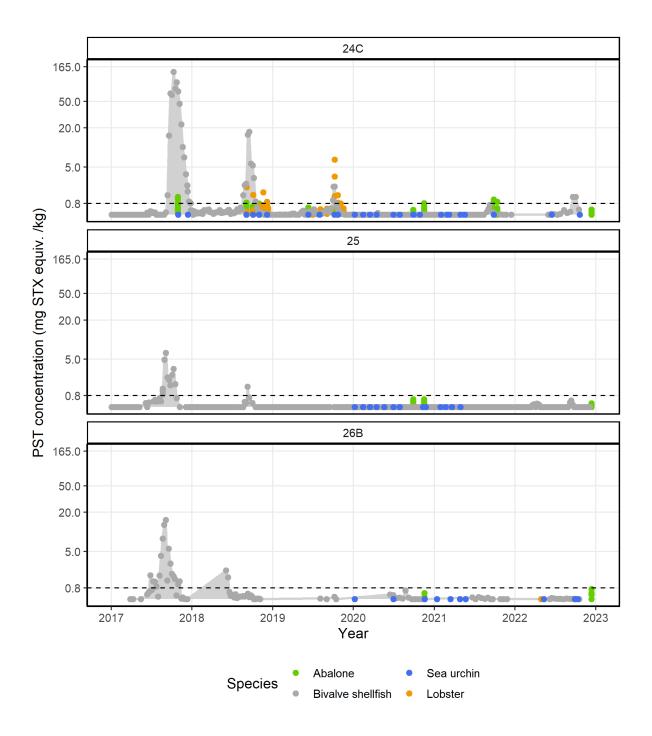


Figure 9 PST in Tasmanian seafood species sampled along the Tasmanian central-east coast. The grey shaded area and circles represent PST levels in bivalve shellfish as an indicator of biotoxin activity. Note that PST concentration is provided on a logarithmic scale on the yaxis.

## **Exposure Assessment**

### Food preparation

Canvassing of 30 online sea urchin recipes revealed multiple different ways of preparing urchins. Higher grades (A & B) are most commonly used as raw garnish on top of food preparations (e.g. soup, pasta, rice, dumplings, canapes, scrambled eggs). Other grades (C-D) are most often used to make sea urchin butter, pâté or cooked into sauces. These end uses align with overseas consumption habits. In Japan, where ~80-90% of the global sea urchin harvest is consumed, sea urchin roe is prepared as fresh roe (raw), cooked (steamed, baked) or may be used in pastes or soups [51, 69]. Yokota gives multiple examples of sea urchin recipes, which highlight the urchin as a luxury "garnish" to the meal [69]. In Japan, sea urchin roe is most frequently consumed at restaurants & wedding banquets, with only a small portion for household consumption [51]. Similarly, sea urchin roe is considered a luxury product on the Chinese/Hong Kong markets (main export destination of Tasmanian sea urchin roe) [70].

### Consumption

The <u>Australian or New Zealand National Nutrition surveys</u> broadly group seafood into one category and do not provide specific information on the consumption of sea urchins or echinoderms. No dietary intake or serving size information could be found for sea urchins or echinoderms from overseas markets. There are currently no recommended daily intake or serving sizes associated with Tasmanian sea urchin roe products. Anecdotal statements, captured during the processor/wholesaler survey, indicate that a person may consume 2-3 lobes (also referred to as tongues) or 20-30 g of premium urchin roe in a sitting.

In the absence of more detailed information on serving sizes, online recipes for sea urchin products were canvassed to approximate the quantity of urchin roe consumed in a single setting. The initial search focused on Australian websites (n=18) and was expanded to include available recipes from overseas (.com) recipes until 30 recipes were canvassed (2 of these recipes did not provide the number of servings and were excluded). While it cannot be excluded that some individuals might consume the entire sea urchin viscera (i.e. complete test content), all recipes found in the online search utilised the roe only. The amount of roe required for each recipe differed, with some given as the number of urchins, number of lobes/tongues or weight of urchin roe (g). To arrive at an indicative serving size, the number of lobes/urchins was approximated to represent a "worst-case-scenario" of the peak gonad yield (10% of total body weight) of a large (130 mm test diameter) C. rodgersii urchin (higher yield and larger than H. erythrogramma urchins). The test diameter represents the maximum size harvested and was converted to body weight as per Cresswell et al. [40], using the formula derived from the fisheries assessments conducted during May 2020 at the peak of the Tasmanian C. rodgersii fishing season. For a 130 mm urchin, this equates to a body weight of ~850 g, a roe yield of 85 g during peak condition and individual roe weights of ~17 g per tongue/lobe (i.e. each urchin contains five tongues). A more conservative estimate of the individual lobe/tongue weight is relating the number of lobes in each package to the total weight. Tasmanian processors generally produce 5 cavity trays with 2 lobes of sea urchin per cavity (often one lobe of Grade A on top of a single Grade B) and a total roe weight of 90-100 g per tray. This equates to an individual lobe/tongue weight of approximately 10 g.

Using the two different tongue/lobe weights as described above, the number of urchins and lobes was converted to a weight (g) for each of the 28 recipes and divided by the number of servings to obtain an indication of serving size (Table 9). Maximum serving sizes were 100 – 170 g of urchin roe per serving. For comparison, the European Food Safety Authority risk assessment of PST in bivalve molluscs was based on a large serving size of 400 g of shellfish [3]. The 2004 FAO/World Health Organization (WHO) risk assessment for marine biotoxins in shellfish used a small medium and large serving size of 100g, 250g and 380g respectively [4].

Table 9 Estimated sea urchin roe serving sizes based on quantities used in 28 online recipes. The assumptions for converting different units of urchin roe quantity provided in each recipe are summarised and discussed in detail in the text. The mean is provided  $\pm$  1 standard deviation.

Estimated	A	Serving Size				
lobe weight	Assumption	Mean	Min	Max	Median	
10 g per lobe/tongue	2 lobes/cavity in 5 cavity tray with total roe weight of 100 g	36±20 g	6 g	100 g	30 g	
17 g per lobe/tongue	130 mm test ( <i>C. rodgersii</i> ), equivalent to 850 g body weight and 85 g of roe (roe yield = 10% body weight), 5 lobes per urchin	55±20 g	6 g	170 g	50 g	

A large meal of urchin roe (0.170 kg) containing the maximum PST concentration recorded in this survey (0.12 mg STX equiv. /kg) would contain 0.020 mg of PST (STX equiv.). For a small adult (60kg) this equates to 0.34  $\mu$ g STX equiv. /kg body weight (bw).

### Hazard characterisation

### Mode of action

The main target for PST are voltage gated sodium channels. These channels are essential for conducting Na $^+$  ions across the plasma cell membrane and have a critical role in generating action potentials in neurons, myocytes and other excitable cells. The binding of STX to the voltage gated sodium channel at site one of the  $\alpha$  subunit within the cellular membrane blocks the inward flow of Na $^+$  to the cell [71]. This inhibits action potential generation and prevents nerve transmission impulses being passed from cell to cell, leading to the reported paralytic effects of PSTs in humans e.g. muscular paralysis, respiratory distress etc. (see acute effects below in Table 10 below). Different forms of the  $\alpha$  subunits of the sodium channel exist in humans. These have different binding affinities to PSTs [72] and it has been suggested that differences in sensitivity to the PSTs may occur as a result [3]. This may indicate that some groups of people have immunity to PSTs; however further research is required to fully evaluate this possibility.

While PST have been traditionally thought of as sodium channel blockers, recent research suggests that PSTs may also interfere with the function of potassium [73] and calcium channels [74]. However, the concentration of PST required to impair the function of these channels is much higher than that required to block sodium channels and therefore the biological relevance of this interaction remains unknown.

#### Acute effects

PSP results in a variety of symptoms in humans, ranging from mild to severe, including death as a worst-case scenario (Table 10). Following the consumption of seafood contaminated with PST, the time to onset of PSP symptoms can be as short as several minutes (paraesthesia and numbness around the lips, tongue, and mouth), but may begin within 12 hours following a latent period. Mild cases often develop gastrointestinal distress (vomiting, diarrhoea), but neurological symptoms can range from benign tingling and numbness sensations to difficulty swallowing and breathing in more severe cases [75, 76]. While there are no antidotes, the majority of patients recover without treatment, which is supportive only (intubation & artificial respiration in severe cases) [75-78]. Several case studies are available detailing the severity of symptoms experienced by individuals after consuming PST contaminated shellfish. A retrospective analysis of 54 outbreaks of PSP involving 117 persons in Alaska over the period 1973 to 1992 shows the time from ingestion of contaminated shellfish to recovery from illness ranged from 30 minutes to 24 hours [78]. However, in a study of a large outbreak of PSP caused by the consumption of scallops in Hong Kong in 2005 (58 cases), the duration of symptoms in some cases was found to be much longer, with a reported range of 1 to 228 hours [79].

In the review of PSP outbreaks in Alaska (1973 – 1992) it was found that death occurred in 0.85 % of affected people [78]. However, in some outbreaks the fatality rate has been higher - for example in Guatemala in 1987, 187 cases of PSP resulted from the consumption of PST contaminated clams (meat and soup), causing 26 people to die. The overall fatality rate was 14%. The fatality rate for victims under the age of 6 was 50% and for those older than 18 the fatality rate was 7% [80]. In fatal cases, death is caused by respiratory paralysis. Several reviews note that patients surviving beyond 24 hours have a higher probability of full recovery [3, 81].

Table 10 Symptoms of Paralytic Shellfish Poisoning in humans [3, 78, 82].

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

#### Chronic effects

No data derived from studies employing standard tests have been reported on long-term toxicity (chronic toxicity or carcinogenicity) of PST [15, 83, 84]. The lack of repeat oral dosing studies in animals and humans led the EFSA's Contaminants in the Food Chain (CONTAM) Panel to conclude that a tolerable daily intake (TDI) could not be established [3].

#### **Toxicity**

The toxicity of individual PST analogues varies depending on the configuration of the side chains of the saxitoxin parent molecule. These differences in toxicity relate to the structural changes in the hydroxyl and carbamoyl side chains, which affects the binding to sodium channels and/or biological activity of the PST analogues. As noted previously, TEFs are employed to relate the toxicity of individual PST analogues to saxitoxin and report total toxicity as saxitoxin equivalents per kg of tissue weight [21].

In 2004 and 2009 the World Health Organisation, Intergovernmental Oceanographic Commission, and Food and Agricultural Organisation (WHO/IOC/FAO, [4]), and the EFSA ([3]) respectively, reviewed data related to human poisonings from PSTs in order to develop an acute reference dose (ARfD) for PSTs. This involved reviewing approximately 20 illness outbreaks in Canada [4, 85, 86] and around 500 reported cases of illness [3]. Data from the illness cases were used to establish a lowest-observed-adverse-effect-level (LOAEL). The LOAELs derived by the WHO/IOC/FAO and EFSA were slightly different i.e. 2.0 and 1.5 µg kg<sup>-1</sup> bw, respectively.

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 an ARfD. The EFSA Panel [3] described how this was done: "From the available reports on intoxications in humans, comprising more than 500 individuals, a LOAEL in the region of 1.5  $\mu$ g STX equiv. /kg bw 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 NOAEL of 0.5  $\mu$ g STX equiv. /kg bw." Table 10 below shows the LOAEL's and ARfD's estimated by the EFSA Panel and the WHO/IOC/FAO Expert Consultation.

Both these reviews expressed concern over the ARfD in comparison to current regulatory levels, with EFSA stating "there is concern for the health for the consumer at the present regulatory limit". The reviews note that in order to avoid exceeding the derived ARfD, the current regulatory level would need to be reduced more than 10-fold. However, an alternative approach for risk assessment was conducted by Finch et. al [5], who examined oral toxicity of PSTs to mice in a sub-acute feeding study. This study determined a substantially higher ARfD of 7.3  $\mu$ g/kg bw/day, meaning a 60kg human would need to consume 540 g shellfish contaminated with PST at the regulatory level to become ill. The authors concluded the current regulatory level is protective of consumer health.

Table 10 Lowest observed adverse effect levels (LOAELs), acute reference doses (ARfD) and tolerated daily intake for PST.

	Food and Agricultural Organisation 2004 [4]	European Food Safety Authority 2009 [3]	Arnich and Thébault, 2018 [18]	Finch et al. 2021 [5]
LOAEL	2.0 μg/kg bw	1.5 μg STX equiv. /kg bw	0.33 μg STX equiv. /kg bw*	
ARfD	0.7 μg STX equiv. /kg bw	0.5 μg STX equiv. /kg bw		0.73 μg STX.2HCl <sup>1</sup> equiv. /kg bw**
TDI	Insufficient data on			
*Determined fro consuming this o **Based on oral				

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<sup>&</sup>lt;sup>1</sup> It has recently been highlighted that the term STX equivalents has previously been used as shorthand for STX.2HCl equivalents [88]. STX equiv in the context of this table refers to the need to determine toxic equivalencies for each analogue before totalling toxin concentration.

# Summary Evaluation of risk (i.e. risk characterisation)

### Severity of hazard

- The consequence of human exposure to PST ranges from mild to severe illness, with fatalities a rare endpoint.
- The LOAEL and ARfD for PST have been determined, but not the TDI, as insufficient data on the chronic effects of PST is available.

## Likelihood of exposure of urchins to PST phytoplankton

- The likelihood of exposure of urchins to PST producing phytoplankton is high:
  - o urchins are harvested year-round from areas where regular blooms of toxic *G. catenatum* and *A. catenella* are known to occur;
  - other species of seafood from the same fishing areas are known to accumulate PST.
- Whilst the normal urchin diet is macro-algae, it is possible they could shift to a more detritus based diet on urchin barrens, which could potentially influence their feeding/exposure to microalgae.

### Propensity for urchins to accumulate and retain PST

- In Tasmanian sea urchin roe surveyed to date, only 3 confirmed and 14 screen samples had detectable levels of PST reported from a total of 228 samples collected on 156 sampling occasions (equivalent to at least 353 individual urchins analysed as either pooled or individual samples).
- Of the positive detections:
  - o one sample was analysed from a *G. catenatum* bloom (n=1/1)
  - o sixteen samples were analysed from A. catenella blooms (n=16/227).
- The maximum confirmed concentration detected was 15% of the regulatory level (0.12 mg STX equiv. /kg), whilst the maximum unconfirmed screen result was 38% of the regulatory level (0.30 mg STX equiv. /kg).
- Many urchin samples containing no or trace levels of PST were collected during periods of heightened biotoxin activity (71 urchins in total, consisting of, 30 *C.* rodgersii, 22 *H. erythrogramma*, and another 19 where the urchin species was unspecified).
- There is currently no evidence to suggest that the PST risk differs between *C. rodgersii* or *H. erythrogramma* urchins during *A. catenella* blooms.
- No PST was detected in sea urchins sampled after blooms, while other grazers (abalone) still contain significant levels of PST.
- Evidence does exist of high (>8 mg STX equiv. /kg) PST levels in non-commercial Chilean/Argentinian sea urchins, where the whole animal has been analysed instead of the roe only.
- Elevated PST levels (<0.35 mg STX equiv. /kg) have also been reported during random surveys of urchins in North Sea, and Madeira/Azores, again when the whole animal has been analysed instead of the roe only.

### Human exposure post-processing

- Urchin roe is the commercial product sold and consumed. Some very limited sale of live urchins occurs domestically, but only the roe is exported. No evidence of consumption of urchin viscera was found during literature searches.
- Urchin roe is largely consumed raw or raw in brine, and either stored refrigerated or frozen.
- Considering the roe processing steps, storage temperatures, and stability of PST during cooking/refrigeration or frozen storage, there is no evidence to suggest that the PST risk is exacerbated or decreased by any of these steps, although there is some evidence of PST leaching/concentrating during steaming in bivalves.
- Preliminary assessment of serving size indicates 100-200 g of urchin roe consumed in a sitting (based on anecdotal reports and online recipes).
- A small adult consuming a large portion of roe at the maximum PST concentration found in this report will consume 0.34 μg STX equiv. /kg bw. This exposure level is less than both the EFSA and FAO/WHO ARfDs of 0.5 and 0.7 μg STX equiv. /kg bw respectively, and considerably lower than the ARfD estimated by Finch et al. of 7.3 μg STX.2HCl equiv. /kg bw.

### Reported linkages with illness

• There have been no reports linking incidences of paralytic shellfish poisoning to the consumption of sea urchins or their products.

# Uncertainty and knowledge gaps

- No information is available on the potential mechanisms of PST accumulation in sea urchins. Postulated exposure routes include consumption of phytoplankton settled on seaweed or in detritus or direct contact with phytoplankton suspended in seawater.
- It is unknown if there is a higher risk of urchin exposure to PST on urchin barrens due to a shift to a more detritus-based diet.
- There is no information/data on uptake/depuration rates of PST in sea urchins.
- There is evidence that under the conditions tested (including extensive, high level, prolonged *A. catenella* blooms) that PST does not accumulate to significant concentrations in urchin roe. However, we have only collected 1 sample from an area known for *G. catenatum* blooms and PST were detected in this sample.
- Few (n=5) urchins were collected during extreme *A. catenella* blooms where PST in bivalves exceeded 10 mg STX equiv. /kg.
- Several international studies have detected PST in urchins, however these studies have mostly analysed the entire urchin contents roe and viscera. A notable outlier is the Chilean sea urchin, *Loxechinus albus*, where a maximum PST concentration of 1.86 mg STX equiv./kg was found in the roe during sampling in an area where intense algal blooms frequently recur (bivalves regularly exceed 100 mg STX equiv./kg in this area, [65]). The viscera (other than the roe) of Tasmanian urchins has not been analysed for PST.

### Control measures

- Urchins currently do not have a separate biotoxin management plan in Tasmania, but as grazers are loosely linked to Abalone Biotoxin Management Plan [10].
   Processors test urchins monthly from abalone sub-blocks that are closed for the taking of abalone for biotoxin reasons as part of their Food Safety Management System. For regulatory purposes, the bivalve PST ML is applied.
- The focus on closed abalone blocks has meant that PST testing has occured on east coast where abalone block closures have persisted in some sub-blocks since 2017 bloom. Some urchin harvest occurs in Channel region where *G. catenatum* recurrently blooms, but generally urchins are not analysed for PST from this area. This has severely limited the information of PST risk in urchins during *G. catenatum* blooms.
- Abalone appear to hold on to significant levels of PST between biotoxin seasons [87]
  which has resulted in an increased risk perception in sea urchins and a concomitant
  requirement for testing.

The current controls are highly conservative for the urchin harvest on the east coast of Tasmania, i.e. there is no evidence that controls are needed to mitigate PST risk during low to moderate *A. catenella* blooms. This is based on extensive sampling (101 sea urchins) during high-risk periods, where PST in bivalve shellfish exceeded 0.1 mg STX equiv./kg at the time and location of urchin sampling. Among the urchin samples collected during these periods, 70% were collected when bivalve PST levels had exceeded the ML (i.e. 71 urchins, including 45 *H. erythrogramma*, 7 *C. rodgersii* and 19 urchins of unknown species). These animals were collected on 15 different sampling occasions and analysed for PST as 42 individual and 4 pooled samples. Monitoring during more extensive blooms may be appropriate, as few urchin samples have been collected during *A. catenella* blooms when PST in bivalves exceeded 10 mg STX equiv./kg.

# Conclusions/recommendations

There is considerable evidence that Tasmanian urchins do not accumulate PST to levels of concern in the roe (the consumed tissue for urchins) during *A. catenella* blooms. Whilst this may also be the case during *G. catenatum* blooms, we cannot rule out PST accumulation in this circumstance due to a lack of sampling effort during these blooms.

The current controls are highly conservative for the urchin harvest on the east coast of Tasmania, i.e. there is no evidence that controls are needed to mitigate PST risk during low to moderate *A. catenella* blooms. This is based on extensive sampling (101 sea urchins) during risk periods, where PST in bivalve shellfish exceeded 0.1 mg STX equiv./kg at the time and location of urchin sampling. Among the urchin samples collected during these periods, 70% were collected when bivalve PST levels had exceeded the ML (i.e. 71 urchins, including 45 *H. erythrogramma*, 7 *C. rodgersii* and 19 urchins of unknown species). These animals were collected on 15 different sampling occasions and analysed for PST as 42 individual and 4 pooled samples.

We recommend a review of the current risk controls based on the information presented in this risk profile. In particular:

- 1. Consideration of when risk controls are necessary.
- 2. De-linking urchin testing from PST results in abalone on east coast.
- 3. Using risk monitoring results from other seafood biotoxin monitoring in Tasmania to indicate potential PST risk associated with *G. catenatum*, considering both where and when harvest activity is occurring.

We also recommend consideration of the following activities to address the current knowledge gaps:

- 1. Testing of urchins for PST during any elevated PST activity associated with *G. catenatum* and during high *A. catenella blooms* when PST in bivalves exceed 10 mg STX.equiv. /kg, with consideration given to more frequent (e.g. weekly monitoring) during and after these blooms.
- 2. Testing urchin viscera during all toxic algal blooms to ascertain why some international and local results differ, maintaining a record of where urchins were sampled (healthy reef vs. urchin barrens).

This risk profile is focused on public health risk, in accordance with the agreed scope. Some overseas markets apply a PST regulatory level to all aquatic product (e.g. major export markets in China and Hong Kong). The level of testing of imported product that occurs in these markets is unknown. On the basis of the results presented in this risk profile, the probability of Tasmanian urchin roe exceeding import standards is low (noting unknown risk during *G. catenatum* blooms, but low harvest and export volumes in *G. catenatum* affected areas).

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# **Glossary**

ARfD: acute reference dose

bw: body weight

EFSA: European Food Safety Authority

EFSA CONTAM: European Food Safety Authority's Contaminants in the Food Chain Panel

FAO: Food and Agricultural Organisation

HILIC MS/MS: hydrophilic interaction chromatography coupled to tandem mass

spectrometry

IMAS: Institute for Marine and Antarctic Studies

IOC: Intergovernmental Oceanographic Commission

LC-FLD: liquid chromatography fluorescence detection

LOAEL: lowest-observed-adverse-effect level

NOAEL: no-observed-adverse-effect level

NRE: Natural Resources and Environment Tasmania

PSP: paralytic shellfish poisoning

PST: paralytic shellfish toxin

ShellMAP: Shellfish Market Access Program

TAC: total allowable catch

TDI: tolerable daily intake

TEF: toxin equivalency factor

WHO: World Health Organisation

## References

- 1. Flukes, E., C. Johnson, and S. Ling, *Forming sea urchin barrens from the inside out: an alternative pattern of overgrazing.* Marine Ecology Progress Series, 2012. **464**: p. 179-194.
- 2. Condie, S.A., E.C.J. Oliver, and G.M. Hallegraeff, *Environmental drivers of unprecedented Alexandrium catenella dinoflagellate blooms off eastern Tasmania, 2012–2018.* Harmful Algae, 2019. **87**: p. 101628.
- 3. EFSA, Scientific opinion of the panel on contaminants in the food chain: marine biotoxins in shellfish saxitoxin group. The EFSA Journal, 2009. **1019**: p. 1-76.
- 4. FAO/WHO/IOC, *Joint ad hoc expert consultation on biotoxins in bivalve molluscs*. 2004: Oslo, Norway. p. 1-31.
- 5. Finch, S.C., et al., Sub-Acute Feeding Study of Saxitoxin to Mice Confirms the Effectiveness of Current Regulatory Limits for Paralytic Shellfish Toxins. Toxins, 2021. **13**(9): p. 627.
- 6. Tasmanian Department of Natural Resources and Environment. *Commercial dive fishery*. 22/02/2023]; Available from: https://nre.tas.gov.au/sea-fishing-aquaculture/commercial-fishing/commercial-dive-fishery.
- 7. Turnbull, A., N. Malhi, and S. Pahl, *Risk ranking for marine biotoxins in Tasmanian seafood*. 2015, South Australian Research and Development Institute, Food Safety and Innovation.
- 8. Turnbull, A., Dorantes-Aranda, J.J., Malhi, N., Stone, D., Bansemer, M., Jolley, J., Hallegraeff, G. and Seger, A. , *Improving risk management of paralytic shellfish toxins in Blacklip Abalone (Haliotis rubra rubra)*. 2020, South Australian Research and Development Institute: Adelaide.
- 9. Department of Primary Industries, Parks, Water and Environment Wild Fisheries Management,, *Rock Lobster biotoxin monitoring program and decision protocols*. 2019.
- 10. Lisson, D., Abalone biotoxin management plan: a management plan for commercially -caught abalone in eastern Tasmania. 2017.
- 11. Tasmanian Shellfish Market Access Program ShellMAP, *Biotoxin management plan. Version 5.1.* 2019.
- 12. FSANZ., Australia New Zealand Food Standards Code, Schedule 19: Maximum levels of contaminants and natural toxicants. 2015, Food Standards Australia New Zealand: Australia. p. 1-7.
- 13. European Union. *Rapid Alert System for Food and Feed (RASFF)*. 24/03/2022; Available from: https://webgate.ec.europa.eu/rasff-window/screen/search.
- 14. US Center for Disease Control and Prevention. *National Outbreak Reporting System* (NORS). 24/03/2022; Available from: https://wwwn.cdc.gov/norsdashboard/.
- 15. EFSA, Marine biotoxins in shellfish domoic acid. Scientific opinion of the Panel on Contaminants in the Food Chain. The EFSA Journal, 2009. **1181**: p. 1-61.
- 16. Wiese, M., et al., *Neurotoxic alkaloids: saxitoxin and its analogs*. Mar Drugs, 2010. **8**(7): p. 2185-211.
- 17. van Egmond, H.P., M.E. van Apeldoorn, and G.J.A. Speijers, eds. *Marine biotoxins*. FAO Food and Nutrition Paper 80, ed. FAO. 2004, Food and Agriculture Organization of the United Nations: Rome, Italy. 278.

- 18. Arnich, N. and A. Thébault, *Dose-response modelling of paralytic shellfish poisoning* (*PSP*) in humans. Toxins, 2018. **10**(4): p. 141.
- 19. Munday, R., et al., *Acute toxicities of saxitoxin, neosaxitoxin, decarbamoyl saxitoxin and gonyautoxins 1&4 and 2&3 to mice by various routes of administration.* Toxicon, 2013. **76**: p. 77-83.
- 20. Oshima, Y., *Postcolumn derivatization liquid chromatographic method for paralytic shellfish toxins.* Journal of AOAC International, 1995. **75**(2): p. 528-532.
- 21. FAO/WHO, Technical paper on toxicity equivalency factors for marine biotoxins associated with bivalve molluscs. 2016, Food and Agriculture Organization of the United Nations, World Health Organization: Rome.
- 22. Boundy, M.J., et al., *Development of a sensitive and selective liquid chromatography—mass spectrometry method for high throughput analysis of paralytic shellfish toxins using graphitised carbon solid phase extraction.* Journal of Chromatography A, 2015. **1387**: p. 1-12.
- 23. Turner, A.D., et al., Single-laboratory validation of a multitoxin ultra-performance LC-hydrophilic interaction LC-MS/MS method for quantitation of paralytic shellfish toxins in bivalve shellfish. Journal of AOAC International, 2015. **98**(3): p. 609-621.
- 24. Negri, A.P., et al., Widespread presence of hydrophobic paralytic shellfish toxins in Gymnodinium catenatum. Harmful Algae, 2007. **6**(6): p. 774-780.
- 25. Lawrence, J., et al., FAO Fisheries and Aquaculture Technical Paper 551, Assessment and Management of Biotoxin Risks in Bivalve Molluscs. 2011, Food and Agriculture Organisation of the United Nations: Rome, Italy.
- 26. Mulvenna, V., et al., *Health risk assessment for cyanobacterial toxins in seafood.* International Journal of Environmental Research and Public Health, 2012. **9**(3): p. 807-20.
- 27. Pearson, L., et al., On the Chemistry, Toxicology and Genetics of the Cyanobacterial Toxins, Microcystin, Nodularin, Saxitoxin and Cylindrospermopsin. Marine Drugs, 2010. **8**(5): p. 1650-1680.
- 28. McLeod, C., et al., *Semi-quantitative risk assessment of paralytic shellfish toxins in canned Australian abalone*. 2010, South Australian Research and Development Institute: Australia. p. 1-57.
- 29. Farrell, H., et al., *Distribution of the genus Alexandrium (Halim) and paralytic shellfish toxins along the coastline of New South Wales, Australia.* Marine Pollution Bulletin, 2013. **72**(1): p. 133-45.
- 30. Bolch, C.J.S. and M.F. de Salas, A review of the molecular evidence for ballast water introduction of the toxic dinoflagellates Gymnodinium catenatum and the Alexandrium "tamarensis complex" to Australasia. Harmful Algae, 2007. **6**(4): p. 465-485.
- 31. Seger, A., et al., *Uptake of paralytic shellfish toxins by Blacklip Abalone (Haliotis rubra rubra Leach) from direct exposure to Alexandrium catenella microalgal cells and toxic aquaculture feed.* Harmful Algae, 2020. **99**: p. 101925.
- 32. Bolch, C., et al. Alexandrium tamarense Group I as the cause of PST on the east coast of Tasmania, Australia. (Abstract Only). in The 16th International Conference on Harmful Algae. 2014.
- 33. McLeod, C., et al., Accumulation and depuration of paralytic shellfish toxins by Australian abalone Haliotis rubra: Conclusive association with Gymnodinium catenatum dinoflagellate blooms. Food Control, 2017. **73**(Part B): p. 971-980.

- 34. Oshima, Y., S. Blackburn, and G. Hallegraeff, *Comparative study on paralytic shellfish toxin profiles of the dinoflagellate Gymnodinium catenatum from three different countries.* Marine Biology, 1993. **116**(3): p. 471-476.
- 35. Hallegraeff, G., et al., Global toxicology, ecophysiology and population relationships of the chainforming PST dinoflagellate Gymnodinium catenatum. Harmful Algae, 2012. **14**: p. 130-143.
- 36. Hallegraeff, G., et al., *Improved understanding of Tasmanian harmful algal blooms* and biotoxin events to support seafood risk management. 2018, Institute for Marine and Antarctic Studies: Australia.
- 37. Bolch, C. and G. Hallegraeff, *Dinoflagellate cysts in recent marine sediments from Tasmania*, *Australia*. 1990.
- 38. Hallegraeff, G.M., et al., *Overview of Australian and New Zealand harmful algal species occurrences and their societal impacts in the period 1985 to 2018, including a compilation of historic records.* Harmful Algae, 2021. **102**: p. 101848.
- 39. Byrne, M. and N. Andrew, *Centrostephanus rodgersii*, in *Developments in Aquaculture and Fisheries Science*. 2013, Elsevier. p. 243-256.
- 40. Cresswell, K., et al., *Tasmanian Longspined Sea Urchin fishery assessment 2018/19*. 2019, Institute for Marine and Antarctic Studies.
- 41. Ling, S., et al., Climate-driven range extension of a sea urchin: inferring future trends by analysis of recent population dynamics. Global Change Biology, 2009. **15**(3): p. 719-731.
- 42. Jones, G. and N. Andrew, *Herbivory and patch dynamics on rocky reefs in temperate Australasia: the roles of fish and sea urchins.* Australian Journal of Ecology, 1990. **15**(4): p. 505-520.
- 43. Keesing, J.K., *Heliocidaris erythrogramma*. Developments in Aquaculture and Fisheries Science, 2020. **43**: p. 537-552.
- 44. Growns, J. and D. Ritz, *Colour variation in southern Tasmania populations of Heliocidaris erythrogramma (Echinometridae: Echinoidea).* Marine and Freshwater Research, 1994. **45**(2): p. 233-242.
- 45. Sanderson, J.C., M. Le Rossignol, and W. James, *A pilot program to maximise Tasmania's sea urchin (Heliocidaris erythrogramma) resource*. 1996: Fisheries Research & Development Corporation.
- 46. Wright, J.T., et al., Density-dependent sea urchin grazing: differential removal of species, changes in community composition and alternative community states. Marine Ecology Progress Series, 2005. 298: p. 143-156.
- 47. Vanderklift, M.A., G.A. Kendrick, and A.J. Smit, *Differences in trophic position among sympatric sea urchin species*. Estuarine, Coastal and Shelf Science, 2006. **66**(1-2): p. 291-297.
- 48. Tasmanian Department of Natural Resources and Environment, *Recreational sea fishing guide 2021-22*. 2021.
- 49. Tasmanian Department of Natural Resources and Environment, *Fishing zone borders, TACs and mnagement provisions for 2022/23 season*, S. Rainer, Editor. 2022: Hobart. p. 2.
- 50. Keane, J., et al., Can commercial harvest of long-spined sea urchins reduce the impact of urchin grazing on abalone and lobster fisheries? 2019.
- 51. Eddy, S. and N. Brown, *Echinoderm Aquaculture*. 2015, John Wiley & Sons Inc.

- 52. Institute for Marine and Antarctic Studies. *Tasmanian Wild Fisheries Assessments*. 21/03/2022]; Available from: https://tasfisheriesresearch.org/long-spined-urchinassessment/.
- 53. EFSA, Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for food of animal origin. Official Journal of the European Union, 2004. **226**: p. 22-82.
- 54. National Standard of the People's Republic of China Standard GB 2733-2015 Fresh, Frozen Aquatic Products of Animal Origin.
- 55. Australian Department of Agriculture Fisheries and Forestry, *Market Access Advice No: 11/9 Exporting Abalone to China*, Export Standards Branch, Editor. 2009.
- 56. FDA, Fish and Fishery Products Hazards and Controls Guidance, in US Department of Health and Human Services, Food and Drug Administration 2011.
- 57. South Korean Ministry of Food and Drug Safety, Food Code. 2019. p. 1-345.
- 58. Singapore Food Authority. Mycotoxins and marine toxins in food. III Maximum limits for marine biotoxins. 5/07/2022]; Available from:

  <a href="https://www.sfa.gov.sg/docs/default-source/default-document-library/mycotoxins-and-marine-biotoxins.pdf">https://www.sfa.gov.sg/docs/default-source/default-document-library/mycotoxins-and-marine-biotoxins.pdf</a> Accessed 23/06/2021.
- 59. New Zealand Government, Animal Products Notice: regulated control scheme bivalve molluscan shelfish for human consumption, in Ministry for Primary Industries. 2022: Wellington, New Zealand.
- 60. CODEX STAN 292-2008: Standard for Live and Raw Bivalve Molluscs.
- 61. CODEX STAN 312-2013 Standard for live abalone and for raw fresh chilled or frozen abalone for direct consumption or for further processing.
- 62. Terrazas, J.O., H.R. Contreras, and C. García, *Prevalence, Variability and Bioconcentration of Saxitoxin-Group in Different Marine Species Present in the Food Chain.* Toxins, 2017. **9**(6): p. 190.
- 63. Silva, M., et al., *Paralytic shellfish toxins occurrence in non-traditional invertebrate vectors from north Atlantic waters (Azores, Madeira, and Morocco).* Toxins, 2018. **10**(9): p. 362.
- 64. Dean, K.J., et al., Multiple new paralytic shellfish toxin vectors in offshore North Sea benthos, a deep secret exposed. Marine Drugs, 2020. **18**(8): p. 400.
- 65. Oyaneder Terrazas, J., H.R. Contreras, and C. García, *Prevalence, variability and bioconcentration of saxitoxin-group in different marine species present in the food chain.* Toxins, 2017. **9**(6): p. 190.
- 66. Silva, M., et al., *New invertebrate vectors for PST, spirolides and okadaic acid in the North Atlantic.* Marine Drugs, 2013. **11**: p. 1936-1960.
- 67. Montoya, N., *Toxinas paralizantes de moluscos en el Mar Argentino: impacto, transferencia trófica y perspectiva (in Spanish).* Marine and Fishery Sciences, 2019. **32**(1): p. 47-69.
- 68. Department of Agriculture Fisheries and Forestry. *Abalone from Tasmania:* sourcing/harvesting obligations (2018-06). Industry Notices 2018; Available from: https://www.agriculture.gov.au/biosecurity-trade/export/controlled-goods/fish/industry-advice-notices/2018/2018-06.
- 69. Yokota, Y. Fishery and consumption of the sea urchin in Japan. in The Sea Urchin: Proceedings of the Workshop at the International Marine Centre, Torregrande, Sardinia, Italy 2000. 2002. CRC Press.

- 70. Liu, H. and Y.-q. Chang, *Sea urchin aquaculture in China*, in *Echinoderm aquaculture*, N. Brown and S. Eddy, Editors. 2015. p. 12-146.
- 71. Zhang, F., et al., *Shellfish toxins targetting voltage-gated sodium channels*. Marine Drugs, 2013. **11**(12): p. 4698-4723.
- 72. Xie, W., et al., Accumulation and depuration of paralytic shellfish poisoning toxins in the oyster Ostrea rivularis Gould Chitosan facilitates the toxin depuration. Food Control, 2013. **30**(2): p. 446-452.
- 73. Wang, J., J.J. Salata, and P.B. Bennett, *Saxitoxin is a gating modifier of hERG K channels*. Journal of General Physiology, 2003. **121**(6): p. 583-598.
- 74. Su, Z., et al., *Saxitoxin blocks L-type ICa.* Journal of Pharmacology and Experimental Therapeutics, 2004. **308**(1): p. 324-329.
- 75. Etheridge, S.M., *Paralytic shellfish poisoning: Seafood safety and human health perspectives.* Toxicon, 2010. **56**: p. 108-122.
- 76. Hurley, W., et al., *Paralytic shellfish poisoning: a case series.* Western Journal of Emergency Medicine, 2014. **15**(4): p. 378.
- 77. de Carvalho, M., et al., *Paralytic shellfish poisoning: clinical and electrophysiological observations.* Journal of Neurology, 1998. **245**(8): p. 551-4.
- 78. Gessner, B.D. and J.P. Middaugh, *Paralytic shellfish poisoning in Alaska: A 20- year retrospective analysis*. American Journal of Epidemiology, 1995. **141**(8): p. 766-770.
- 79. 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.* Journal of Tropical Medicine and Public Health, 2006. **37**(1): p. 120-125.
- 80. Rodrigue, D.C., et al., *Lethal paralytic shellfish poisoning in Guatemala*. American Journal of Tropical Medicine and Hygiene, 1990. **42**: p. 267-271.
- 81. Sumner, J., Food safety plans for abalone farms, in Assessment of Risk: Biotoxins/Abalone. 2000.
- 82. van Dolah, F.M., *Marine algal toxins: Origins, health effects, and their increased occurrence.* Environmental Health Perspectives, 2000. **108**(SUPPL. 1): p. 133-141.
- 83. Daneshian, M., et al., A roadmap for hazard monitoring and risk assessment of marine biotoxins on the basis of chemical and biological test systems. Altex, 2013. **30**(4): p. 487-545.
- 84. Munday, R. and J. Reeve, *Risk assessment of shellfish toxins*. Toxins 2013. **5**(11): p. 2109-37.
- 85. Toyofuku, H., *Joint FAO/WHO/IOC activities to provide scientific advice on marine biotoxins (research report).* Marine Pollution Bulletin, 2006. **52**: p. 1735-1745.
- 86. FAO, *Marine Biotoxins. Food and Nutrition Paper 80*. 2004, Food Agriculture Organization: Rome.
- 87. Seger, A., T. Jordan, and A. Turnbull, *Review of Paralytic Shellfish Toxin monitoring data for Tasmanian Blacklip Abalone (2011-2022)*. 2022, Institute for Marine and Antarctic Studies: Hobart.
- 88. Turnbull, A.R., Harwood, D.T., Boundy, M.J., Holland, P.T., Hallegraeff, G., Malhi, N., Quilliam, M.A., 2020. *Paralytic shellfish toxins—call for uniform reporting units*. Toxicon 178, 59-60.