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Improving risk management of paralytic shellfish toxins in Blacklip Abalone (*Haliotis rubra rubra*)

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Abbreviations

ACA	Abalone Council of Australia Limited
AST	Analytical Services Tasmania
dcSTX	decarbamoylsaxitoxin
doSTX	deoxydecarbamoylsaxitoxin
DPIPWE	Department of Primary Industries, Parks, Water and Environment, Tasmania
GTX	gonyautoxin
IMAS	Institute of Marine and Antarctic Studies
ML	maximum level (regulatory level[1])
STX.2HCl equiv.	saxitoxin di-hydrochloride equivalents
Neo	neosaxitoxin
PST	paralytic shellfish toxin
SRL	Southern Rock Lobster
STX	saxitoxin
ShellMAP	Shellfish Market Access Program
TACL	Tasmanian Abalone Council Limited
TEF	Toxin equivalency factor

[1] The Codex bivalve and abalone regulatory level for PST is 0.8 mg STX.2HCl equiv. kg $^{-1}$ (Codex Standards 292-2008 and 312-2013, respectively).

Executive Summary

The accumulation of paralytic shellfish toxins (PST) of microalgal origin in abalone tissues causes a trade and human health risk that requires active management. Toxic algal blooms of the genus *Alexandrium* have recently caused several abalone harvest closures on the east coast of Australia. Risk management is hampered by a scarcity of knowledge on the mechanisms and rates of accumulation and depuration of the associated PST. A collaborative effort by researchers from the South Australian Research and Development Institute (SARDI), the Institute of Marine and Antarctic Science (IMAS) and Cawthron Institute conducted field studies on the east coast of Tasmania during toxic blooms in 2018 and 2019, and experimental studies in South Australia in 2018 to investigate uptake and depuration of PST from *A. catenella* in *Haliotis rubra rubra*, Blacklip Abalone. Several key results from the study will be used to inform improved risk management of this issue. In particular, we showed for the first time that: abalone can accumulate PST from exposure to toxic algal cells; the predominant accumulation is in the foot tissue; and toxins depurate extremely slowly from this tissue. We conducted a successful laboratory validation of a rapid screening test for PST analysis in foot tissue for future use during *A. catenella* blooms that should lead to significant savings in PST monitoring in Tasmania.

Background

In 2013, Codex released a food standard for abalone that required all countries exporting abalone to have an appropriate marine biotoxin risk assessment and, where necessary, implement risk management programs. In order to provide adequate human health risk management and to maintain access to abalone export markets (currently valued at A$187 M), the Australian abalone industry conducted a risk assessment that demonstrated a low risk for most production areas, with the exception of southern Tasmania during *Gymnodinium catenatum* microalgal blooms.

Since the initial risk assessment, *Alexandrium* spp. blooms have also caused cessation of abalone harvest in New South Wales, and on the east coast of Tasmania. These species are known to occur in all abalone producing states. A precautionary risk management approach is undertaken for abalone harvest during *Alexandrium* blooms in Australia, due to: the paucity of information on PST accumulation in abalone from these blooms; the expense of the intensive monitoring required for finer scale risk management (approximately A$5,000 in analysis for each site sampled); and the adverse impact of any PST detection in overseas markets. The precautionary approach has led to long term closures of some abalone fishing blocks on the east coast of Tasmania (up to two years).

Aims

This research project aims to improve the risk management of PST in abalone during and following *A. catenella* blooms, particularly to reduce the economic impact on fishers. It will increase the understanding of the risk that *Alexandrium* species pose to accumulation of PST in abalone. Specific objectives are to:

1. Determine PST uptake by abalone from two routes: exposure to cultures of *Alexandrium catenella* and contaminated feed
2. Determine the relative risk of PST accumulation in abalone compared to Southern Rock Lobster (SRL) at two field sites
3. Validate the Neogen rapid test kits for PST analysis in abalone foot and viscera tissues.

Methods

The research was conducted as three separate projects. The experimental work was conducted at the South Australian Aquatic Biosecurity Centre in Roseworthy, South Australia. Toxic *A. catenella* cultures sourced from Tasmania were bulk cultured at the Centre. Blacklip Abalone were collected from South Australian waters and acclimated to feed on pellets in tank systems. In one experiment, abalone were fed pellets contaminated with toxins produced by the *A. catenella* cultures. In another experiment,

abalone were exposed directly to field relevant concentrations of toxic algal cells, with a daily exchange of fresh culture in the abalone tanks. On harvest, the abalone were dissected into epipodium, foot and viscera tissues, and PST measured in each tissue.

Field sampling occurred on the east coast of Tasmania in conjunction with a similar project examining PST risk in Southern Rock Lobster. Blacklip Abalone (5 animals at each site) were collected by IMAS dive teams from three sites in 2018 and one in 2019 in response to *A. catenella* bloom events. Abalone were separated into foot and viscera tissues and analysed for PST.

A single laboratory validation of the Neogen rapid test kit for PST analysis in abalone foot and viscera tissues was undertaken, following international guidelines. Field and experimental data from Blacklip Abalone contaminated with PST from *A. catenella* and *G. catenatum* blooms were reviewed, and a stock contaminated matrix was produced for each tissue to represent the PST profile normally seen during contamination from each bloom species. A dilution series was then created, with PST concentrations ranging from no contamination to contamination above the regulatory level (0.8 mg STX.2HCl equiv. kg^{-1}). Multiple replicates of each dilution were tested with the Neogen rapid test kit to produce a probability of detection curve across these PST concentrations.

Key findings and implications

The field and experimental research undertaken demonstrated that Blacklip Abalone do accumulate PST toxins from *A. catenella* blooms to levels of concern for both trade and human health. Accumulation occurred through both consumption of toxic material and direct exposure to algal blooms: in the field this is likely to be through exposure to, and grazing of, algal cells and cysts that have sunk out of the water column and are present on the rock and seaweed surfaces.

The PST are concentrated primarily in the epipodium of the foot tissue, thus removing the epipodium will substantially reduce the toxicity of the foot. Processed abalone are therefore a lower risk than live abalone, although less valuable for the export market.

The total toxicity of the foot (including the epipodium) was always higher than that of the viscera. This important finding, which needs to be confirmed by additional field sampling, could reduce monitoring costs by half as it negates the need to sample both viscera and foot tissues.

Depuration of PST is very slow from the Blacklip Abalone foot: in one case an approximate decrease of 0.1 mg STX.2HCl equiv. kg^{-1} over 6 months. As a result, toxins may carry over from previous bloom seasons resulting in animals exceeding the regulatory level for long periods. Bivalve shellfish and Southern Rock Lobster (SRL) are not good sentinel species to indicate toxicity of the abalone, as they accumulate and depurate toxins more rapidly in response to the algal bloom peaks and troughs.

The Neogen rapid screen test performed well to indicate PST toxicity in abalone foot tissue following *A. catenella* blooms, with a 98% probability of detection at the regulatory level. Combined with the fact that foot tissue is the most at risk of PST accumulation, this result provides good potential for a cost-effective risk management regime. We therefore recommend the Neogen rapid screen test in Blacklip Abalone foot should undergo a field validation. However, the Neogen rapid screen test for PST in its current form is not appropriate for use with Blacklip Abalone viscera, or for Blacklip Abalone exposed to *G. catenatum* blooms. This is most likely because the PST analogues accumulated in the viscera during *A. catenella* blooms, or in the foot and viscera during *G. catenatum* blooms, are poorly detected by the Neogen rapid screen test kit. Care should be taken when relying on this test kit that the PST profile remains predominantly saxitoxin and neosaxitoxin (as normally occurs following *A. catenella* blooms), else the results cannot be assured.

Recommendations

Importantly, this project has highlighted substantial differences between the uptake and depuration of the PST analogues produced by *A. catenella* and those produced by *G. catenatum*. The situation during *A.*

catenella blooms in Tasmania seems to be more similar to international observations of PST in abalone in Spain and South Africa than the observations made during *G. catenatum* blooms in south east Tasmania. Improved risk management on the east coast will require a better understanding of PST uptake and depuration in Blacklip Abalone. Future research required in order to design an effective field monitoring program needs to focus on understanding: residual toxin levels between blooms; the uptake and depuration rates; variability between animals at each site; variability in PST profiles within and between seasons; and geographic variability. The slow depuration rate in particular is of concern and has the ability to cause long-term disruption to harvest.

Of high priority to improve the cost effectiveness of the current biotoxin monitoring is confirmation of the higher concentration of PST in foot tissues, better understanding of the range of profiles seen in foot tissue across blooms, and a field validation of the Neogen rapid screening tool for Blacklip Abalone foot tissue.

We recommend that a working group consisting of industry, regulators and the researchers from this project be formed to review the Tasmanian abalone marine biotoxin management plan in light of the results presented here. The slow depuration rate is of particular concern and needs to be considered carefully when determining a sampling regime. Further research (particularly field work) is required to confirm the findings of this report and will allow substantial improvements to the biotoxin management plan. The report should be disseminated to all states (industry groups, food safety and fisheries regulators, shellfish quality assurance programs) to highlight the risk of PST accumulation in abalone following toxic algal blooms.

Keywords

Marine biotoxin, paralytic shellfish toxin, *Alexandrium catenella, Haliotis rubra rubra*

Chapter 1 - Introduction

The risk management of phytoplankton derived biotoxins in aquacultured and wild caught marine produce requires an understanding of toxin uptake and depuration mechanisms to inform effective monitoring and implementation of strategies that ensure consumer health and safety. Consequences of human ingestion of seafood contaminated with Paralytic, Amnesic or Diarrhetic shellfish toxins (PST, AST and DST) can be severe, with symptoms ranging from mild discomfort, paraesthesia, diarrhoea and vomiting to, in extreme cases, death (James et al., 2010). Overseas detection of PST in exported marine produce can result in prolonged trade closures (multiple years in some cases) and indirectly impact sales of other seafood commodities due to reduced consumer confidence.

Perhaps one of the most notorious among the shellfish poisoning syndromes, paralytic shellfish poisoning, is caused by the ingestion of saxitoxin and its congeners, collectively referred to as paralytic shellfish toxins. The natural producers of these toxins belong to the widespread microalgal genus *Alexandrium*, the species *Gymnodinium catenatum* and the tropical *Pyrodinium bahamense*. Naturally occurring blooms of these dinoflagellate species can span over large coastal areas (up to 100s of km^2 in case of *Alexandrium*) and persist for several months (Anderson et al., 2012a; Condie et al., 2019; Hallegraeff et al., 2012). The PST produced by these species tend to rapidly (within days) build up to significant levels in filter feeding bivalve molluscs (Gueguen et al., 2012; Kwong et al., 2006) and have been demonstrated to subsequently accumulate in higher trophic levels. Predatory species, such as cephalopods (Braid et al., 2012; Robertson et al., 2004), crustaceans (Desbiens et al., 1995; McLeod et al., 2018) and certain carnivorous gastropods (Compagnon et al., 1998; Lin and Hwang, 2012) have all been documented to contain PST at concentrations exceeding the regulatory maximum allowable level (ML) of 0.8 mg STX.2HCl equiv. kg^{-1}. Consequential closures of commercial and recreational harvest areas following detection of elevated PST levels, as well as the cost of ongoing monitoring during bloom periods, provide a strong economic incentive to better understand PST uptake and depuration mechanisms in species with high market value, such as abalone.

In 2013, Codex released a food standard for abalone that required all countries exporting abalone to have an appropriate marine biotoxin risk assessment and, where necessary, implement risk management programs (Codex Alimentarius Commission, 2013). This standard has been adopted by many trading countries, including China, which stipulates a maximum level for marine biotoxins in all aquatic products. Australia produces 3,400 t of abalone per annum (Mobsby, 2018), with an export value of A$187 M (live, frozen and processed). An Australian marine biotoxin risk assessment in 2014 indicated that most abalone production was not at risk from accumulation of high levels of marine biotoxins, with only southern Tasmania having to implement a marine biotoxin management program to manage PST accumulation during *Gymnodinium catenatum* microalgal blooms.

Since the initial risk assessment, *Alexandrium* spp. blooms have also caused cessation of abalone harvest in New South Wales and on the east coast of Tasmania. However, limited information currently exists on the ability of Australian abalone to accumulate toxins from *Alexandrium* species. This information would be a valuable addition to the original risk assessments to both demonstrate that Australia is maintaining up-to-date risk assessments, and to improve risk management activities in response to this new threat.

Alexandrium species are known to occur in Western Australia, South Australia, New South Wales, Victoria and Tasmania (McLeod et al., 2014), with the highly toxic cold-water *A. catenella* forming recurrent blooms on the east coast of Tasmania since 2012. Prior to 2017, only low levels of PST were detected in abalone in association with *Alexandrium* blooms (max. 0.3 mg STX.2HCl equiv. kg^{-1}), in contrast to elevated levels of PST found during *G. catenatum* blooms in southern Tasmania. However,

during the 2017 Tasmanian *A. catenella* bloom, Blacklip Abalone were found to contain 1.2 mg STX.2HCl equiv. kg^{-1} (1.5 times the Codex regulatory level).

Currently, a precautionary risk management approach is taken to cease abalone harvest during *Alexandrium* blooms in Australia, with harvest closures based on information arising from bivalve shellfish monitoring programs. At the time this proposal was developed (2017), the impact on the abalone fishery on the east coast of Tasmania was more than 25 block closures, some of which had been continuous for over 2 years. This conservative approach was adopted due to: the paucity of information on PST accumulation in abalone from *A. catenella* blooms; the expense of the intensive monitoring required for finer scale risk management; and the high consequence of any PST detection in overseas markets[2].

This research project aims to improve the risk management of PST in abalone during and following *A. catenella* blooms, particularly to reduce the economic impact on fishers. It will increase the understanding of the risk that *Alexandrium* species pose to accumulation of PST in abalone. The project includes:

- experimental studies of PST uptake during exposure to toxic feed and toxic algae in the water[3]
- field studies examining the PST concentration in Blacklip Abalone foot and viscera tissues during *A. catenella* blooms
- validation of a rapid screening tool for PST in abalone tissues, with potential to significantly reduce monitoring costs.

The experimental, field and validation studies are each presented as a separate chapter in this report, with a combined discussion and conclusion following. The experimental work was undertaken in the South Australian Aquatic Biosecurity Centre, alongside similar experimental work on PST uptake in Southern Rock Lobster. The field component of the work leveraged off field work concurrently occurring with Southern Rock Lobster, as both high value seafood species are impacted by *A. catenella* blooms on the east coast of Tasmania. As such, there is potential to share risk management costs if consistent relationships in toxin accumulation by lobster and abalone can be determined.

[2] Following the detection of PST in Tasmanian mussels by Japan in 2012, shellfish from all Australian states were barred from export to Japan. Each state then had to submit their biotoxin management plan and recent testing results for assessment prior to re-entry to the Japanese market. This process took several months (NSW and SA) to years (Tas) to complete.

[3] Costs for this study were significantly offset by a grant from the Australian Seafood CRC (FRDC Project 2017-051) covering hire of the South Australian Aquatic Biosecurity Centre at Roseworthy, South Australia; hardware such as pumps, tanks, and filtration systems; saltwater delivery; production of algal cultures; and full-time technical staff to maintain the systems and conduct the daily animal husbandry.

Chapter 2 - Objectives

1. Determination of PST uptake by abalone from two routes: exposure to cultures of *A. catenella* and contaminated feed
2. Determination of relative risk of PST accumulation in abalone compared to Southern Rock Lobster at two field sites
3. Validation of rapid test kits for PST analysis in abalone foot and viscera tissues

Chapter 3 - Investigation of the uptake of PST from microalgal and toxic aquaculture feed pellet sources by abalone

3.1 Introduction

Paralytic, diarrhetic and amnesic shellfish toxins have all been documented to accumulate in a range of wild caught and aquacultured abalone species (Malhi et al., 2014; Martínez et al., 1996; Pitcher et al., 2001). To the best of our knowledge, no human intoxications have been confirmed to date, although PST at concentrations exceeding the bivalve ML have previously been reported in *Haliotis midae* from South Africa (Pitcher et al., 2001), *Haliotis tuberculata* from Spain (Bravo et al., 1999) and in Blacklip Abalone (*Haliotis rubra rubra*) from Australia (McLeod et al., 2017). The detection of toxic abalone in the apparent absence of PST producing phytoplankton, along with either no detection of PST in mussels (Spain, Bravo et al., 1999; Martínez et al., 1996) or significantly different PST profiles between abalone and mussel tissues (South Africa, Pitcher et al., 2001), has cast some doubt on microalgae as the source of PST in these areas.

In Australian waters, however, PST concentrations in both abalone foot and viscera have been conclusively linked to seasonal phytoplankton blooms, with PST concentrations following bloom development, and *G. catenatum* DNA detected in abalone viscera (McLeod et al., 2017). Field sampling of Blacklip Abalone over a two-year period in south eastern Tasmania showed that PST levels closely followed the seasonal bloom dynamics of *G. catenatum*, with low background toxicity being retained in the foot between bloom episodes. While *G. catenatum* blooms in Tasmanian waters have usually been confined to estuarine areas (Anderson et al., 2012b; Hallegraeff et al., 1995), recurrent large-scale blooms of *A. catenella* along the east coast of Tasmania have repeatedly caused regional harvest closures since 2012 (Hallegraeff et al., 2018). In the absence of monitoring for PST in abalone, precautionary Tasmanian abalone fishery closures are currently initially based on PST levels in bivalve shellfish. A better understanding of the uptake of PST from *Alexandrium* by abalone promises improvements to existing management and monitoring protocols (certain Tasmanian harvest areas have remained closed for more than two consecutive years). The Tasmanian abalone fishery represents the largest wild abalone resource in the world, supplying close to 25% of the annual wild-caught global abalone harvest (TACL, 2017), providing a strong economic incentive to effectively manage the impact of PST on the fishery.

We here present the results from Blacklip Abalone exposures to either live *A. catenella* microalgal cultures or a PST contaminated food source with known toxin profile (feed pellet). PST levels in viscera, epipodium and foot tissue were analysed separately via LC-MS (Boundy et al., 2015; Turner et al., 2020, 2015) to investigate potential PST uptake by Blacklip Abalone.

3.2 Methods

3.2.1 Abalone stock

Wild Blacklip Abalone were collected from Pelican Point near Mt. Gambier, South Australia on the 12th of October 2018 under ministerial exemption ME9903010. Animals were transported in seawater in 50 L containers (20 animals/container) with temperature as well as dissolved oxygen (DO; Oxyguard Polaris 2) monitored at hourly intervals during the 8 h journey to the experimental facility. Ambient air was supplied via battery operated aquarium pumps (Aqua One, 250 L h^{-1}) and supplemented with a 30-60 s burst of medical grade oxygen as required (if <85% DO).

Husbandry

Upon arrival, abalone were weighed and transferred to 450 L holding tanks (50 animals/tank) to acclimate to experimental conditions and wean abalone from their natural seaweed diet onto specially formulated pellet feed (see below). Water in the holding tanks was constantly circulated (~1000 L h^{-1}) through canister filters (Aqua One Nautilus CF2700) loaded with preconditioned K1 media. Temperature, pH (HACH KTO sensION), DO (Oxyguard, Polaris 2), conductivity (HACH KTO sensION), ammonia (API Fishcare, LR8600) and nitrite concentrations (Hach, NitriVer® 3) were measured daily. Solid waste (faeces and uneaten feed) was removed via siphon and daily water exchanges (10-100%) were conducted in response to the recorded water parameters to maintain the following: ammonia <1 ppm, nitrite <0.4 ppm, salinity 35, temperature 15.1 – 18.1°C and pH 8-8.2.

Weaning of abalone

Abalone were weaned off their natural diet over a period of five weeks by slowly reducing the amount of seaweed and increasing the amount of feed pellets provided. Green (*Ulva* spp.) and red seaweed species (*cf. Platoma* and *Ploclamium* spp) were initially harvested at the site of abalone collection. To continuously supply fresh seaweed during the weaning phase, *Ulva* spp. was subsequently collected from Outer Harbor, Adelaide. After an initial acclimatisation period of 2 days without feed, abalone were fed a diet consisting of a 50% *Ulva* and red seaweed presented at 1.7% abalone body weight (BW, based on wet in shell weight). Once abalone were feeding on the seaweed, each tank received ~1.3% BW (160 g) of seaweed and 0.08% BW (10 g) of small brood-stock pellets (circular, 3 mm diameter; Aquafeeds Australia, Mount Barker). At the peak of the weaning process, abalone were fed the brood-stock pellet at 0.25% BW, supplemented with 0.4% BW *Ulva*. Prior to experiments commencing, seaweed additions ceased and abalone weaned from the brood-stock pellet onto the non-toxic basal experimental pellets.

3.2.2 *Alexandrium* cultures

Alexandrium catenella strain AT.TR/F (previously known as *A. tamarense* Group 1) was obtained from the Institute for Marine and Antarctic Studies, Hobart, Australia (originally isolated from Triabunna, Tasmania in 2012 by Chris Bolch). The microalga was cultivated in batch cultures (15 L carboys) with 120 µmol photons m^{-2} s^{-1} of light supplied by a custom array of low temperature LEDs on a 12:12 h light:dark cycle. Cultures were maintained at 18 ± 1 °C and the seawater culture medium (0.22 µm sterile filtered coastal seawater) supplemented with modified GSe nutrient concentrations (final media = 3/4 GSe nutrients, 5 mM sodium bicarbonate and 7.5 pM H_2SeO_3 to replace the soil extract in the basal recipe). The growth vessels were gently aerated (0.15 L min^{-1}) with ambient air during the dark period and 1.5 - 2.5% (v/v) CO_2 in the light.

3.2.3 Toxic feed preparation

To study the direct dietary uptake of PST by abalone, an aquaculture feed pellet was especially formulated to contain in-house produced PST harvested from *A. catenella* cultures. Feed pellets were produced using cold extrusion methods at Aquafeeds Australia (Mt Barker, South Australia, Australia).

Extraction of PST

Alexandrium catenella cultures were aerated with ambient air (no CO_2), but otherwise grown under the conditions described above. Using a here quantified average PST quota of 9.86 pg STX.2HCl equiv. $cell^{-1}$, the volume to be harvested was calculated to yield theoretical final PST concentrations of 1.2 and 12 mg STX.2HCl equiv. kg^{-1} for the low and high toxin diet, respectively (see below). A volume of 350 L of algal culture (~5,200 cells mL^{-1}) was harvested in batches during the stationary growth phase

via centrifugation (1,500 *x g* for 10 min, Beckman Avanti J-25). For each batch, the supernatant was discarded and the cell pellet resuspended in the remaining 10 mL of supernatant to be stored at -20°C for up to one month to allow the required algal culture volume to be processed. One day prior to pellet manufacture, the concentrated cell suspensions were defrosted and placed in a waterbath (80°C, 10 min) to ensure complete lysis of algal cells and release of intracellular PSTs. The lysed cell preparation was immediately cooled on ice before being centrifuged (3500 *x g*, 5 min, ScanSpeed 1580R) to pelletise cell fragments. The resulting cell fragment free supernatant (from here on referred to as the extract) was collected and stored at 4°C for feed pellet preparation the following day. A subsample of the extract was immediately stored at -80°C and later shipped to the Cawthron Institute, New Zealand, for PST analysis via LC-MS (see below).

Pellet preparation

Diets were formulated to contain 35% crude protein, 5% crude lipid and 17.5 MJ kg^{-1} gross energy (Bansemer et al., 2016, Table 3.1). Experimental diets were prepared by first mixing the required dry ingredients in a Hobart mixer (5 min; Hobart Corp., Troy, USA) before adding in the wet ingredients (water to ∼30 % of the total ingredient weight, fish oil, sodium alginate and calcium carbonate). The water addition was spiked with *A. catenella* PST extract to yield a control, low and high PST diet corresponding to theoretical final PST concentrations of 0, 1.2 and 12 mg STX.2HCl equiv. kg^{-1} of diet. Combined dry and wet ingredients were mixed for an additional 5 min and the pellets manufactured using a TR110 pasta machine (Macchine Per Pasta SRL, Molina Di Malo, Italy). The resulting wet pellets were dried at 45°C until an average moisture content of 9.5% was reached and a flat, sinking pellet produced (10 x 2 x 0.52 mm). A subsample of each diet was stored at -80°C at the start and end of the main exposure experiment before being shipped to the Cawthron Institute, New Zealand, for PST analysis by the LC-MS (Boundy et al. 2015, Turner et al. 2015; 2020; see Section 3.2.6).

Table 3.1 Diet formulation for the preparation of abalone feed pellets after Bansemer et al. (2016).

Ingredient	**g/100g**
Salmon fish meal (fish meal 65 % protein)	6.00
Soy protein concentrate	8.00
Soybean meal (solvent extracted)	28.44
Wheat flour	27.53
Fish oil	1.00
Vitamin/mineral premix	0.50
Sodium alginate	0.30
Lupins (de-hulled)	22.40
Ulva meal	5.00
Calcium carbonate	0.22
Monosodium phosphate	0.61
Total	100.0

3.2.4 Pilot trials

The experimental set-up was tested in two separate trials to determine the suitability of static tanks for housing abalone (i.e. no water flow, 100% water exchanges every 24 hours), as well as feed palatability by examining the feed rejection behaviour of the three different experimental diets (control, low and high PST).

Static trial

Abalone were transferred from the 450 L acclimation tanks to 35 L plastic tubs (390 x 300 x 300 mm) filled with either 10 or 20 L of seawater (one abalone per tank, three replicates for each volume). Air was supplied through a sponge biofilter that had previously been conditioned for 3 weeks with nitrifying *Nitrosomonas* spp. and de-nitrifying *Nitrobacte*r spp. As abalone were still in the weaning phase of acclimation, both seaweed (2 g wet weight of *Ulva* seaweed) and feed pellets (1.2 g of broodstock diet and 1.2 g of control diet) were provided to better observe the influence of tank volume on abalone feeding behaviour. Animals were fed at the end of each day (3:00-4:00 pm). As described for the acclimation tanks above, temperature, DO, salinity, ammonia, nitrite and pH were recorded at the start of each day (9:00 am) and left-over feed collected by siphoning onto a fine filter mesh. For the duration of the experimental period (5 days), the uneaten feed was cumulatively collected from individual tanks and stored frozen until analysis. Immediately after feed collection, each tank received a 100% water exchange.

Palatability trial

To inform the choice of feed pellet for the PST treatment in the main experiment (low or high PST content), a 7-day palatability trial was conducted. Abalone were randomly allocated to 20 L static tanks to receive 2.4 g of either the control, low or high PST feed pellet (equivalent to 1% biomass dry weight (BDW) of the heaviest abalone in the trial). A fourth treatment received the control pellet in addition to 3 mL of the GSe nutrient supplement (Blackburn et al. 1989) to test the response of abalone to the *Alexandrium* culture media (Section 3.2.2). Each treatment consisted of five animals housed in individual tanks that were maintained as described above for the static trial. For each experimental diet, a single 20 L tank (biofilter, no abalone) served as a feed study control. These tanks received 2.4 g of the respective diet (control, low or high PST) to estimate the loss of left-over feed to the system. Uneaten feed was collected daily from all tanks and dried at 105 °C for 16 h. To determine the amount of pellet consumed by each individual abalone, the resulting dry weight of the unconsumed feed from the experimental tanks was subtracted from the dry weight of the feed collected from the respective feed study tanks (control, low and high PST).

3.2.5 Exposure of abalone to PST

Abalone were exposed to two different PST sources: PST containing feed pellets or live *A. catenella* microalgal culture. Abalone were chosen at random from the two acclimation tanks and transferred to individual 25 L tanks (390 x 300 x 300 mm) containing 20 L of seawater. These static tanks (no seawater flow, 100% water exchange every 24 h) were arranged in 8 rows of 5 tanks. Seawater for exchanges was held in a continuously circulated, chilled reservoir (16 ± 0.5°C, 4000 L). To buffer any potential pH fluctuations during the 24 h static period, the seawater was supplemented with sodium bicarbonate as required. As described for the pilot trials above, air was supplied through a preconditioned sponge biofilter. The tanks were randomly allocated to four different treatments: control diet (no PST; 9 replicates), toxic diet (high PST pellet; 9 replicates), microalgal exposure (control diet + *A. catenella* culture; 12 replicates), as well as a GSe nutrient control (control diet + algal culturing nutrients; 5 replicates). The respective diets (control or high PST) were fed at 1% BDW (2.6 ± 0.05 g per animal) of the heaviest abalone in the trial (262 g in shell weight). To take the loss of uneaten feed to the system into account, three 25 L tanks were set up identical to the experimental

tanks, although without abalone, to serve as feed study controls for each of the three diets (control diet, toxic diet and microalgal exposure).

Abalone husbandry

Abalone were maintained in seawater at a salinity of 35 - 36 and temperature of 16.0 ± 2.0°C throughout the experimental period (4 weeks). Tanks were covered with perforated, opaque lids (~40% perforation) and light supplied at an average intensity of 13 Lux by shadecloth covered fluorescent lights on a 12:12 h light:dark cycle. All routine husbandry operations were carried out under red light. Daily operations commenced by measuring DO and temperature directly in the tank (Oxyguard, Polaris 2) and collecting a 50 mL water sample from each tank. The pH of these samples was immediately recorded (Meterlab, PHM210) and nutrient concentrations determined through colorimetric tests (ammonia, nitrite and nitrate) according to the following regime: ammonia (API Fishcare, LR8600) and nitrite (Hach, NitriVer® 3) concentrations were assayed every 3 days for each tank and nitrate (Hach, NitraVer® 5) every two weeks. Salinity was recorded daily (Amzdeal, ATC) from one of the five tanks in each row on a rotating basis (i.e. each tank was tested every 5 days).

Feed consumption

After routine environmental parameter measurements were completed, any uneaten feed was removed from the tanks by spot siphoning onto a fine filter mesh. The daily collected feed from each tank, including the feed study tanks, was cumulatively stored in weekly intervals to allow for later assessment of feed consumption by each animal throughout the experimental period. Immediately upon completion of feed collection, all seawater and faeces were removed by inversion of the tanks that were immediately refilled with fresh seawater from the reservoir tank. Collected feed was dried at 105 °C for 16 h. The resulting dry weight of the unconsumed feed from the experimental tanks was subtracted from the dry weight of the feed collected from the respective feed study tanks (control, low and high PST) to yield the weekly pellet consumption of each individual abalone in the experiment. Feed intake was corrected for abalone body weight (in-shell) and expressed as g of feed consumed per kg of abalone per day.

Microalgal exposure

Upon completion of the daily husbandry and feed collection, *A. catenella* culture was added to each tank in the microalgal exposure treatment group. Algal cultures were selected based on cell density (>2 x 10^7 cells L^{-1}) determined by preserving a subsample in Lugol's solution (1%) and subsequent microscopic enumeration (Olympus CX31, Sedgewick-Rafter chamber). The amount of culture to be added to each tank was calculated to yield a final cell concentration of approximately 2 x 10^5 cells L^{-1} in the experimental tanks. This concentration was chosen to represent peak bloom concentrations at depth (*A. catenella* is known to reach up to 3 x 10^5cells L^{-1} in Tasmanian waters; integrated surface samples, Condie et al. 2019). The volume of algal culture to be added to each tank was displaced prior to addition and the corresponding amount of GSe media nutrients added to each tank in the nutrient control group.

Abalone dissection

Abalone were harvested and dissected into viscera, epipodium and foot tissues for PST analysis. To estimate baseline PST levels in abalone (sourced from the wild), nine individual animals were randomly selected from the two acclimation tanks when the main PST exposure experiment commenced. Shell length (measured across widest point), in-shell and shucked weight were recorded before separating the viscera from the foot. The viscera were weighed, and the head and mantle discarded. The epipodium (here defined as the outer black layer of the frill and ~1 mm of the ventral covering of the foot) was carefully sliced/cut from the white foot tissue. The foot and epipodium

were weighed and the foot thoroughly washed to remove all traces of mucous. All tissues (viscera, epipodium and foot) were homogenised (Velp Scientifica, OV5 homogenizer) to yield a well-mixed, smooth paste suitable for PST analysis.

3.2.6 Toxin analysis

All abalone PST samples were analysed at Cawthron Institute, New Zealand, by LC-MS/MS (Waters Acquity UPLC i-Class system coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer with electrospray ionization) following the method described by Boundy et al. (2015) and Turner et al. (2015), with results reported in mg STX.2HCl equiv. kg^{-1} calculated using FAO toxicity equivalency factors (FAO/WHO, 2016). Sample preparation used a different solvent-to-sample ratio than detailed in the methods described above and involved weighing 2.0 ± 0.1 g of homogenised abalone tissue (epipodium, abalone viscera or foot) into a 50 mL centrifuge tube followed by the addition of 18 mL of 1% acetic acid. The mixture was vortex mixed before being placed into a boiling water bath for 5 min. Samples were then cooled for 5 min in an ice bath before further vortex mixing. Insoluble debris was pelleted by centrifugation at 3,200 x g for 10 min before a 1 mL aliquot was transferred into a 1.5 mL polypropylene tube, followed by the addition of 5 µL of ammonium hydroxide (NH_4OH; 25% ammonia) before clean-up. The solid-phase extraction procedure was performed manually with amorphous graphitized polymer carbon Supelco ENVI-Carb 250 mg/3 mL cartridges. The cartridges were conditioned with 3 mL of acetonitrile–water–acetic acid (20:80:1, v/v/v), followed by 3 mL of water–25% NH_4OH (1,000:1, v/v). Sample extracts (400 µL) were loaded onto the conditioned cartridges and then washed with 700 µL of MilliQ water. PST was then eluted with 2 mL of acetonitrile–water–acetic acid (20:80:1, v/v/v) into a labelled 15 mL polypropylene tube. The eluent was mixed and diluted by transferring 100 µL to a polypropylene autosampler vial and adding 300 µL of acetonitrile. Sample analysis used hydrophilic interaction LC-MS/MS, as described by Boundy et al. (2015) and Turner et al. (2015). Method performance was assessed by fortifying PST-free blank matrix generated from control animals and determining recovery. Results reported as part of this study were corrected based on average spike recoveries observed for the different sample matrices analysed. The limit of reporting for each PST analogue differed for each matrix tested.

3.2.7 Statistical analysis

All data were tested for normality and homogeneity of variance with Kolmogorov-Smirnov and Levene's test, respectively. Differences between experimental groups were tested using Student's T-test or where appropriate analysis of variance (ANOVA). PST concentration data was log transformed to test for the effect of the treatments between different abalone tissues (2-way factorial ANOVA with interaction effect). Significant differences between treatment groups detected by ANOVA were followed up with Tukey's HSD test. Unless otherwise stated, all values represent the mean ± 1 standard deviation. All analyses were performed, and graphs constructed, with the statistical package R (www.r-project.org).

3.3 Results

3.3.1 Acclimation and weaning of abalone

Abalone were transported from Pelican Point, Mt. Gambier to the South Australian Aquatic Biosecurity Centre with minimal mortalities occurring throughout the acclimation phase (3% mortality). Within five days of arrival, abalone were fed 1.7% BDW of seaweed. At this point, the broodstock pellet was successfully introduced to supplement seaweed additions and abalone were observed to be actively feeding on the pellets 15 days after entering the Centre. Seaweed additions were gradually decreased over time and completely ceased on day 24. The colour of abalone faeces was subsequently observed to change from black to brown (colour of pellet). The animals readily

took to the control pellet diet that was used to gradually (25% increments) replace the broodstock pellet in preparation for the pilot trials.

3.3.2 Pilot trials

Pellet preparation

A theoretical calculation based on an average PST quota of 9.86 pg cell^{-1} determined that 350 L of *A. catenella* culture at a cell density of 5,200 cells mL^{-1} would yield final PST concentrations of 1.2 and 12.0 mg STX.2HCl equiv. kg^{-1} of feed. PST concentrations in the final pellet product were confirmed to be very close to the desired concentrations (1.5 and 10.7 mg STX.2HCl equiv. kg^{-1} for the low and high PST pellet formulations, respectively).

Static trial

Over the 5-day trial period, no significant differences in the environmental parameters were observed between the 10 and 20 L static tanks. Water parameters predominantly stayed within the desired ranges (ammonia <1 ppm, DO > 85%, nitrite <0.4 ppm, salinity 35, temperature 12.0-16.3°C and pH 8-8.2). A single 10 L tank deviated considerably from these on day 4 of the experiment (ammonia = 1 ppm, nitrite = 0.69, DO = 72%) and the animal was observed to actively crawl out of the water (partially submerged) to only return below the waterline 5 min after water exchange and biofilter air stone replacement. However, pellet consumption in this tank (0.34 g) proved similar to the average consumption in the other two replicates of the same treatment (0.68 ± 0.24 g as fed kg abalone^{-1} day^{-1}). No significant difference in the amount of feed consumed was observed between the 10 and 20 L tanks ($t_{(4)}$ =-2.56, p = 0.087). To reduce the likelihood of water parameter fluctuations, future experiments were conducted with a tank volume of 20 L.

Palatability trial

Water parameters throughout the palatability trial (7 days) stayed within their respective acceptable ranges: salinity = 35-37, temperature = 16.0 ± 0.6°C, ammonia = <0.25 ppm, nitrite = 0.13 ± 0.02 ppm and pH = 8.14 ± 0.06. Dissolved oxygen concentrations did not drop below 85% saturation except for one instance (day 2), where biofilter air supply remained disconnected overnight in one replicate exposed to the high PST pellet (53 % DO). This tank was still included in further statistical analysis, as the feed consumed by this individual abalone (0.6 g as fed kg abalone^{-1} day^{-1}) was close to the average of the remaining four replicates within the same treatment (1.0 ± 0.39 g as fed kg abalone^{-1} day^{-1}).

Abalone actively fed throughout the entire experimental period. Abalone consumed significantly different amounts of feed pellets between the four diet/nutrient exposure treatments (ANOVA: $F_{(3,16)}$ = 9.20, p = <0.001). While no significant differences in feed consumption were found among the control pellet (no PST), GSe nutrient exposure and high PST diet (Tukey's HSD: p > 0.55), animals fed the low PST pellet consumed up to twice the amount of feed over the experimental period (2.0 ± 0.6 g day^{-1} kg abalone^{-1}) than in any other treatment (Tukey's HSD: p <0.01, Figure 3.1).

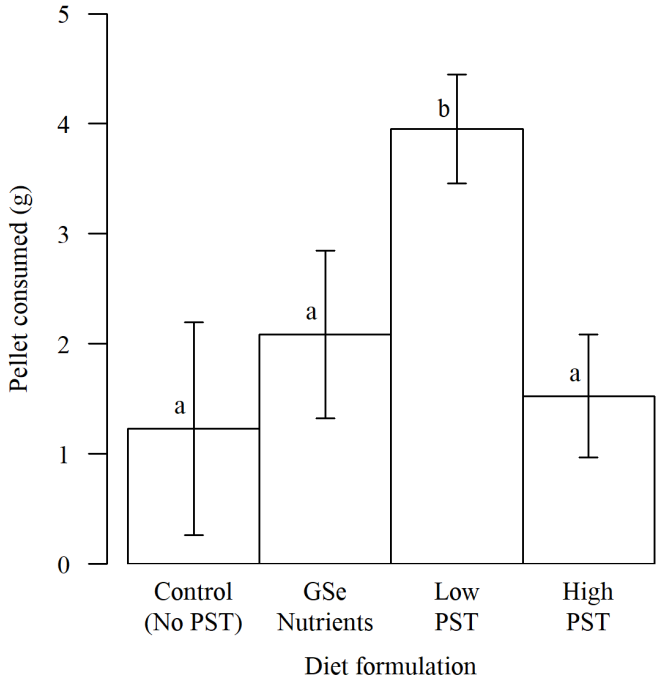

Figure 3.1 Feeding of abalone on experimental feed pellets of either high or low PST concentration or when exposed to algal culturing media nutrients (control pellet + GSe nutrients). Error bars represent 1 standard deviation around the mean and letters denote significant differences between treatments (Tukey's HSD).

3.3.3 Main experiment

During the three-day acclimation phase leading up to the experimental exposure treatments, all abalone were actively feeding on the non-toxic control pellets (average feed intake of 1.72 ± 0.8 g of feed kg abalone^{-1} day^{-1} across all treatments). No significant differences in feed intake were observed between the individuals that constituted the subsequent treatment groups (ANOVA: $F_{(3,28)} = 0.275$, $p = 0.84$). Comparison of feed consumption by abalone exposed to the GSe nutrient treatment (1.72 ± 0.65 g of feed kg abalone^{-1} day^{-1}) with that of the five *a priori* selected control animals (2.26 ± 1.24 g of feed kg abalone^{-1} day^{-1}) revealed no significant impact of algal culturing nutrients on feed intake over the four week trial (Student's T-test: $T_{(38)} = 1.73$, $p = 0.091$, data not shown). An ANOVA investigating the effect of treatment (control, toxic, *A. catenella* exposure) and week (1-4) on feed consumption by abalone, revealed no significant interaction between treatment and week (ANOVA: $F_{(6,96)} = 2.1$, $p = 0.066$) and no significant effect of the treatments (ANOVA: $F_{(2,96)} = 1.8$, $p = 0.174$). However, feed consumption did not remain constant over the experimental period, with abalone in all treatments consuming marginally more feed during the first two weeks of the experiment (2.17 ± 0.84) when compared to the final two weeks (1.36 ± 0.76 g kg abalone^{-1} day^{-1}; Tukey's HSD: $p = < 0.03$).

PST exposure

Total PST concentrations differed significantly between the abalone exposure treatments and tissue types at the end of the 28 day exposure period (ANOVA: $F_{(9,128)} = 12.04$, $p = < 0.0001$). Across all treatments, the highest PST concentrations were detected in the epipodium, followed by the viscera and foot tissues (Figure 3.2). When taking into account the contribution of individual tissues to the whole animal, only the total PST concentration in the *Alexandrium* exposure treatment proved significantly different from the control group (Tukey's HSD: $p = < 0.0001$). After the 28-day exposure,

the animals exposed to the toxic microalga accumulated up to 0.128 mg STX.2HCl equiv. kg^{-1} (mean = 0.101 ± 0.017 mg STX.2HCl equiv. kg^{-1}). In this treatment group, 72 ± 8% of the total PST was situated in the epipodium.

Significantly higher PST concentrations were detected in the epipodium of animals directly exposed to the *Alexandrium* cells (0.718 ± 0.167 mg STX.2HCl equiv. kg^{-1}) than in any of the other three treatments (Tukey's HSD: p = < 0.00001). The total PST concentration in the epipodium did not differ between the toxic pellet and control treatment or tissues of control animals on day 0 (Tukey's HSD: p = >0.9994). Indeed, control abalone (collected from the wild and held for 2 months of acclimation prior to dissection on day 0) presented with up to 0.296 mg STX.2HCl equiv. kg^{-1} in the epipodium (mean = 0.204 ± 0.072), but was much lower levels in the foot (0.008 ± 0.003) and viscera (0.005 ± 0.002), totalling 0.026 ± 0.008 mg STX.2HCl equiv. kg^{-1} in the whole animals (all tissues). The PST profile in the epipodium across all four treatments was dominated by saxitoxin (STX, 69-90 % between treatments) and relatively lower amounts of neosaxitoxin (NEO, 7-18%), decarbamoylsaxitoxin (dcSTX, 3-11%) and gonyauxtoxins (GTX, 0.3-11%).

PST concentrations in the foot tissues of the control (day 0 and day 28) and toxic pellet treatments did not exceed a maximum of 0.0128 mg STX.2HCl equiv. kg^{-1}. Although PST concentrations in the foot tissue of animals exposed to the *Alexandrium* statistically differed from the other groups (Tukey's HSD: p = < 0.001), they only proved marginally higher (0.016 ± 0.004 mg STX.2HCl equiv. kg^{-1}). The PST profile was again dominated by STX (72-90%), with dcSTX (day 0 and 28 control only) and GTX2&3 (toxic pellet and *Alexandrium* treatments) detected as the only other notable PST analogues.

In the viscera, minor levels of PST were detected in the day 0 and day 28 control groups (<0.008 mg STX.2HCl equiv. kg^{-1}), with those in the toxic pellet treatment proving only marginally higher (0.015 ± 0.008 mg STX.2HCl equiv. kg^{-1}). Once again, the highest viscera PST concentrations were observed in animals exposed to *Alexandrium* (0.093 ± 0.034 mg STX.2HCl equiv. kg^{-1}: Tukey's HSD: p = < 0.001). Only STX was detected in day 0 and day 28 control animals, whereas the PST profile in the toxic pellet and *Alexandrium* treatments proved more complex. Here, STX only made up 17-18 % of the profile, with substantial proportions of GTX1&4 (29 and 50%) and GTX2&3 (24 and 47%) detected. The remainder of these profiles is made up of minor contributions from c-toxins (C1&2) and dcSTX, with trace levels of deoxydecarbamoyl-saxitoxin (doSTX) and GTX5 detected in the toxic pellet and *Alexandrium* exposure treatments, respectively.

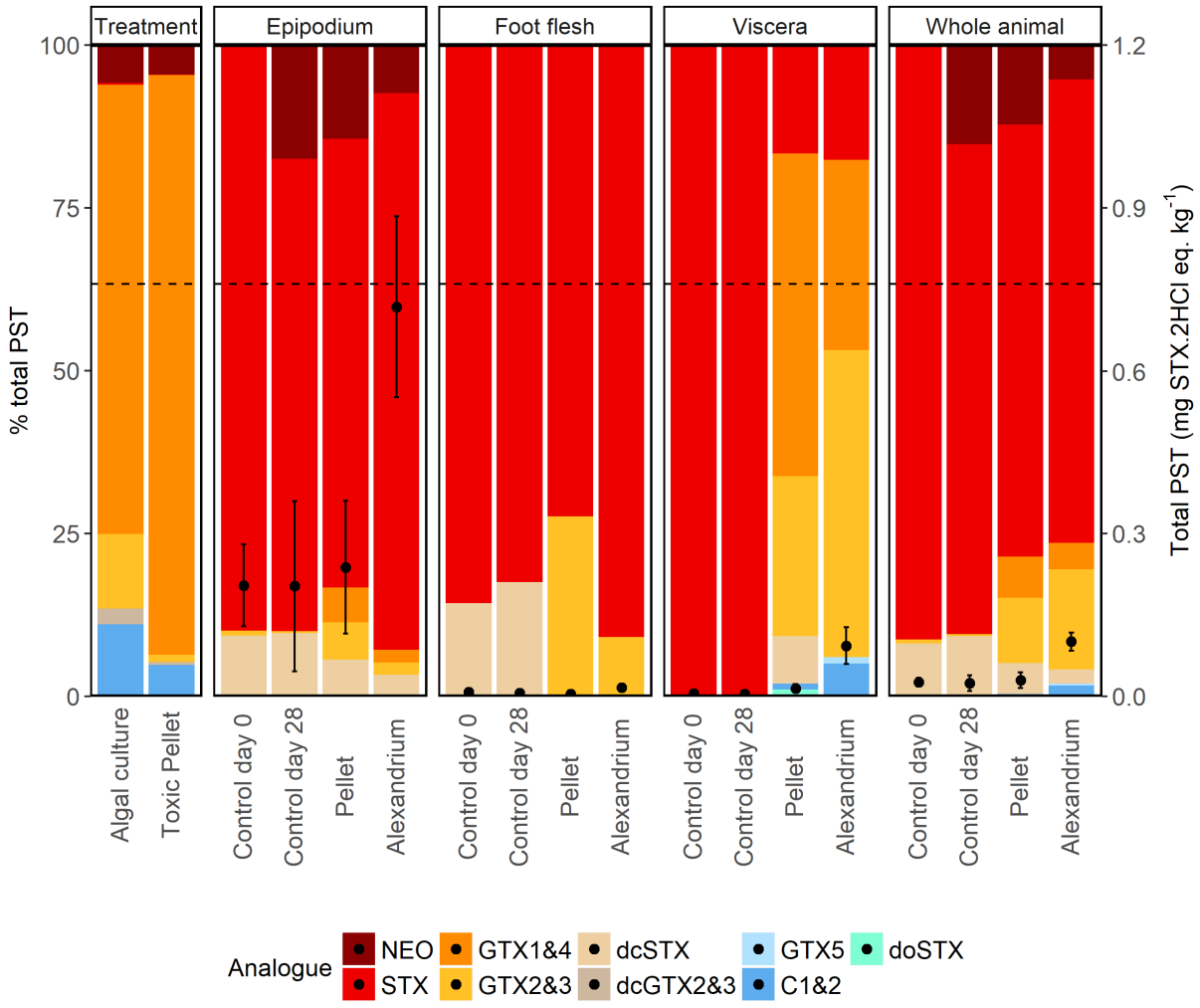

Figure 3.2 PST profiles (percentage toxicity) in *Alexandrium* algal culture and the toxic feed pellets prepared from algal extracts. PST profiles for epipodium, foot and viscera tissues as well as the whole abalone are given for each treatment group. The control treatment group is split into animals harvested on day 0 and day 28 (start and end of experiment). Total PST concentration is represented by the black points and expressed as mg STX.2HCl equiv. kg^{-1} on the secondary y-axis on the right, with error bars representing 1 standard deviation around the mean (n= 9). The CODEX abalone maximum level (0.8 mg STX.2HCl equiv. kg^{-1}) is represented by the dashed horizontal line.

3.4 Discussion

We successfully demonstrated uptake of microalgal PST in Blacklip Abalone via feed consumption, as well as via direct exposure to live PST producing phytoplankton. Historically, the source and uptake mechanisms of PST have remained elusive in abalone and the subject of much speculation (Bravo et al., 2001; McLeod et al., 2017). Our controlled tank experiments add to the growing body of evidence that abalone are able to take up PST from direct exposure to phytoplankton producers, albeit in our case, limited amounts (up to 128 µg STX.2HCl equiv. kg^{-1} over a 28-day period at simulated peak bloom concentrations of 2 x 10^5 cells L^{-1}).

No adverse effects of PST exposure to either the diet (toxic pellet) or *A. catenella* cells was observed on abalone behaviour (i.e. no reduced feed intake or other avoidance behaviour). Interestingly, the pilot palatability trial showed preferred feed intake of the low PST pellet over the control diet and no

difference in feed intake between non-toxic control and high PST pellet (10.7 mg STX.2HCl equiv. kg^{-1}). However, it should be noted that PST levels exceeding 1 mg STX.2HCl equiv. kg^{-1} have been known to paralyse (i.e. prevent attachment or righting) both aquacultured and wild South African abalone *Haliotis midae* (Pitcher et al., 2001).

The relative contribution of viscera and foot tissues to total PST appears to be microalgal species specific. While Blacklip Abalone sampled during *G. catenatum* blooms in the Huon Estuary (Tasmania, Australia) presented with up to four times higher PST levels in the viscera than the foot (McLeod et al., 2017), we here detected higher PST concentrations in the combined foot tissues (epipodium + foot muscle) after exposure to *A. catenella* (on average four times higher than in the viscera). This coincides with the PST analysis of Blacklip Abalone collected from the east coast of Tasmania during *A. catenella* blooms, where higher toxicity was also reported in the foot tissues relative to the viscera.

In our tank experiments, the higher toxicity in the combined foot tissues (epipodium + foot muscle) is driven by the distinctly higher concentration of PST accumulated in the epipodium (up to a maximum of 1.084 mg STX.2HCl equiv. kg^{-1} over 28 days of *A. catenella* exposure). This accumulation of PST in the epipodial tissues of the foot has also been reported in Spanish Orma (*H. tuberculata*) and South African abalone *(H. midae)* collected from the field (Bravo et al., 1999; Pitcher et al., 2001), raising the possibility of direct uptake of PST through the outer layers of the foot epidermis (McLeod et al., 2017). PST have been demonstrated to be concentrated in glandular epidermal cells of Spanish Orma and their mucilage covering, leading Bravo et al. (2001) to suggest that the epipodium may serve as a storage organ for PST and/or a PST secretory function.

Although the epipodium constitutes only a small percentage of the overall weight of the abalone, its high PST level contributes substantially to the overall toxin burden. Removal of the majority of the epipodium through scrubbing before market could therefore significantly reduce toxicity of contaminated animals and may be employed as a potential mitigation strategy. However, despite considerable PST reductions (up to 70% removal: this report; Dowsett et al., 2011; Pitcher et al., 2001), scrubbing of the foot is associated with a significant reduction in product value from \$80 kg^{-1} for live, wild abalone compared to \$40 kg^{-1} for scrubbed and canned product (beach prices, Tasmanian Abalone Council, 2018). Current risk management therefore aims to allow live harvest, implementing a closed/open status of individual harvest blocks (TACL, 2017). Rather than canning product that could otherwise be sold at premium prices overseas, industry preference at this stage is to invest in the validation of a rapid PST screening test for abalone tissues, such as the Neogen lateral immuno-flow assay (Neogen Corporation, Lansing, USA). An effective pre-screening test could significantly reduce the amount and thereby cost of analytical testing required to inform management of individual abalone blocks.

Furthermore, our laboratory results and limited field data (SARDI, unpublished data) suggest that the cost of analytical testing may be halved by preferentially testing the foot and not the viscera of abalone suspected to be contaminated with PST from *A. catenella*. Currently, PST are monitored through separate analysis of the viscera and foot tissue of individual abalone, costing around A\$5,000 per sampling interval per harvest block. Additional field data will be needed to determine if the observed higher PST concentration in the foot tissue after *A. catenella* exposure also hold true across multiple bloom seasons in the field.

The oral uptake efficiency of PST delivered via the feed pellet appears to be very low. Previous work feeding PST contaminated pellets (0.15 mg STX.2HCl equiv. kg^{-1}) to Greenlip Abalone (*Haliotis laevigata*) showed limited uptake over a 50-day exposure period (0.0198 mg STX.2HCl equiv. kg^{-1} in the foot and concentrations below the limit of quantification in the viscera; Dowsett et al. 2011). Even though we have fed a more concentrated PST pellet by almost two orders of magnitude (10.7 mg STX.2HCl equiv. kg^{-1}, comparable daily feed intake), significant concentrations of PST (in statistical

terms) were only found in the viscera (not the foot or epipodium) after 28 days of exposure to the highly toxic pellet (0.015 ± 0.008 in viscera compared to control 0.004 ± 0.002 mg STX.2HCl equiv. kg^{-1}). This low uptake of PST indicates a low oral uptake efficiency of PST by Blacklip Abalone (at least for this particular food source).

However, PST of microalgal origin appear to be more readily taken up. Following exposure to *Alexandrium*, PST concentrations in the viscera were observed to be on average 6 times higher than in the pellet treatment (up to a maximum of 0.149 mg STX.2HCl equiv. kg^{-1}), with substantially higher PST levels found in both foot and epipodial tissues (up to 0.718 mg STX.2HCl equiv. kg^{-1}). In both treatments (algal and pellet exposure), the PST profile of the viscera most closely resembled the respective PST source (>80% GTX analogues, no STX), whereas foot and epipodium tissues contained almost exclusively STX and NEO, despite the algal cultures and toxic pellets containing very low proportions of these toxins (< 5%). The question of biotransformation of PST was raised during investigations into the putative source of PST in Spanish and South African abalone (Bravo et al., 1999; Pitcher et al., 2001). While in these scenarios, additional evidence further questioned involvement of PST producing microalgae (e.g. absence of PST in bivalves in the region), the laboratory results obtained in the present work closely align with PST profiles found in Blacklip Abalone from the Tasmanian east coast collected during *A. catenella* blooms (Section 4 below), which also presented with higher percentages of GTX in the viscera, whereas the foot was dominated by STX & NEO. From our studies we cannot state whether this variation in PST profiles in abalone tissues is due to biotransformation or variable uptake and depuration rates.

The maximum PST concentration of whole animals after the experimental *A. catenella* exposure (0.128 mg STX.2HCl equiv. kg^{-1}) were lower than PST levels occasionally observed in the field (>0.8 mg STX.2HCl equiv. kg^{-1}). These differences may be explained by the position of microalgal cells in the water column and differing lengths of exposure. *A. catenella* blooms in Tasmanian waters generally persist longer than our 28-day exposure period (3-6 months, Hallegraeff et al., 2017), with periods of increased exposure to sedimented algae during bloom termination. Suspension of live *A. catenella* cells in the water column (as in our experimental exposures) certainly would have reduced the amount of microalgal cells available for grazing and/or epipodial uptake. Furthermore, prolonged retention of PST in wild South African abalone (>7 months; Etheridge et al., 2002; Pitcher et al., 2001) suggest slow depuration rates, and the potential for cumulative PST uptake over subsequent bloom seasons. This, however, does not appear to be the case for wild Tasmanian Blacklip Abalone populations exposed to bloom episodes of *G. catenatum*, which are known to depurate PST and only retain low levels between bloom episodes (McLeod et al., 2017). Surprisingly, we here detected such low background PST levels in wild South Australian Blacklip Abalone sourced from an area where no PST monitoring currently occurs. While PST levels were low (<0.038 mg STX.2HCl equiv. kg^{-1}), this detection serves as a reminder for industry to consider how to manage the risk of biotoxin accumulation in areas not traditionally associated with blooms, and to develop biotoxin response/management plans for implementation during novel bloom events.

Chapter 4 - Determination of relative risk of PST accumulation in abalone compared to Southern Rock Lobster in two field sites

4.1 Introduction

Export of Australian wild abalone is valued at approximately A$187 M per annum (Mobsby, 2018). The majority of this product is exported to China. In order to realise the value of this product, abalone must comply with Chinese food standards, one of which covers marine biotoxins in aquatic products (GB 5009.213-2016). Whilst abalone are understood to be a lower risk for marine biotoxins than bivalve shellfish (Deeds et al., 2008; McLeod et al., 2014; Shumway, 1995), they can accumulate biotoxins and have been known to exceed regulatory levels in the past (Bravo et al., 2001; Bravo et al., 1999; Harwood et al., 2014; Martínez et al., 1996; McLeod et al., 2017; Pitcher et al., 2001). The Codex Standard (312-2013) for live abalone and for raw fresh chilled or frozen abalone for consumption or for further processing (Codex Alimentarius Commission, 2013) states:

"Abalone from some geographical areas have been found to accumulate certain marine biotoxins. It is up to the Competent Authority (using a Risk Assessment) to determine whether a risk exists in any geographical areas under its control and if so, put in the necessary mechanisms to ensure that the part of the abalone to be consumed, meets with the marine biotoxins level in the Standard for Live and Raw Bivalve Molluscs (CODEX STAN 292-2008)".

The Australian abalone industry pro-actively addressed the biotoxin amendment to Codex Standard 312-2013 by finalising risk assessments for biotoxins in 2014 (Turnbull et al., 2014). The risk assessments found that PST was a risk for abalone from the south-east of Tasmania in association with *G. catenatum* blooms. Subsequently a marine biotoxin management plan was put in place in this zone in 2014. This was extended to cover the east coast of Tasmania in 2018, following the detection of PST in abalone from this region.

The current abalone biotoxin management plan is based around the abalone blocks used for fisheries management. Monitoring of abalone in each block is triggered when the commercial bivalve monitoring program detects toxic microalgal cells in the water and/or biotoxins in bivalve shellfish. Closures of abalone blocks may then follow if no sampling of abalone occurs or if toxins are detected in abalone at levels exceeding the bivalve regulatory level. Sampling is required to indicate it is safe to resume harvest. Given the large number of abalone blocks (more than 30 sub-blocks on the east coast of Tasmania), sampling to re-open is costly and some abalone blocks have remained closed for prolonged periods following blooms (up to two years).

Concurrent with this abalone project, a large research project has been underway to better understand PST accumulation in Southern Rock Lobster *Jasus edwardsii* (FRDC Project 2017-086). As part of that project, rock lobster and potential prey species were collected from four sites along the east coast of Tasmania during *A. catenella* blooms; Binalong Bay, Georges Bay, Okehampton Bay and Pirates Bay. At the same time, abalone samples have been collected for the field component of the present project.

The aim of the field component of this project was to improve the understanding of PST accumulation in Blacklip Abalone from *A. catenella* blooms on the east coast of Tasmania. Knowledge of uptake and maximum levels, as well as the relative risk compared to lobster, will assist in developing a cost effective risk management strategy for abalone during these blooms. The

information obtained will be used to update the Tasmanian Abalone Marine Biotoxin Management Plan.

4.2 Method

4.2.1 Site selection

A steering committee was set up to oversee both the Southern Rock Lobster and Blacklip Abalone biotoxin projects. The steering committee consisted of representatives from the Tasmanian Abalone Council, the Tasmanian Department of Primary Industries Parks Wildlife and Environment (DPIPWE) Fisheries Branch, DPIPWE Biosecurity Food Safety, the Department of Agriculture, Water and Environment Export Branch, FRDC, IMAS and SARDI. The steering committee considered sites for the fieldwork, based on biotoxin history, production levels and ease of boat access for the dive team. In the first year the focus was on Binalong Bay/Georges Bay (Block 30A), Okehampton Bay (Block 24C) and Pirates Bay (Block 22C), as shown in Figure 4.1. In the second year, more frequent sampling was conducted in Okehampton Bay only.

4.2.2 Abalone sampling and processing

Sampling was triggered on information from the Tasmanian commercial bivalve biotoxin monitoring program (including phytoplankton and shellfish testing) and the Tasmanian Southern Rock Lobster (SRL) biotoxin monitoring program sentinel mussel samples. All abalone blocks were closed during all sampling events for fisheries management reasons.

On each sampling occasion, a dive crew from IMAS Taroona aimed to collect five Blacklip Abalone at each site, using SCUBA tanks (0-12m depth), along with the other marine species collected for the lobster study. Abalone were kept on ice overnight or frozen (-30°C) and processed on return to Taroona, generally the following day or within four weeks. Shell length (maximum width of each shell) and wet weight of each animal (without shell) was measured. The animals were shucked and foot and viscera separated. Each tissue was homogenised thoroughly using a domestic blender (viscera) and a Vitamix blender (Vita Prep 3; foot) and stored frozen at -30°C. At the end of each season, tissues were sent to Cawthron Laboratories in New Zealand for PST analysis (frozen and kept in dry ice during shipping).

4.2.3 Blacklip Abalone PST analysis

Tissues for each animal were either analysed separately (during strong blooms), or the five abalone from the one site were pooled into one foot and one viscera sample (all other times). If the pooled sample was ≥ 0.16 mg STX.2HCl equiv. kg^{-1}, all individual animals were analysed, as this level could contain at least one animal at or above the bivalve regulatory level of 0.8 mg STX.2HCl equiv. kg^{-1}.

PST analysis occurred by LCMS-MS using the method of Boundy et al. (2015) and Turner et al. (2015; 2020), as detailed in Section 3.2.6 above.

4.2.4 Biotoxin information collected from other programs

Much of the real-time information on algal blooms on the east coast of Tasmania is collected from the commercial bivalve marine biotoxin monitoring program, managed by the Shellfish Market Access Program (ShellMAP), in DPIPWE. The closest commercial bivalve sites to Binalong Bay are the 6 harvest zones in the Moulting Bay growing area, St Helens, approximately 7 km away (Figure 4.1). The closest commercial bivalve site to Okehampton Bay is Spring Bay, a long-line mussel growing area approximately 7 km away (Figure 4.1). Spring Bay is an area that has experienced recurrent toxic

blooms of *A. catenella* at high cells concentrations, and for extended periods (Condie et al. 2019). There are no commercial bivalve farms in the vicinity of Pirates Bay.

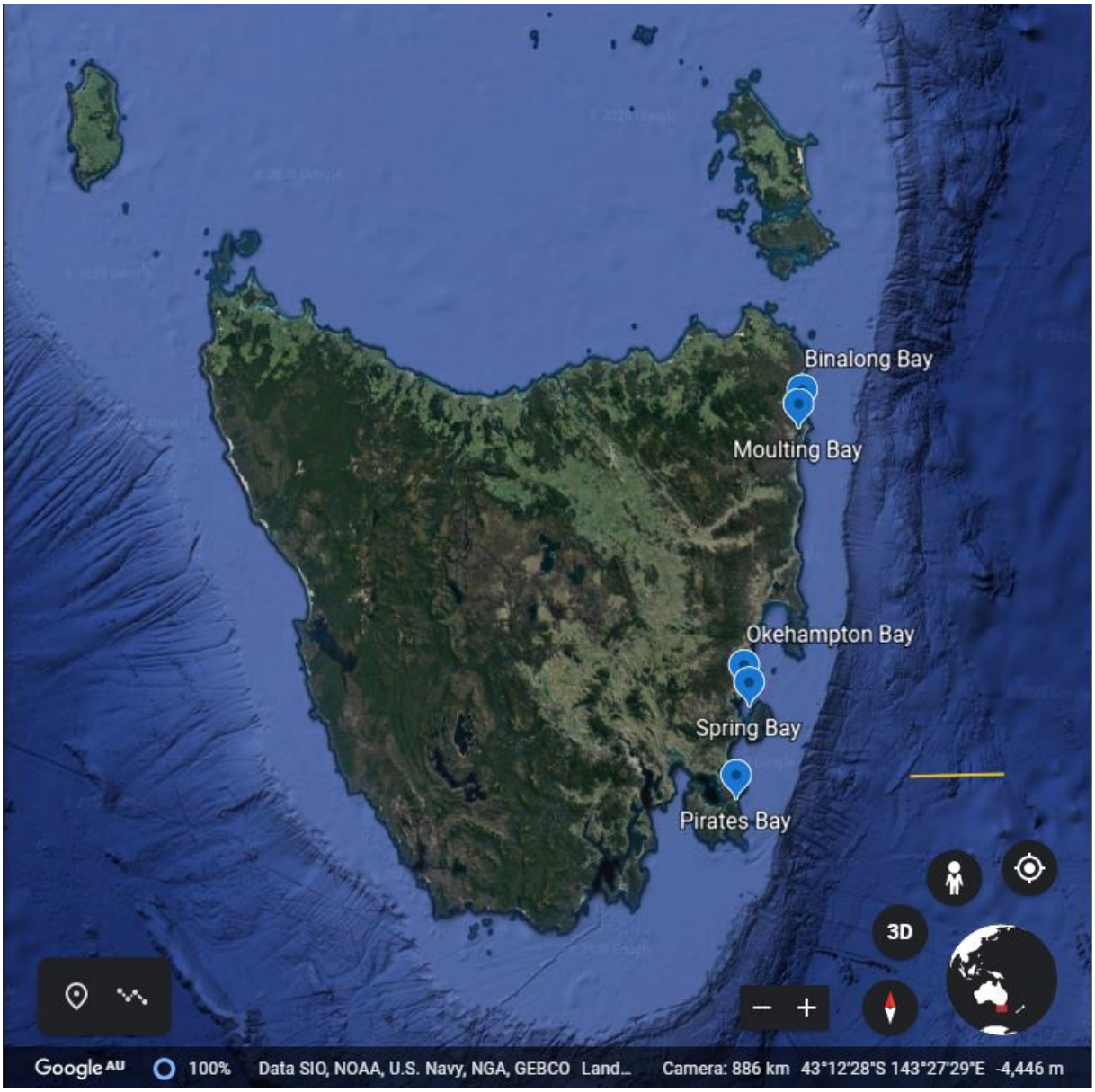

Figure 4.1 Map of Tasmania showing abalone sample sites and associated bivalve monitoring sites. Yellow Line is 50 km.

The ShellMAP collects monthly algal samples in commercial bivalve growing areas. Water samples are collected via an integrated sampler or bottle sample (inter-tidal zones only); fixed with Lugols Iodine; concentrated gravimetrically in a measuring cylinder; and analysed by microscopy at Analytical Services Tasmania (AST).

Both Moulting Bay and Spring Bay are regarded as high biotoxin risk, thus ShellMAP collects bivalve tissue samples on a weekly basis. One dozen oysters or mussels were homogenised in a pooled sample from each area. The pooled samples were analysed for PST via the Lawrence method (AOAC, 2011), either at Symbio in Sydney (pre-Jan 2018), or at AST (post Jan 2018). Data from relevant

bivalve monitoring sites (Moulting Bay and Spring Bay) were provided from ShellMAP for the period October 2017 to November 2019.

The SRL biotoxin monitoring program is managed by DPIPWE Wild Fisheries. The program draws on information from ShellMAP: sampling of lobster and sentinel mussels from selected sites on the east coast of Tasmania begins when the ShellMAP results indicate bloom activity on the east coast. During bloom activity, sentinel mussels are collected on a fortnightly basis (or weekly when PST levels start increasing), and when they exceed the bivalve regulatory level, sampling of lobsters is undertaken, continuing until bloom activity has passed. Mussel samples are collected by contractors from lines set up at the sentinel sites in advance on Flinders Island, Binalong Bay, and Bicheno, and by IMAS staff from jetties in Pirates Bay, White Beach (Nubeena) and Adventure Bay (Bruny Island). Each sample consists of ~12 pooled mussels. When required, lobsters are collected and processed by IMAS staff at their Taroona facilities. The hepatopancreas of each lobster is homogenised and analysed individually. During this study DPIPWE lobster and mussel samples were analysed at Symbio and AST as for the ShellMAP samples. Data from this program were provided from October 2017 to November 2019.

Southern Rock Lobster hepatopancreas samples from the east coast of Tasmania were collected concurrently with the abalone samples in this project, through a related FRDC project to improve biotoxin risk management in SRL (FRDC 2017-086). Lobsters were collected by IMAS on the same dives as the abalone collection (5 animals at each site). Animals were kept on ice overnight and processed on return to Taroona. During 2018 hepatopancreas samples were stored frozen at -30°C prior to sending to Cawthron Laboratories in New Zealand for PST analysis via the Boundy method (Boundy et al., 2015; Turner et al., 2015; 2020). In 2019 however, hepatopancreas samples were sent to AST for immediate analysis via the Lawrence method (AOAC, 2011).

4.2.5 Data analysis

All statistical analyses were performed using R Software (R Core Development Team version 3.6, April 2019). Data were checked for homoscedasticity using Levene's test and normality using the Shapiro-Wilk test, and appropriate transformations made if required prior to testing for significant differences using analysis of variance. Differences were considered statistically significant when p <0.05. Correlation between abalone tissue and viscera PST concentrations was tested using linear regression.

The mean and standard deviation of the percentage contribution of each analogue to total toxicity of the foot and viscera tissues was calculated for the subset of tissues where the individual PST concentration was >0.2 mg STX.2HCl equiv. kg^{-1}, by dividing the toxicity equivalent of each analogue by the total toxicity and multiplying by 100.

4.3 Results

4.3.1 Bloom development 2017-2019 based on bivalve PST monitoring data

Data from the commercial bivalve monitoring program showed that there was minimal toxic activity in the north and south of the Tasmanian east coast during 2018 and 2019, however algal blooms did develop in the Spring Bay area during the winter/spring of both years, impacting Okehampton Bay (Figure 4.2, Table 4.1).

Bivalve harvest from Zone 6, Moulting Bay, an inter-tidal oyster zone at the mouth of the bay, was halted due to PST from *A. catenella* in late October 2017, and again in August and September 2018. The maximum PST concentration recorded in oysters from Zone 6 in 2018 was 1.1 mg STX.2HCl equiv. kg^{-1}. Cells of *A. catenella* were recorded in the 2018 May, July and August

monthly phytoplankton samples, with the maximum observed cell concentration being 2,100 cells L^{-1} in July. No biotoxin closures occurred in 2019.

A bloom of *A. catenella* in 2017 resulted in a long-term closure of the Spring Bay growing area, from mid-September 2017 until 5th January 2018. Further closures occurred during 2018 and 2019 (see Table 4.1 for details). The maximum PST concentration recorded in mussels was 21.1 mg STX.2HCl equiv. kg^{-1} on the 17th September 2018. Cells of *A. catenella* were seen in the March, June, July, August and September 2018 monthly phytoplankton samples, with the maximum cell concentration being 6,700 cells L^{-1} in September. The commercial bivalve farm was closed from the 4th to the 30th October in 2019.

Table 4.1 Summary of paralytic shellfish toxin related bivalve harvest closures and sentinel mussel data (indicating exceedance of the maximum allowed PST level) from sites adjacent to the abalone sampling sites used in this study.

Sample Site	Closest commercial bivalve harvest area	Approximate distance to sample site	Closure dates	Closed days
Binalong Bay & Georges Bay	Zone 6, Moulting Bay	7 km	13/10/17 - 27/10/17	14
			10/8/18 – 7/9/18	30
Okehampton Bay	Spring Bay	7 km	15/9/17 – 5/1/18	112
			22/8/18 – 30/10/18	69
			4/10/19 – 30/10/19	26
Pirates Bay	DPIPWE sentinel mussel site for SRL monitoring	0 – 2.5 km	16/10/17 – 8/12/17*	NA

*Dates when sentinel mussels exceeded bivalve ML

Whilst there are no commercial bivalve farms in the vicinity of Pirates Bay, sentinel mussel sampling does occur in high-risk periods through the DPIPWE SRL biotoxin monitoring program. A bloom in late 2017 saw elevated PST concentrations in mussels from Pirates Bay from the 16th October to the 8th December 2017, reaching a maximum PST screen concentration of 52 mg STX equiv. kg^{-1}. Fortnightly mussel samples between August and late November in 2018 showed a maximum level of 0.24 mg STX.2HCl equiv. kg^{-1}, well below the Codex bivalve ML of 0.8 mg STX.2HCl equiv. kg^{-1}.

4.3.2 Blacklip Abalone monitoring data

At total of 91 Blacklip Abalone were collected during the study, 61 animals during 2018 and 30 during 2019. The mean abalone length at Binalong & Georges Bay, Okehampton Bay and Pirates Bay was 13.1 ± 0.9, 10.4 ± 1.3 and 13.6 ± 1.5 cm respectively, whilst the mean abalone weight was 192.7 ± 43.6, 96.5 ± 36.9 and 221.4 ± 72.0 g respectively. There was a significant difference in the length and weight of abalone between sites (p values <0.001), with both measurements being smaller at Okehampton Bay.

Sample collection occurred on 12 different sampling occasions. From these animals, 106 Blacklip Abalone tissue samples were analysed for PST (12 composite foot samples, 44 individual foot, 1

composite viscera sample, 49 individual viscera). Raw results (including PST analogue data) are given in Appendix 3 and summarised in Table 4.2, alongside PST data from SRL sampled concurrently. The individual and pooled abalone tissue PST data are displayed for each site in Figure 4.2, with PST concentrations from concurrent samples of bivalves and SRL hepatopancreas overlaid for comparison.

Table 4.2 Summary of Blacklip Abalone and Southern Rock Lobster field PST concentrations (mg STX.2HCl equiv. kg^{-1}) collected from the east coast of Tasmania during 2018 and 2019.

		H. rubra rubra **foot**		***H. rubra rubra*** **viscera**		***J. edwardsii*** **hepatopancreas**	
Site	**Date**	**Mean ± SD**	**Max**	**Mean ± SD**	**Max**	**Mean ± SD**	**Max**
Binalong Bay, Block 30A	8/10/2018	0.15 ± 0.05	0.23	0.03 ± 0.01	0.04	0.04 ± 0.03	0.06
	5/11/2018	0.08		0.02 ± 0.01	0.04	0.02	0.04
Georges Bay, Block 30A	9/10/2018	0.30 ± 0.28	0.86	0.05 ± 0.03	0.09	0.04	0.05
Okehampton Bay, Block 24C	4/09/2018	0.72 ± 0.25	1.07	0.26 ± 0.09	0.41	0.64 ± 1.06	2.74
	4/10/2018	0.56 ± 0.14	0.76	0.23 ± 0.04	0.29	0.55 ± 0.65	1.83
	31/10/2018	0.51 ± 0.27	0.99	0.11 ± 0.05	0.16	0.38 ± 0.27	0.73
	20/11/2018					0.69 ± 0.51	1.64
	6/12/2018	0.46 ± 0.21	0.72	0.09 ± 0.04	0.12	0.42 ± 0.34	0.88
	12/12/2018					0.32 ± 0.17	0.46
	11/6/2019	0.35 ± 0.22	0.63			0.11 ± 0.08	0.14
	7/10/2019	0.34 ± 0.08	0.47	0.15		2.36 ± 2.53	6.70
Pirates Bay, Block 22C	5/09/2018	0.13		0.04 ± 0.01	0.06	0.07 ± 0.05	0.16
	11/10/2018	0.10		0.03 ± 0.02	0.06	0.05 ± 0.03	0.09
	8/11/2018	0.31 ± 0.18	0.65	0.03 ± 0.01	0.04	0.10 ± 0.12	0.34

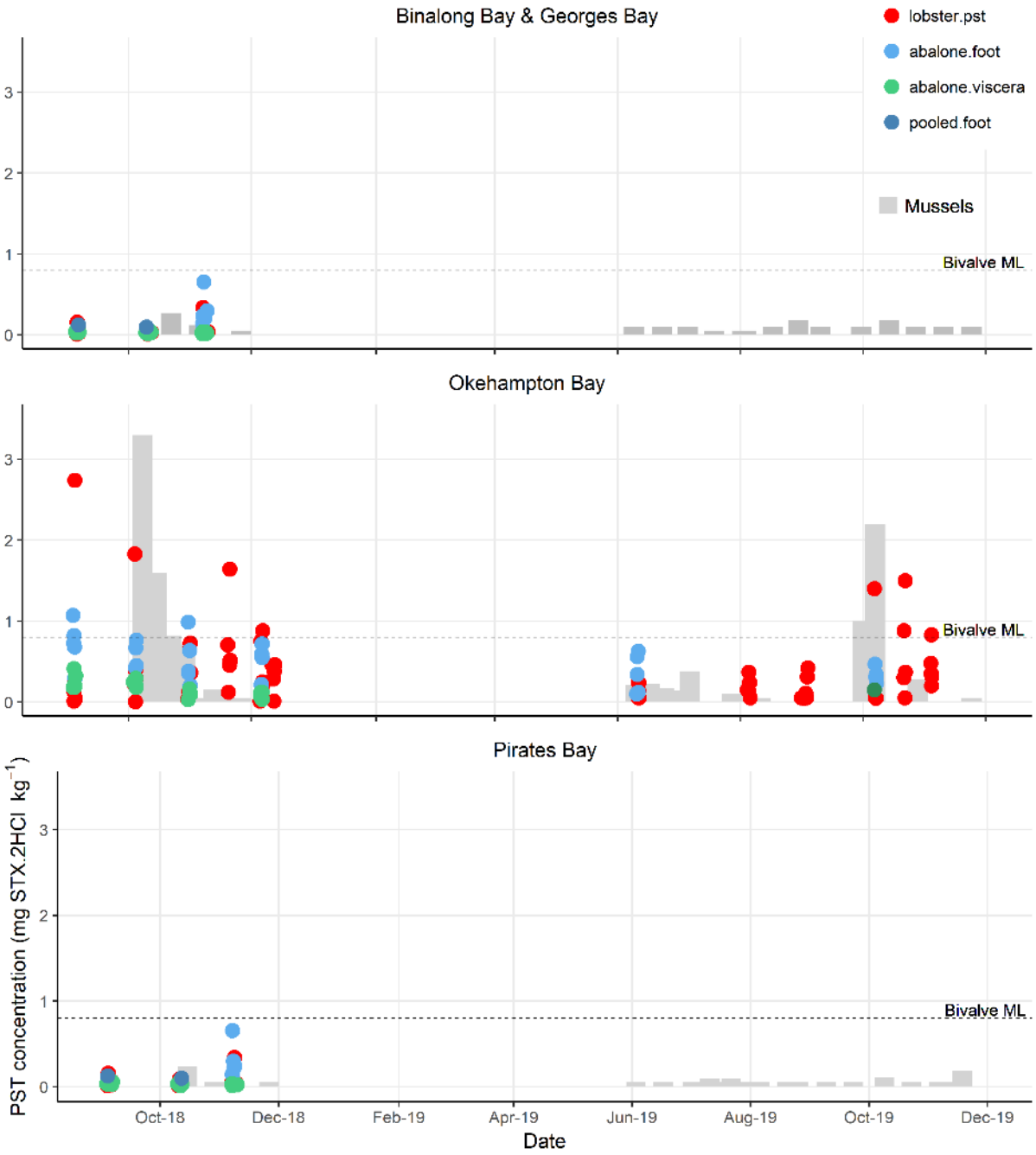

Figure 4.2 Paralytic shellfish toxin concentrations in abalone foot (light blue circle for individual and dark blue for pooled samples) and viscera (green circle) tissues from three sites on the east coast of Tasmania collected during 2018 and 2019, in comparison with Southern Rock Lobster hepatopancreas (red dot) and bivalve shellfish (grey polygon) PST concentrations. Bivalve data is a compilation of DPIPWE mussel sentinel data and data from the commercial bivalve monitoring program. The PST regulatory level is shown as the black dotted line

The majority of sampling occurred in Okehampton Bay, in response to the stronger algal blooms that occurred there, although all sites showed elevated PST concentrations in abalone. In fact, PST concentrations in abalone foot exceeded the maximum allowable level (ML) of 0.8 mg STX.2HCl equiv. kg^{-1} on three of the twelve sampling occasions (maximum value 1.07 mg STX.2HCl equiv. kg^{-1}), and was above 0.6 mg STX.2HCl equiv. kg^{-1} on further four occasions. In contrast, the abalone viscera

remained well under the bivalve ML, with a maximum concentration of 0.41 mg STX.2HCl equiv. kg^{-1}. The abalone viscera PST concentration was significantly different to the foot PST concentration (p value <0.05), but the two were correlated (Figure 4.3). Viscera PST concentration was lower than the foot concentration on all occasions that foot and viscera tissue were analysed for individual animals (seven sampling occasions, 34 animals).

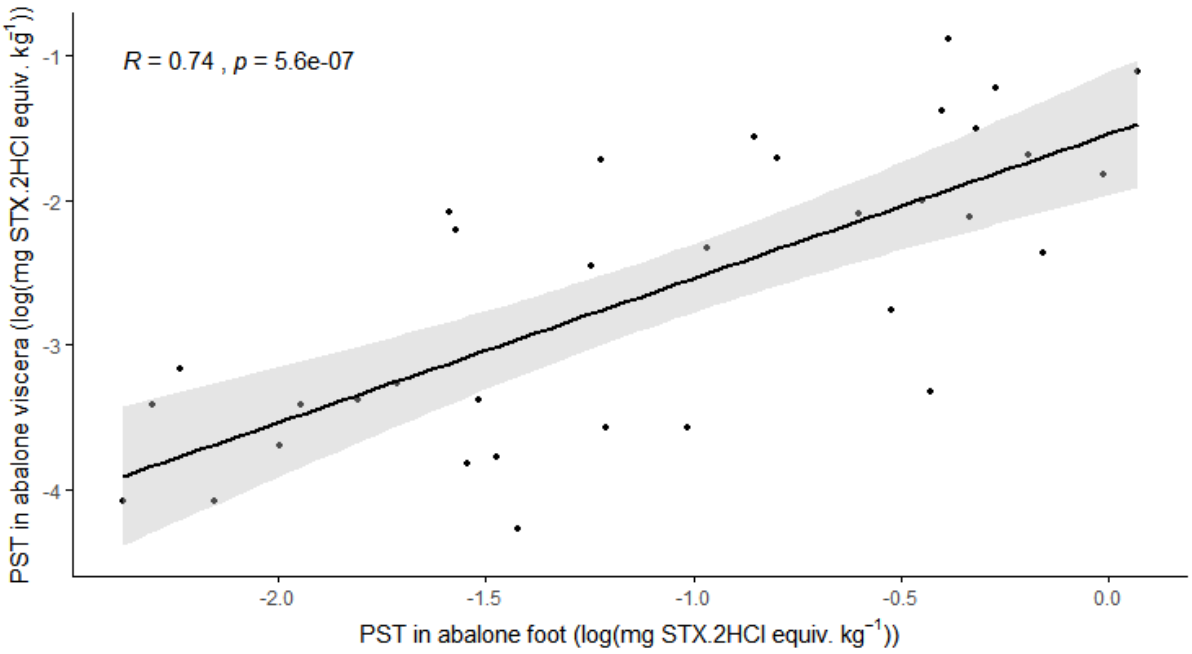

Figure 4.3 The correlation between PST concentration in abalone foot and viscera tissues.

At the start of the 2018 bloom, the PST concentration in abalone foot tissue from both Binalong Bay and Okehampton Bay was above the ML. Concurrent mussel samples showed only slight elevation of PST in Moulting Bay, adjacent to Binalong Bay, and the second abalone sample from this area showed only low levels of toxin. In contrast, mussel data from Spring Bay and Okehampton Bay showed continued high levels of toxins and PST concentrations in abalone foot tissue remained elevated at Okehampton Bay until the end of December 2018. PST concentrations in the foot were still elevated in June 2019, despite low bloom activity in the intervening period.

Blacklip Abalone tissues from Pirates Bay showed low toxin levels in October 2018, increasing slightly in November most probably due to increasing concentrations of toxic algal cells.

The percentage contribution of each PST analogue to total PST was relatively consistent. Foot tissue toxicity was comprised mainly of STX and NEO (69 ± 18% and 22 ± 21 % respectively) with GTX2 and GTX3 each contributing an average of 1 - 5% of total toxicity. In the viscera, STX again was responsible for the majority of the toxicity (70 ± 5%), whilst NEO was absent, and GTX2 and GTX3 accounted for 13 ± 4 and 14 ± 3% of toxicity respectively.

The PST analogue percentages in the foot found in the field were generally consistent with the experimental results; STX was the dominant analogue in both, followed by NEO. However, the experimental viscera toxicity varied between that found in the field and that in the experimental work, where STX accounted for only 17-18% of total toxicity. The percentages of GTX2 and GTX3 were considerably higher in viscera in the experimental work (combined toxicity of 24 and 47% for the pellet and algal diet respectively).

Due to the variation in weight and length with site, data from Okehampton Bay only was used to test for differences between size and PST concentration in the foot (n=29). No significant difference was seen with PST concentration across both length and weight (p = 0.67 and 0.49 respectively).

4.3.3 Comparison with Southern Rock Lobster PST data

The PST concentrations found in abalone foot were not significantly different to PST concentrations in SRL hepatopancreas (p = 0.37). On some occasions, abalone foot exceeded the ML when SRL hepatopancreas did not, and on other occasions, the reverse was true. Variability in abalone foot PST concentrations was lower than the variability of PST concentrations in SRL hepatopancreas (Table 4.2, Figure 4.2).

4.4 Discussion

Blacklip Abalone tissues accumulated PST from *A. catenella* blooms to concentrations that exceeded the bivalve ML, and therefore presented a trade and human health risk. Without exception, the foot tissue (the tissue most commonly consumed) showed higher PST levels than the viscera. This is the reverse of the situation generally found in Blacklip Abalone during *G. catenatum* blooms in the south-east of Tasmania (McLeod et al., 2017). It is, however, consistent with the detection of PST in Spanish and South African abalone, the latter following potential contamination with comparable genetic strains of *A. catenella* (Bravo et al., 2001; Bravo et al., 1999; Pitcher et al., 2001). It is also consistent with the experimental uptake results detailed in Section 3 of this report. Analysing foot tissue only is thus likely to be protective of public health and trade as well as potentially saving half the analytical costs of the monitoring program, although more samples collected during bloom periods are required to confirm this finding.

The field data demonstrate the slow depuration of PST from abalone foot tissues. The first samples taken in 2018 showed a surprisingly elevated level of PST, with the foot of one animal from Georges Bay and two from Okehampton Bay exceeding the ML on the first sampling date. On both occasions, there had been a bloom of *A. catenella* present for several weeks, making it difficult to distinguish if this was residual toxin from the large 2017 bloom, or toxins rapidly accumulated during 2018. To resolve this issue, sampling in 2019 was targeted prior to bloom commencement at Okehampton Bay. Despite 6 months depuration between the last 2018 sampling event in December and the first sampling event in June 2019, the mean and maximum PST concentrations at Okehampton Bay had decreased by only 0.1 mg STX.2HCl equiv. kg^{-1}. This slow depuration was consistent across all toxin analogues, with the proportion of each analogue remaining approximately the same. Similarly, slow depuration of toxins was found in *H. tuberculata* from the Galician coast (Bravo et al. 1996 found no depuration over 3 months), and in *H. midae* from South Africa (Pitcher et al. 2001 found no depuration over 7 months).

Bivalves showed increased levels of PST during *A. catenella* blooms, with rapid accumulation and depuration of toxins in line with cell numbers (ShellMAP data). Bivalve PST levels have effectively been used as an early warning tool for PST in abalone in south-eastern Tasmania at the start of *G. catenatum* blooms (McLeod et al. 2017), and for PST in SRL in eastern Tasmania during *A. catenella* blooms. However, the data collected during this study suggest that bivalves are not an effective tool for indicating toxicity in Blacklip Abalone during or following *A. catenella* events. This could be due to a combination of two issues: the slow depuration rate of PST from Blacklip Abalone foot, and the distance between the sentinel bivalves and abalone dive sites. Whilst there is good connectivity

between the Spring Bay mussel farm and Okehampton Bay, the fine scale variation of the *A. catenella* blooms is not fully understood and could be contributing to the poor relationship seen here.

There was no significant difference between the PST concentration of SRL hepatopancreas and that of abalone foot tissues, although it needs to be acknowledged that variation of the PST concentration in SRL hepatopancreas is high. On two of the three occasions when abalone foot exceeded the ML, PST concentrations in lobster hepatopancreas were below the regulatory level, indicating that SRL were not a good sentinel for abalone toxicity. This was particularly evident in samples from Georges Bay on the 9th October 2018 when the maximum lobster hepatopancreas concentration found was only 0.05 mg STX.2HCl equiv. kg^{-1}, whilst the maximum abalone foot PST concentration was 0.86 mg STX.2HCl equiv. kg^{-1}.

Comparison of PST analogue contributions to total toxicity in abalone samples collected during 2017 (TACL data, unpublished) and the data described here are made in section 5.2. For foot data, STX is commonly the dominant analogue, contributing to over 50% of toxicity. During 2017, a higher proportion of GTX2&3 was found in the foot, although it should be noted that the PST analysis was completed by different laboratories, using different analytical methods. A similarly higher contribution by GTX analogues was found in the viscera during 2017.

The limited data collected during this study suggest that abalone size does not impact PST accumulation. This is not consistent with research conducted on Spanish Orma by Bravo et al. (1991), who found that larger abalone accumulated PST to higher levels.

Whilst the data collected during the fieldwork align with that found in the experimental work (Blacklip Abalone can take up PST from *A. catenella*, and PST are primarily concentrated in the epipodium/foot tissue), there remain many unanswered questions from the limited study conducted here. Future research required in order to design an effective field monitoring program needs to focus on understanding: the uptake and depuration rates; residual toxin levels between blooms; variability between animals at each site; and geographic variability. The slow depuration rate in particular is of concern and has the ability to cause long-term disruption to harvest.

Chapter 5 - Validation of Neogen rapid PST test kits in abalone viscera and foot tissues

5.1 Introduction

Effective management of paralytic shellfish toxin (PST) risk in seafood is critical to ensure public safety and access to both overseas and domestic markets. Routine analysis of shellfish samples for the presence/absence of paralytic shellfish toxins forms an integral part of any shellfish quality assurance program. Naturally, the analytical methods employed for such purposes are required to meet certain performance criteria to ensure delivery of accurate and reliable results.

In Tasmania, two microalgal species, *G. catenatum* and *A. catenella*, have been associated with elevated PST levels in abalone (McLeod et al., 2017, TAS DPIPWE data and field data from this project). Historically, *G. catenatum* blooms affecting abalone have been documented predominantly in the Huon Estuary and D'Entrecasteaux Channel, whereas *A. catenella* blooms present a recurring PST risk on the east coast of Tasmania. Abalone viscera and foot tissues collected during blooms of these two species have presented with distinct PST profiles that further vary between viscera and foot.

Under the current abalone biotoxin management plan for Tasmania, the closed/open status of abalone harvest blocks is decided based on the quantification of PST levels via HPLC analysis (Lawrence method) of both viscera and foot tissues of at least 5 individual abalone per sampling interval (10 samples total). These analyses are costly ($500-600 per sample) and require a lengthy turnaround time (~3 days). An attractive option to reduce analytical costs and time is the use of the Neogen PST rapid test kit. While not designed to deliver quantification of PST concentrations, this qualitative assay (positive/negative result) offers the opportunity to rapidly (~25 min per sample) pre-screen tissue samples at a fraction of the cost (~$30 per sample). Positive detections could then be followed up with HPLC analysis to compare sample results to the regulatory level (0.8 mg STX.2HCl equiv. kg^{-1}) and implement the decision-making tree defined in the biotoxin management plan.

Before employing the Neogen test kit, it is of critical importance to validate/verify its suitability for the intended use. This includes consideration of a range of naturally encountered PST profiles (the test exhibits different cross-reactivities between PST analogues; Jawaid et al., 2015) in each tissue matrix (abalone viscera and foot tissue). It is generally assumed that the more closely related a new food matrix (abalone tissues) is to a previously validated matrix (e.g. bivalve molluscs), the greater the probability that a method will perform similarly with the new matrix (Weitzel et al., 2007). Full validations of the Neogen PST standard method have successfully been conducted previously for Pacific Oyster, Blue Mussel and rock lobster hepatopancreas tissues (Turnbull et al., 2018; Dorantes-Aranda et al. in press). These studies included detailed analysis of ruggedness (repeatability between test kit production batches) and specificity (cross-reactivity with other potentially encountered biotoxins), as well as an international validation with 16 participating laboratories (Dorantes-Aranda et al., 2018).

Rather than conducting a full scale validation repeating the above listed performance criteria that are unlikely to differ between molluscan matrixes, we here followed the National Association of Testing Authorities (NATA 2018), Food and Drugs Administration (FDA, 2015) and Analytical Laboratory Accreditation Criteria Committee guidance on qualitative analytical methods (Weitzel et al., 2007) to conduct a verification of the Neogen test kit in Blacklip Abalone. The probability of PST detection around the regulatory limit (0.8 mg STX.2HCl equiv. kg^{-1}) was determined for four different PST

profile/tissue mixes representing common PST profiles found in viscera and foot tissues of abalone during *A. catenella* and *G. catenatum* blooms in Tasmanian waters.

5.2 Methods

5.2.1 PST profiles

To determine the most suitable PST profiles representing PST accumulation in Tasmanian Blacklip Abalone tissues typically found during Tasmanian harmful algal bloom (HAB) events, all available data on PST concentrations in abalone tissues during *A. catenella* and *G. catenatum* blooms in Tasmanian waters was reviewed. Foot and viscera tissues presented with unique PST profiles when exposed to either toxic species, requiring the preparation of four different master-mixes spiked with differing amounts of PST reference materials (Table 5.1). The profiles chosen to represent abalone foot and viscera tissues from *G. catenatum* blooms were based on Harwood et al. (2014). *A. catenella* profiles were selected based on 2017 and 2018/2019 field data collected from the east coast of Tasmania (TAS DPIPWE data and field data from this project).

PST profiles in abalone foot tissue from *A. catenella* blooms were found to vary significantly between 2017 and 2018/19, requiring careful profile selection to account for the different cross-reactivities of the Neogen antibodies towards various PST analogues (Jawaid et al., 2015). Foot tissues were dominated by readily detected neosaxitoxin (NEO) and saxitoxin (STX) in 2018/2019, with lower concentrations of gonyauxtoxins 2&3 (0.8-15.5% total toxicity). A larger proportion (up to 56% toxicity) of the not as readily detected GTX2&3 was found in the 2017 samples, with PST levels close to the regulatory level. Consequently, the average profile of these (2017) samples was selected as the target profile.

Similarly, PST profiles in abalone viscera from *A. catenella* blooms differed significantly between the sampling years. While samples from 2017 presented with a high proportion of STX, the samples from the 2018/2019 bloom were characterised by a high GTX content. A single sample from 2019 presented with extraordinarily high GTX2&3 content and no GTX1&4. As Neogen cross-reactivities for the latter are lower (i.e. not as readily detected), it was decided to treat this sample as an outlier and challenge the Neogen test kit against the average profile of the remaining 2017 samples (i.e. GTX2&3 and the harder to detect GTX1&4).

As no contaminated abalone tissues from *G. catenatum* blooms were available, these profiles had to be entirely generated from non-toxic tissues. Consequently, decarbamoyloxysaxitoxin (doSTX) toxicity in tissues representing 40% total toxicity (as % STX.2HCl equiv.) of the *G. catenatum* profiles could not be included. Decarbamoyloxysaxitoxin exhibits low toxicity and due to the concentration of available doSTX reference material would have necessitated the addition of uneconomical volumes that would have substantially changed the matrix consistency (>220% v/w liquid addition). Instead, doSTX was omitted in the *G. catenatum* viscera and foot tissue profiles. However, standard doSTX reference material was sent to Queen's University Belfast, Ireland, to the researchers who developed the Neogen antibodies, so that the cross-reactivity of doSTX with the Neogen test kit antibodies could be separately analysed.

Table 5.1 Selected representative PST profiles (% toxicity) from *A. catenella* and *G. catenatum* in Blacklip Abalone viscera and foot tissues.

Tissue type	C1&2	dcGTX2&3	GTX2&3	GTX1&4	doSTX	dcSTX	STX	NEO
A.catenella viscera	6.8	2.8	41.2	37.9	-	-	10.7	-
A.catenella foot	-	-	33.2	-	-	-	45.2	21.6
G. catenatum foot	-	-	-	-	-	52.4	47.6	-
G. catenatum viscera	3.0	12.1	-	-	-	60.6	24.2	-

5.2.2 PST contaminated tissues

To generate PST contaminated master-mixes of the four selected profiles, abalone tissues were processed with a stick type homogeniser (Velp Scientifica, OV5) to produce a consistent paste (abalone foot) and dense slurry (abalone viscera). Care was taken to avoid overheating of tissue samples during homogenisation. PST concentrations and desired profiles were achieved through the addition of certified PST reference materials (National Research Council, Canada). Three subsamples of each of the four unique PST profiles (*G. catenatum* foot and viscera and *A. catenella* foot and viscera) were analysed via LC-MS to confirm PST profiles and thorough mixing of these master-mixes. Once confirmed, these mixes were diluted with non-toxic tissues to yield 0, 0.25, 0.4, 0.6, 0.8 and 1.1 mg STX.2HCl equiv. kg^{-1}, with 4, 10, 10, 38, 38 and 10 replicates at each of the respective concentrations. An additional seven replicates at 1.5 mg STX.2HCl equiv. kg^{-1} were analysed for the *A. catenella* foot tissue matrix. The non-toxic tissues utilised for the purpose of diluting the PST master-mixes originated from the previously conducted tank experiments and were confirmed via LC-MS to contain insignificant levels of PST (<0.03 mg STX.2HCl equiv. kg^{-1}). After the final mixes were prepared, one analyst weighed out 1 ± 0.05 g of sample for each replicate. Replicates were then stored at -80°C for two weeks before a second analyst conducted the Neogen PST analysis.

5.2.3 PST analysis via LC-MS

PST analysis occurred by LCMS-MS using the method of Boundy et al. (2015) and Turner et al. (2015; 2020), as detailed in Section 3.2.6 above.

5.2.4 Neogen analysis

Neogen rapid test kits were sourced from Cell Biosciences Pty Ltd. across multiple production lots, as previous validation work had shown little variation between production runs (Turnbull et al., 2018). The test procedure followed the standard method outlined in detail in previous validation works by Turnbull et al. (2018), which also followed the protol recommended by the manufacturer.

5.2.5 Data analysis

PST concentrations determined through LC-MS analysis were converted to STX.2HCl equiv. kg^{-1} using internationally accepted toxin equivalency factors (FAO/WHO, 2016). All PST concentration values and toxicity percentages given here are expressed as STX.2HCl equiv. Statistical analysis of the

probability of detection was based on the methods outlined in Labudde and Harnly (2012), Macarthur and von Holst (2012) and Wehling et al. (2011). All statistical analysis and graphing of results was conducted in R (https://www.R-project.org).

5.3 Results

5.3.1 PST profiles

Actual PST profiles were confirmed via LC-MS to be very close to the original target profiles selected from the field data (Figure 5.1). Analysis of triplicate subsamples of each of the four toxic master-mixes verified homogenous PST concentrations prior to dilution with non-toxic material (standard deviation around mean = 0.04 - 0.13 mg STX2.HCl equiv. kg^{-1}).

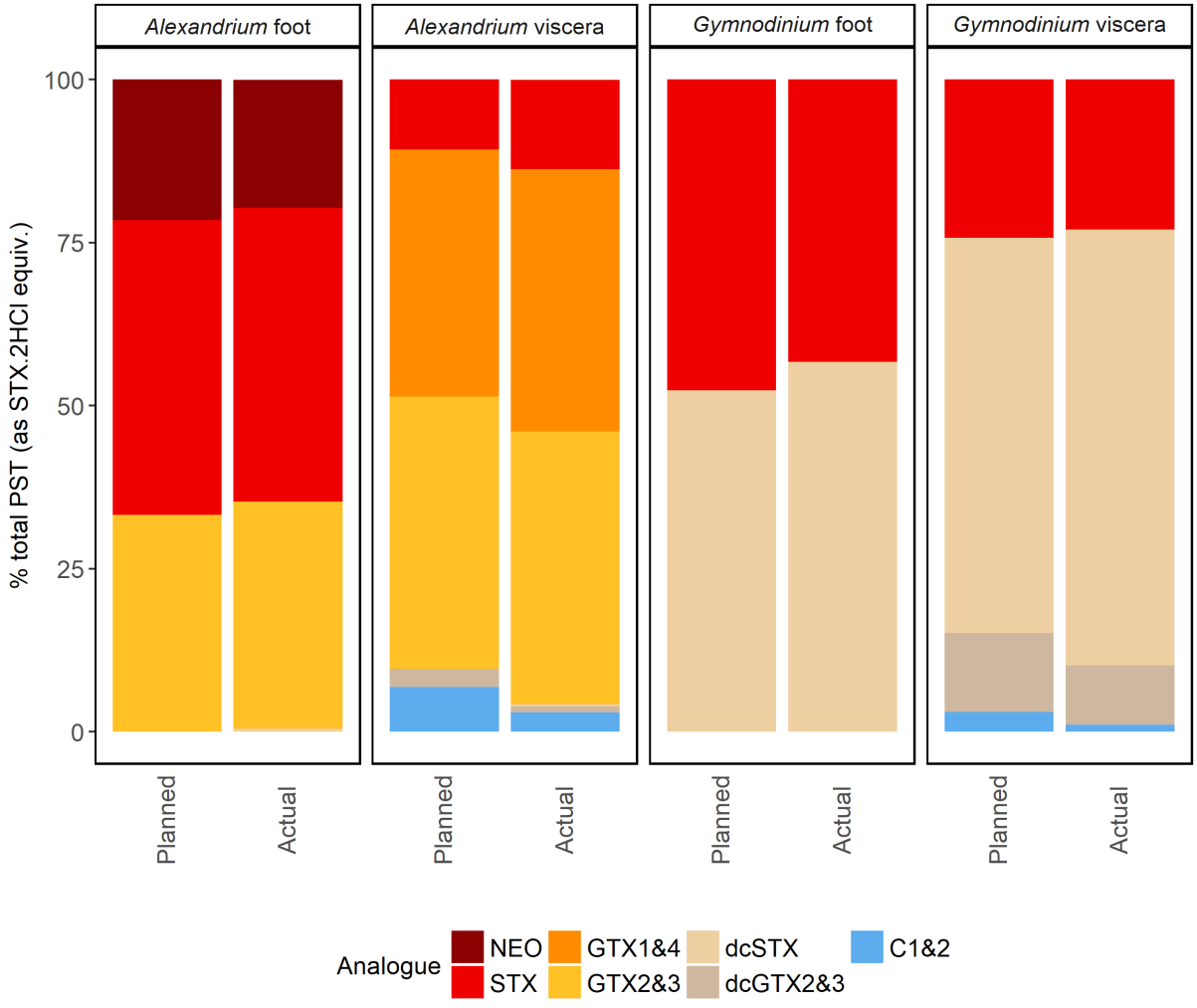

Figure 5.1 Originally planned (target) and actual contribution of individual PST analogues to overall toxicity in abalone viscera and foot tissues used for Neogen verification of profiles commonly encountered during *A. catenella* and *G. catenatum* blooms in Tasmanian waters.

The results for the Neogen detection of PST in the replicates for each profile in viscera and foot tissue (110 replicates for *G. catenatum* toxins in foot and viscera tissues, 110 replicates for *A. catenella* toxins in viscera tissue and 117 replicates for *A. catenella* toxins in foot tissue) are summarised in Table 5.1 and graphically represented in Figure 5.1. While the Neogen test kit reliably returned negative results for PST concentrations < 0.25 mg STX.2HCl equiv. kg^{-1} across all profiles, detection at

the higher PST levels was strongly dependent upon the PST profile. The profile representing abalone foot tissue from *A. catenella* blooms was the only profile for which no negative detections were recorded above 0.8 mg STX.2HCl equiv. kg^{-1} (Figure 5.2a). Even at 1.1 mg STX.2HCl equiv. kg^{-1}, the profiles representing abalone viscera from *A. catenella,* and foot and viscera from *G. catenatum* blooms exhibited low probabilities of detection (POD; 70, 80 and 90%, respectively, Figure 5.2 b-d).

Table 5.2 Probability of detection (POD) at various PST concentrations in abalone foot and viscera tissues spiked with different toxin profiles.

Bloom profile	Abalone tissue	Concentration (mg STX.2HCl equiv. kg^{-1})	No. of samples	No. of detections	POD	95% CI*
A. catenella	Foot	0.03	4	0	0.00	(0.00, 0.49)
A. catenella	Foot	0.18	9	0	0.00	(0.00, 0.30)
A. catenella	Foot	0.29	10	2	0.20	(0.06, 0.51)
A. catenella	Foot	0.41	38	27	0.71	(0.55, 0.83)
A. catenella	Foot	0.56	38	28	0.74	(0.58, 0.85)
A. catenella	Foot	0.83	10	10	1.00	(0.72, 1.00)
A. catenella	Foot	1.50	7	7	1.00	(0.65, 1.00)
A. catenella	Viscera	<0.01	4	0	0.00	(0.00, 0.49)
A. catenella	Viscera	0.17	10	0	0.00	(0.00, 0.28)
A. catenella	Viscera	0.40	10	0	0.00	(0.00, 0.28)
A. catenella	Viscera	0.50	38	7	0.18	(0.09, 0.33)
A. catenella	Viscera	0.87	38	17	0.45	(0.30, 0.60)
A. catenella	Viscera	1.10	10	7	0.70	(0.40, 0.89)
G. catenatum	Foot	0.03	4	0	0.00	(0.00, 0.49)
G. catenatum	Foot	0.27	10	0	0.00	(0.00, 0.28)
G. catenatum	Foot	0.28	10	0	0.00	(0.00, 0.28)
G. catenatum	Foot	0.52	38	16	0.42	(0.28, 0.58)
G. catenatum	Foot	0.76	38	24	0.63	(0.47, 0.77)
G. catenatum	Foot	0.96	10	8	0.80	(0.49, 0.94)
G. catenatum	Viscera	<0.01	4	0	0.00	(0.00, 0.49)
G. catenatum	Viscera	0.26	10	1	0.10	(0.02, 0.40)
G. catenatum	Viscera	0.41	10	1	0.10	(0.02, 0.40)
G. catenatum	Viscera	0.57	38	23	0.61	(0.45, 0.74)
G. catenatum	Viscera	0.84	38	35	0.92	(0.79, 0.97)
G. catenatum	Viscera	1.10	10	9	0.90	(0.60, 0.98)

* CI = confidence interval

Fitting a binomial logistic regression model to the data allowed for calculation of the POD exactly at the regulatory level (0.8 mg STX.2HCl equiv. kg^{-1}). Again, in abalone foot tissue with a typical *A. catenella* bloom profile, the probability to detect the presence of PST is much greater (98% POD) than in viscera tissues from the same bloom (38%) or *G. catenatum* sourced foot (71%) or viscera tissues (88%).

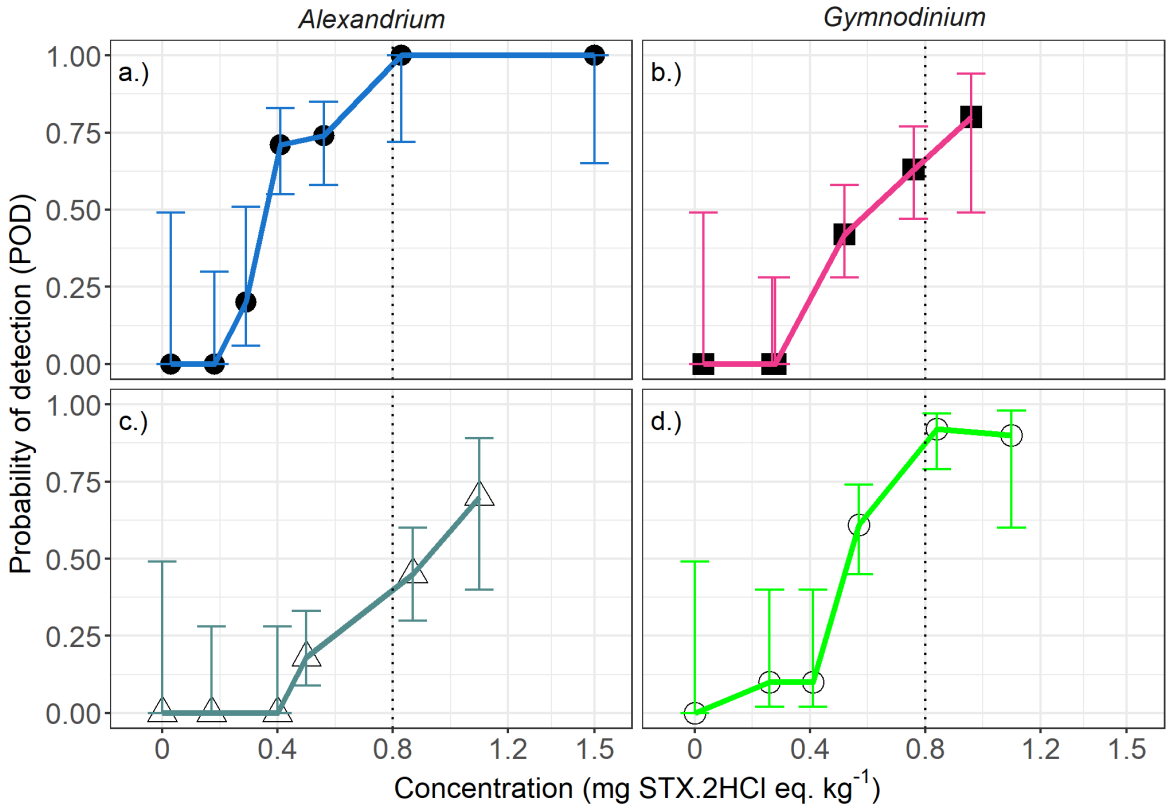

Figure 5.2 Probability of detection (POD) by the Neogen test kit at various PST concentrations in Blacklip Abalone foot (a. & b.) and viscera (c. & d.) tissues containing PST profiles commonly encountered after *A. catenella* and *G. catenatum* blooms in Tasmanian waters. The dashed vertical line represents the PST regulatory limit at 0.8 mg STX.2HCl equiv. kg^{-1}.

5.4 Discussion

We here for the first time challenged the Neogen rapid test kit against four different PST profiles in Blacklip Abalone viscera and foot tissues. The considerable differences in the ability of the Neogen test kit to detect the presence of PST in abalone tissue at the regulatory level between the four different profiles can to a certain extent be explained by their unique PST composition. Certain analogues, such as STX and NEO, have much higher cross-reactivities (100 and 129%, respectively) to the antibodies in the Neogen test kit than gonyau-, c-type or decarbamoyl toxins (3-23%; Jawaid et al., 2015). This becomes most evident when comparing the PST profiles with the highest and lowest PODs at the regulatory level (0.8 mg STX.2HCl equiv. kg^{-1}): the *A. catenella* viscera profile (38% POD) was dominated by harder to detect, low cross reactivity gonyautoxins and a minor component of STX (<14%, Table 5.1). On the other hand, the *A. catenella* foot profile (98% POD) was predominately made up of highly reactive STX and NEO.

However, the abalone tissue matrix appears to have a more pronounced impact on the POD than the PST composition of individual profiles. While the cross reactivity of individual PST analogues may have contributed to the observed differences in POD between the different abalone tissues, previous validation work in mussel and oyster matrixes demonstrated reliable detection at the PST regulatory level, even when profiles were dominated by PST analogues with lower cross reactivities (gonyau- and c-toxins; Turnbull et al., 2018). Furthermore, the observed differences in POD between the two profiles representing abalone tissues from *G. catenatum* blooms suggests that the Neogen test kit performs better in the viscera than in the foot matrix. Based on the PST analogues' cross-reactivities with the Neogen antibodies, the larger STX component in the foot tissue *a priori* should predict a higher POD in the foot rather than in the viscera tissue. However, the opposite was observed (71% POD in foot and 88% in viscera). A possible explanation is a difference in the efficiency of PST extraction between the two different matrixes. Although PST recovery rates are reported to not significantly differ between abalone and mussel tissues during sophisticated LC-MS extraction techniques (Harwood et al., 2014), the comparatively simple extraction step in the Neogen protocol: manual mixing of tissue with water using a roller and a 200-µm mesh bag filter, may not be as effective at extracting PST from the foot matrix as it is in less viscous mussel tissues.

When comparing the overall POD curves (Figure 5.2) to those acquired in previous studies (Turnbull et al., 2018), it quickly becomes apparent that PSTs are not as readily detected in abalone tissues as they are in bivalve shellfish (e.g. 100% POD at 0.6 mg STX.2HCl equiv. kg^{-1} in Blue Mussels). Based on the here tested PST profiles, the Neogen test kit does not appear suited to detect PSTs in abalone impacted by *G. catenatum* blooms. However, it should be noted that a large component of the *G. catenatum* PST profile, doSTX, had to be omitted here during master-mix preparation (see Section 5.2). The cross-reactivity of doSTX with the Neogen antibodies is currently unknown, but experiments by a group in Belfast are currently underway to confirm cross-reactivity.

The Neogen test kit proved ill-suited for the detection of PST in abalone viscera representing PST profiles often encountered on the east coast of Tasmania during *A. catenella* blooms. In the foot tissue, however, the test method was able to consistently detect PST contamination, (98% POD at the regulatory level), without any false negatives. This may be of interest to biotoxin management, as PST levels in Blacklip Abalone after *A. catenella* exposure have historically been higher in the foot tissue than in the viscera, offering the option of pre-screening foot tissues with the Neogen rapid test. However, it should be reiterated that the ability of the Neogen test to detect the presence of PST is highly dependent on the presence, concentration and cross-reactivity of individual PST analogues, as well as the sample matrix. As evident from the here presented work, changes in PST profile composition will impact the POD at the regulatory level. Continued, careful monitoring of PST profiles in abalone foot tissues on the east coast of Tasmania is recommended to help inform decisions on the suitability of the Neogen test for pre-screening of foot tissues for PST.

Chapter 6 - Discussion

During the experimental component of this project we have successfully demonstrated uptake of PST in Blacklip Abalone via feed consumption (i.e. from contaminated pellets), as well as via direct exposure to live PST producing phytoplankton. This is the first time uptake of PST by abalone has been shown from direct exposure to phytoplankton PST producers, although associations between PST in abalone and toxic blooms have been noted previously in field situations in Tasmania and South Africa (Bravo et al., 2001; McLeod et al., 2017), and previous experimental work has shown PST uptake through consumption of contaminated feed pellets (Dowsett et al., 2011).

The fieldwork conducted within this project conclusively demonstrated that Blacklip Abalone tissues can accumulate PST from *A. catenella* blooms to concentrations that exceed the CODEX ML. Appropriate risk management activities are therefore necessary to protect this valuable trade and reduce human health risk.

Without exception, across both the field and experimental work with *A. catenella*, the foot tissue (the tissue most commonly consumed) showed higher PST levels than the viscera. This is in direct contrast to PST accumulation in Blacklip Abalone during *G. catenatum* blooms in south-east Tasmania (McLeod et al., 2017). Whilst the epipodium was not analysed for PST from field samples, we found distinctly higher concentrations of PST accumulated in the epipodium in the experimental studies. This accumulation of PST in the epipodial tissues of the foot has also been reported in Spanish Orma and South African abalone collected from the field (Bravo et al., 1999; Pitcher et al., 2001), raising the possibility of direct uptake of PST through the outer layers of the foot epidermis (McLeod et al., 2017). This appears to be supported by the relatively low oral uptake efficiency of PST delivered via the feed pellet compared with the substantially higher PST levels found in both foot and epipodial tissues exposed to algal cells.

The high PST concentration of the epipodium contributes substantially to the overall toxin burden. Removal of the majority of the epipodium through scrubbing before market could therefore significantly reduce toxicity of contaminated animals (up to 70% removal: this report; Dowsett et al., 2011; Pitcher et al., 2001). However, current risk management is aimed at allowing live harvest to maximise value realised in the market ($80 kg^{-1} for live, wild abalone compared to $40 kg^{-1} for scrubbed and canned product; beach prices, Tasmanian Abalone Council, 2018), and thus removal of the epipodium is not an economically palatable risk management option.

The concentration of PST in foot tissues suggest that the cost of analytical testing may be halved by preferentially testing the foot and not the viscera of abalone suspected to be contaminated with PST from *A. catenella*. Currently, PST are monitored through separate analysis of the viscera and foot tissue of individual abalone (5 animals per site), costing around A$5,000 per sampling interval per harvest block. Furthermore, the encouraging finding of the single-laboratory validation of the Neogen rapid screening tool for PST in Blacklip Abalone foot opens the way for additional savings. If field-testing of this kit is successful, samples testing negative via the Neogen test need not undergo further chemical analysis, further reducing analytical costs to the monitoring program.

The considerable differences in the ability of the Neogen test kit to detect the presence of PST at the regulatory level in different abalone tissues can, to a certain extent, be explained by their unique PST composition. Of the four tissue/PST combinations tested, the only successful validation was for *A. catenella* analogues in Blacklip Abalone foot tissue. This profile was predominately comprised of STX and NEO, for which the Neogen kit is highly reactive. The abalone tissue matrix also appears to have a pronounced impact on the Neogen POD: unlike the work presented here on abalone, previous validation work in bivalve matrixes demonstrated reliable detection at the PST regulatory level, even

when profiles were dominated by PST analogues with lower cross reactivities (gonyau- and c-toxins; Turnbull et al., 2018). The observed differences in POD between the two profiles representing abalone tissues from *G. catenatum* blooms suggest that the Neogen test kit performs better on the viscera than on the foot matrix. A possible explanation is a difference in the efficiency of PST extraction between the two different matrixes. The results provide a note of caution that the detection of PST by the Neogen rapid screening tool is highly dependent on the presence, concentration and cross-reactivity of individual PST analogues, as well as the sample matrix. Changes in PST profile composition will impact the POD at the regulatory level, and continued, careful monitoring of PST profiles in abalone foot tissues on the east coast is recommended to help inform decisions on the suitability of the Neogen test for pre-screening of foot tissues for PST.

The field data demonstrate the slow depuration of PST by abalone foot tissues. Following the large 2017 bloom, the first samples taken in 2018 showed a surprisingly elevated level of PST, exceeding the ML at two of the three sites. Similarly, despite 6 months depuration between the last 2018 sampling event in December and the first sampling event in June 2019, the decrease in PST concentrations at Okehampton Bay were minimal (approx. 0.1 mg STX.2HCl equiv. kg^{-1}). Depuration in the abalone is thus slower than that found in Blue Mussels and SRL, and neither of these organisms were found to be useful sentinel species to indicate PST levels in abalone.

The limited data collected during this study suggest that abalone size does not impact PST accumulation. This is not consistent with research conducted on Spanish Orma by Bravo et al. (1991), who found that larger abalone accumulated PST to higher levels, and needs to be confirmed with additional sampling.

During the experimental work, no adverse effects of PST exposure to either the diet (toxic pellet) or *A. catenella* cells were observed on abalone behaviour (i.e. no reduced feed intake or other avoidance behaviour). However, it should be noted that PST levels exceeding 1 mg STX.2HCl equiv. kg^{-1} have been known to paralyse (i.e. prevent attachment or righting) in both aquacultured and wild South African abalone during *A. catenella* blooms (Pitcher et al., 2001). Furthermore, during an event of high toxicity (16 mg STX equiv. kg^{-1} in abalone by mouse bioassay), brood-stock were found to cease spawning, and then produce unviable spawn for several months following the event (irregular division of eggs and formation of grossly abnormal larvae). It is unknown whether the spawning difficulties were a direct response to PST, or ill-health of the brood stock.

Whilst the data collected during the fieldwork align with that found in the experimental work (Blacklip Abalone can take up PST from *A. catenella*, and PST are primarily concentrated in the epipodium/foot tissue), there remain many unanswered questions from the studies conducted here. Future research required in order to design an effective field monitoring program needs to focus on understanding: the uptake and depuration rates; residual toxin levels between blooms; variability between animals at each site; variability in PST profiles within and between seasons; and geographic variability. The slow depuration rate in particular is of concern and has the ability to cause long-term disruption to harvest.

Chapter 7 - Conclusion

The current field and experimental research have shown that Blacklip Abalone do accumulate PST from *A. catenella* blooms to levels of concern for both trade and human health. Accumulation can occur through both consumption of toxic material and direct exposure to algal blooms. We have shown that abalone can accumulate PST from both consumption and exposure to toxic cells: in the field this is likely to be through exposure to and grazing of algal cells and cysts that have sunk out of the water column and are present on the rock and seaweed surfaces.

The PST are concentrated primarily in the epipodium of the foot tissue: removing the epipodium will substantially reduce the toxicity of the foot. Processed abalone are therefore a lower risk than live abalone, although considered less valuable for the export market.

The total toxicity of the foot was always higher than that of the viscera. This important finding needs to be confirmed by additional field sampling, and could reduce monitoring costs by half as it negates the need to analyse both viscera and foot tissue for PST.

Depuration of PST is very slow by the Blacklip Abalone foot: in one case approximately 0.1 mg STX.2HCl equiv. kg^{-1} over 6 months. As a result, toxins may carry over from previous bloom seasons resulting in animals exceeding the ML for long periods. Bivalve shellfish and SRL are not good sentinel species to indicate toxicity of the abalone, as they accumulate and depurate toxins more rapidly in response to the algal bloom peaks and troughs.

The Neogen rapid screen test performed well to indicate PST toxicity in abalone foot tissues following *A. catenella* blooms, with a 98% probability of detection at the regulatory level. Combined with the fact that foot tissue is the most at risk of PST accumulation, this result provides promise for a cost-effective risk management regime. We therefore recommend the Neogen rapid screen test in Blacklip Abalone foot should undergo a field validation. However, the Neogen rapid screen for PST in its current form is not appropriate for use with Blacklip Abalone viscera, or for Blacklip Abalone foot exposed to *G. catenatum* blooms. Care should be taken when relying on this test kit that the PST profile remains predominantly STX and NEO.

Importantly, this project has highlighted substantial differences between the uptake and depuration of the PST analogues produced by *A. catenella* and those produced by *G. catenatum*. The situation during *A. catenella* blooms on the east coast seems to be more similar to international observations of PST in abalone in Spain and South Africa than the observations made during *G. catenatum* blooms in south east Tasmania. Improved risk management on the east coast will require a better understanding of PST uptake and depuration in Blacklip Abalone.

Chapter 8 - Implications

This project has confirmed the fact that PST accumulates in Blacklip Abalone in association with *A. catenella* blooms, and thus appropriate biotoxin monitoring and management is required during these blooms to control the trade and public health risk. Industry and health and export regulators need to be aware of the issue and take action during *A. catenella* blooms, which have been known to occur in all abalone-producing states. Recreational harvest warnings should occur alongside industry harvest closures.

The project has provided the following information to inform these risk management programs:

- The slow depuration of PST from the abalone means harvest disruptions following bloom events may be long standing, and bivalve molluscs or SRL are not useful sentinel species to indicate the toxicity of abalone
- The data suggests (but needs to be confirmed with further field work) that:
    - the preferential testing of abalone foot tissue could adequately manage the PST risk during *A. catenella* blooms, halving monitoring costs
    - the Neogen rapid test kit for PST screening could be a useful tool for indicating risk in the foot tissue, further reducing monitoring costs (approx. $30 per test cf $250 for the new LCMS testing method)
- The uptake of PST from direct exposure to toxic algal cells implies that abalone in aquaculture systems drawing seawater from bloom affected areas are also at risk of PST accumulation
- The significant reduction (~70 %) in toxicity for processed abalone where the epipodium is removed could provide a risk management option where live trade is not required
- When using the Neogen rapid screening test kit to indicate PST toxicity, PST profiles need to be constantly monitored to confirm the predominance of STX and NEO in the profile.

Whilst we observed no gross impact on abalone health during our exposure experiments at low PST contamination levels, other research at higher levels of contamination has observed paralysis and spawning issues. Observations of impact on abalone health should be made during high toxicity events.

Chapter 9 - Recommendations

A working group consisting of industry, regulators and the researchers from this project should be formed to review the Tasmanian abalone marine biotoxin management plan in light of the results found.

The slow depuration rate is of particular concern and has ability to cause long-term disruption to harvest. Further research (particularly field work) is required to confirm the findings of this report, and will allow substantial improvements to the biotoxin management plan (see Further development below).

The report should be disseminated to all states (industry groups, Food Safety and Fisheries regulators, shellfish quality assurance programs) to highlight the risk of PST accumulation in abalone following toxic algal blooms. A submission has recently been put to Food Standards Australian New Zealand (FSANZ) by state food safety regulators to introduce a specific marine biotoxin level for abalone in the Food Standards Code. Information from this report should be made available to FSANZ for consideration.

Further development

There remain many unanswered questions from the study conducted here. Future research required in order to design an effective field monitoring program needs to focus on better understanding of:

1. the uptake and depuration rates of PST in abalone in the field;
2. residual toxin levels between blooms;
3. variability between animals at each site;
4. variability in PST profiles within and between seasons; and
5. geographic variability.

Of highest priority to improve the cost effectiveness of the current biotoxin monitoring is confirmation of the higher concentration of PST in foot tissues, better understanding of the range of profiles seen in foot tissue across blooms, and a field validation of the Neogen rapid screening tool for Blacklip Abalone foot tissue.

Chapter 10 - Extension and Adoption

The project was officially communicated to stakeholders through the following:

- Steering group meetings
  - 23rd July 2018
  - 30th April 2019
- FRDC milestone reports
  - FRDC 2017-051 (Experimental facility): 23/2/2018; 1/3/2019; 17/4/2019
  - FRDC 2017-225: 14/6/19; 11/11/19
- Abalone Council Australia (ACA) updates
  - FRDC 2017-051: Nov 2018; Feb 2019; Mar 2019
  - FRDC 2017-225: Nov 2018; March 2019; Sept 2019
- Tasmanian Abalone Council Limited (TACL) updates
  - 5/4/2019
- 2018 Field data summary report to DPIPWE and Dean Lisson 17/5/2019

The final report will be disseminated to:

- FRDC
- ACA
- TACL
- Department of Agriculture, Water and Environment
- DPIPWE – Food safety, ShellMAP and Wild Fisheries
- Australian Abalone Growers Association
- Seafood New Zealand
- New Zealand seafood safety program
- The Paua Industry Council
- State abalone fisheries management programs
- Australian Shellfish Quality Assurance Program Managers

The experimental uptake of PST will be submitted as a paper to Harmful Algae, a draft of which is presented in Chapter 3.

Project coverage

The experimental work was reported in Fish Magazine, September 2019, “Joint initiative tackles biotoxins” page 14-16.

The experimental work (in combination with similar research on Southern Rock Lobster and Pacific Oysters) was awarded the South Australian Seafood Industry Research and Development Award for 2019.

Chapter 11 - Project materials developed

A fact sheet on the project was prepared for industry distribution and is attached in Appendix 4.

A draft scientific paper on the experimental work has been prepared for submission to Harmful Algae (as presented in Chapter 3).

Chapter 12 - Appendices

Appendix 1. List of researchers and project staff

SARDI staff assisting with work at the South Australian Aquatic Biosecurity Centre

- Elliot Brown
- Jan Lee
- Geoff Holds
- Dr. Marty Deveney
- Dr. Stephen Pahl
- Grant Mann

IMAS for field sample collection and processing

- Ruari Colquhoun
- Jane Ruckert
- Michael Porteous
- David Faloon
- Simon Talbot
- Sarah-Jane Pyke
- Sowdamini Sesha Prasad
- Hugh Gray
- Lauryn Kitchener

Cawthron Institute New Zealand for marine biotoxin analysis

- Penny Harrison
- Emilie Burger

Aquafeeds Australia

- Joel Scanlon

DPIPWE for provision of ancillary information and assistance with planning

- Hilary Revill
- Grant Pullen
- Matt Bradshaw
- Megan Burgoyne
- Owen Hunt

Steering Committee Members

- Chris Izzo
- Dean Lisson
- Shelly Alderman
- Hilary Revill
- Grant Pullen
- Owen Hunt
- John Sansom
- Tom Cosentino
- Josh Fielding

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Appendix 3. PST analogues in Blacklip Abalone from the east coast of Tasmania, 2018-2019.

Analogues not detected (ND) and therefore not noted here were C3, C4, dcGTX1, dcGTX3, dcGTX4, and GTX5. Units are mg STX.2HCl equiv. kg^{-1}. FAO TEFs applied. TEFs were 0.01 (C1), 0.10 (C2), 0.20 (dcGTX2), 0.40 (GTX2), 0.60 (GTX3), 1.00 (GTX1), 0.70 (GTX4), 0.05 (GTX6), 0.05 (doSTX), 0.50 (dcNEO), 1.00 (STX), 2.00 (NEO).

Sample	Tissue	Sampling date	Origin	Block	C1	C2	dcGTX2	GTX2	GTX1	GTX4	doSTX	dcSTX	dcNEO	STX	NEO	Total PST (mg STX.2HCl eq/kg)
Aba10b,11b,12b,13b,14b	Pooled foot	8/10/18	Binalong Bay	30A	ND	ND	ND	0.009	ND	ND	0.001	0.004	ND	0.140	0.016	0.170
Aba 10b	Foot	8/10/18	Binalong Bay	30A	ND	ND	ND	0.012	ND	ND	0.000	0.004	ND	0.140	0.020	0.180
Aba 11b	Foot	8/10/18	Binalong Bay	30A	ND	ND	ND	0.009	ND	ND	0.000	0.002	ND	0.072	0.011	0.100
Aba 12b	Foot	8/10/18	Binalong Bay	30A	ND	ND	ND	0.012	ND	ND	0.001	0.006	ND	0.190	0.018	0.229
Aba 13b	Foot	8/10/18	Binalong Bay	30A	ND	ND	ND	0.007	ND	ND	0.000	0.004	ND	0.110	0.011	0.136
Aba 14b	Foot	8/10/18	Binalong Bay	30A	ND	ND	ND	ND	ND	ND	0.000	0.002	ND	0.099	0.005	0.107
Aba 46b, 47b, 48b, 49b, 50b	Pooled foot	5/11/18	Binalong Bay	30A	ND	ND	ND	0.001	ND	ND	0.001	0.002	ND	0.074	ND	0.078
Aba16b,17b,18b,19b,20b	Pooled foot	9/10/18	Georges Rocks	30A	ND	ND	ND	0.004	ND	ND	0.001	0.005	ND	0.160	0.320	0.490
Aba 16b	Foot	9/10/18	Georges Rocks	30A	ND	ND	ND	ND	ND	ND	0.000	0.008	ND	0.140	0.140	0.288
Aba 17b	Foot	9/10/18	Georges Rocks	30A	ND	ND	ND	0.004	ND	ND	0.000	0.001	ND	0.079	0.009	0.093
Aba 18b	Foot	9/10/18	Georges Rocks	30A	ND	ND	ND	0.003	ND	ND	0.001	0.002	ND	0.160	0.690	0.856
Aba 19b	Foot	9/10/18	Georges Rocks	30A	ND	ND	ND	ND	ND	ND	0.000	0.005	ND	0.140	0.019	0.164
Aba 20b	Foot	9/10/18	Georges Rocks	30A	ND	ND	ND	0.003	ND	ND	0.000	0.005	ND	0.097	0.011	0.116
Aba 56b, 57b, 58b, 59b, 60b	Pooled foot	6/12/18	Okehampton Bay	24C	ND	ND	ND	0.029	ND	ND	0.001	0.008	ND	0.260	0.042	0.347
Aba 56b	Foot	6/12/18	Okehampton Bay	24C	ND	ND	ND	0.032	ND	ND	0.003	0.013	0.007	0.440	0.095	0.594
Aba 57b	Foot	6/12/18	Okehampton Bay	24C	ND	ND	ND	0.072	ND	ND	0.003	0.022	0.017	0.500	0.087	0.717
Aba 58b	Foot	6/12/18	Okehampton Bay	24C	ND	ND	ND	0.006	ND	ND	0.001	0.006	ND	0.180	0.027	0.219
Aba 59b	Foot	6/12/18	Okehampton Bay	24C	0.000	ND	ND	0.037	ND	ND	0.003	0.019	ND	0.410	0.070	0.548
Aba 60b	Foot	6/12/18	Okehampton Bay	24C	0.000	ND	ND	0.019	ND	ND	0.001	0.005	ND	0.150	0.033	0.208

Sample	Tissue	Sampling date	Origin	Block	C1	C2	dcGTX2	GTX2	GTX1	GTX4	doSTX	dcSTX	dcNEO	STX	NEO	Total PST (mg STX.2HCl eq/kg)
Aba26b,27b,28b,29b,30b	Pooled foot	4/09/18	Okehampton Bay	24C	0.000	ND	0.001	0.058	ND	ND	0.002	0.010	ND	0.680	0.160	0.923
Aba 26b	Foot	4/09/18	Okehampton Bay	24C	0.000	ND	ND	0.018	ND	ND	0.001	0.002	ND	0.250	0.022	0.295
Aba 27b	Foot	4/09/18	Okehampton Bay	24C	ND	ND	ND	0.065	ND	ND	0.001	0.018	ND	0.650	0.320	1.074
Aba 28b	Foot	4/09/18	Okehampton Bay	24C	ND	0.000	ND	0.078	ND	ND	0.001	0.011	ND	0.500	0.062	0.680
Aba 29b	Foot	4/09/18	Okehampton Bay	24C	0.000	0.000	ND	0.049	ND	ND	0.001	0.007	ND	0.600	0.150	0.824
Aba 30b	Foot	4/09/18	Okehampton Bay	24C	0.000	0.001	ND	0.040	ND	ND	0.001	0.020	ND	0.580	0.066	0.727
Aba32b, 33b, 34b, 35b	Pooled foot	4/10/18	Okehampton Bay	24C	ND	ND	ND	0.055	ND	ND	0.002	0.011	ND	0.550	0.071	0.707
Aba 32b	Foot	4/10/18	Okehampton Bay	24C	ND	ND	ND	0.043	ND	ND	0.001	0.016	ND	0.620	0.069	0.764
Aba 33b	Foot	4/10/18	Okehampton Bay	24C	ND	0.000	ND	0.019	ND	ND	0.002	0.008	ND	0.560	0.076	0.670
Aba 34b	Foot	4/10/18	Okehampton Bay	24C	ND	ND	ND	0.052	ND	ND	0.001	0.010	ND	0.320	0.050	0.450
Aba 35b	Foot	4/10/18	Okehampton Bay	24C	ND	ND	ND	0.032	ND	ND	0.001	0.005	ND	0.350	0.026	0.426
Aba36b, 37b, 38b, 39b, 40b	Pooled foot	31/10/18	Okehampton Bay	24C	ND	ND	0.001	0.044	ND	ND	0.003	0.012	ND	0.550	0.190	0.813
Aba 36b	Foot	31/10/18	Okehampton Bay	24C	ND	ND	ND	0.023	ND	ND	0.001	0.016	ND	0.420	0.170	0.638
Aba 37b	Foot	31/10/18	Okehampton Bay	24C	ND	ND	ND	0.019	ND	ND	0.000	0.008	ND	0.260	0.076	0.363
Aba38b	Foot	31/10/18	Okehampton Bay	24C	ND	ND	ND	0.046	ND	ND	0.002	0.019	ND	0.720	0.180	0.987
Aba 39b	Foot	31/10/18	Okehampton Bay	24C	ND	ND	ND	0.021	ND	ND	0.000	0.006	ND	0.160	0.017	0.204
Aba 40b	Foot	31/10/18	Okehampton Bay	24C	ND	ND	ND	0.020	ND	ND	0.001	0.012	ND	0.240	0.100	0.381
Aba 62b, 63b ,64b, 65b, 66b	Pooled foot	11/06/19	Okehampton Bay	24C	ND	ND	ND	0.011	ND	ND	0.001	0.011	ND	0.210	0.022	0.255
Aba 62b	Foot	11/06/19	Okehampton Bay	24C	ND	ND	ND	0.005	ND	ND	ND	ND	ND	0.097	ND	0.103
Aba 63b	Foot	11/06/19	Okehampton Bay	24C	ND	ND	ND	0.013	ND	ND	0.001	0.012	ND	0.470	0.061	0.558
Aba 64b	Foot	11/06/19	Okehampton Bay	24C	ND	ND	0.001	0.009	ND	ND	0.001	0.008	ND	0.290	0.032	0.343
Aba 65b	Foot	11/06/19	Okehampton Bay	24C	ND	ND	ND	0.024	ND	ND	0.001	0.014	ND	0.520	0.056	0.626
Aba 66b	Foot	11/06/19	Okehampton Bay	24C	ND	ND	ND	0.005	ND	ND	ND	0.005	ND	0.110	ND	0.122
Aba 82b, 83b, 84b, 85b, 86b	Pooled foot	7/10/19	Okehampton Bay	24C	ND	ND	ND	0.015	ND	ND	0.007	0.015	ND	0.200	0.027	0.264
Aba 82b	Foot	7/10/19	Okehampton Bay	24C	ND	ND	ND	0.013	ND	ND	0.001	0.005	ND	0.280	0.032	0.336
Aba 83b	Foot	7/10/19	Okehampton Bay	24C	ND	ND	ND	0.012	ND	ND	0.001	0.011	ND	0.280	0.034	0.342

Sample	Tissue	Sampling date	Origin	Block	C1	C2	dcGTX2	GTX2	GTX1	GTX4	doSTX	dcSTX	dcNEO	STX	NEO	Total PST (mg STX.2HCl eq/kg)
Aba 84b	Foot	7/10/19	Okehampton Bay	24C	ND	ND	ND	0.012	ND	ND	0.001	0.005	ND	0.180	0.023	0.226
Aba 85b	Foot	7/10/19	Okehampton Bay	24C	ND	ND	ND	0.030	ND	ND	0.002	0.017	ND	0.360	0.056	0.473
Aba 86b	Foot	7/10/19	Okehampton Bay	24C	ND	ND	ND	0.022	ND	ND	0.001	0.010	ND	0.240	0.031	0.309
Aba1b, 2b, 3b, 4b, 5b	Pooled foot	5/09/18	Pirates Bay	22C	ND	ND	ND	0.008	ND	ND	0.004	0.004	ND	0.100	0.010	0.126
Aba21b, 22b, 23b, 24b, 25b	Pooled foot	11/10/18	Pirates Bay	22C	ND	ND	ND	0.005	ND	ND	0.002	0.002	ND	0.066	0.024	0.099
Aba 41b, 42b, 43b, 44b, 45b	Pooled foot	8/11/18	Pirates Bay	22C	ND	ND	ND	0.004	ND	ND	0.002	0.003	ND	0.070	0.110	0.189
Aba 41b	Foot	8/11/18	Pirates Bay	22C	ND	ND	ND	0.004	ND	ND	0.002	0.002	ND	0.076	0.130	0.213
Aba 42b	Foot	8/11/18	Pirates Bay	22C	ND	ND	ND	0.002	ND	ND	0.002	0.003	ND	0.068	0.068	0.143
Aba 43b	Foot	8/11/18	Pirates Bay	22C	ND	ND	ND	0.002	ND	ND	0.002	0.002	ND	0.082	0.210	0.298
Aba 44b	Foot	8/11/18	Pirates Bay	22C	ND	ND	ND	0.003	ND	ND	0.003	0.004	ND	0.100	0.130	0.241
Aba 45b	Foot	8/11/18	Pirates Bay	22C	ND	ND	ND	0.008	ND	ND	0.006	0.009	ND	0.180	0.450	0.653
Aba 10a	Viscera	8/10/18	Binalong Bay	30A	ND	ND	ND	ND	ND	ND	0.000	0.001	ND	0.037	ND	0.038
Aba 11a	Viscera	8/10/18	Binalong Bay	30A	ND	ND	ND	0.005	ND	ND	ND	0.001	ND	0.027	ND	0.033
Aba 12a	Viscera	8/10/18	Binalong Bay	30A	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.023	ND	0.023
Aba 13a	Viscera	8/10/18	Binalong Bay	30A	ND	ND	ND	ND	ND	ND	ND	0.001	ND	0.019	ND	0.025
Aba 14a	Viscera	8/10/18	Binalong Bay	30A	ND	ND	ND	ND	ND	ND	0.000	ND	ND	0.038	ND	0.042
Aba 46a	Viscera	5/11/18	Binalong Bay	30A	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.012	ND	0.012
Aba 47a	Viscera	5/11/18	Binalong Bay	30A	ND	ND	ND	ND	ND	ND	0.000	ND	ND	0.034	ND	0.036
Aba 48a	Viscera	5/11/18	Binalong Bay	30A	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.038	ND	0.038
Aba 49a	Viscera	5/11/18	Binalong Bay	30A	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.017	ND	0.017
Aba 50a	Viscera	5/11/18	Binalong Bay	30A	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.019	ND	0.019
Aba 16a	Viscera	9/10/18	Georges Rocks	30A	ND	ND	ND	ND	ND	ND	0.000	0.002	ND	0.060	0.024	0.086
Aba 17a	Viscera	9/10/18	Georges Rocks	30A	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.017	ND	0.017
Aba 18a	Viscera	9/10/18	Georges Rocks	30A	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.050	0.044	0.094
Aba 19a	Viscera	9/10/18	Georges Rocks	30A	ND	ND	ND	ND	ND	ND	0.000	ND	ND	0.034	ND	0.034
Aba 20a	Viscera	9/10/18	Georges Rocks	30A	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.017	ND	0.017
Aba 56a	Viscera	6/12/18	Okehampton Bay	24C	ND	ND	ND	0.006	ND	ND	ND	0.003	ND	0.051	ND	0.063

Sample	Tissue	Sampling date	Origin	Block	C1	C2	dcGTX2	GTX2	GTX1	GTX4	doSTX	dcSTX	dcNEO	STX	NEO	Total PST (mg STX.2HCl eq/kg)
Aba 57a	Viscera	6/12/18	Okehampton Bay	24C	ND	ND	ND	0.017	ND	ND	ND	0.004	ND	0.095	ND	0.121
Aba 58a	Viscera	6/12/18	Okehampton Bay	24C	0.000	ND	ND	0.005	ND	ND	ND	0.002	ND	0.027	ND	0.034
Aba 59a	Viscera	6/12/18	Okehampton Bay	24C	ND	ND	ND	0.012	ND	ND	0.001	0.005	ND	0.100	ND	0.123
Aba 60a	Viscera	6/12/18	Okehampton Bay	24C	ND	ND	ND	0.013	ND	ND	0.000	ND	ND	0.094	ND	0.110
Aba 26a	Viscera	4/09/18	Okehampton Bay	24C	0.000	0.004	ND	0.033	ND	ND	0.000	0.001	ND	0.098	ND	0.179
Aba 27a	Viscera	4/09/18	Okehampton Bay	24C	0.000	0.002	ND	0.031	ND	ND	0.001	0.006	ND	0.250	ND	0.327
Aba 28a	Viscera	4/09/18	Okehampton Bay	24C	0.001	0.004	ND	0.063	ND	ND	0.001	0.006	ND	0.280	ND	0.413
Aba 29a	Viscera	4/09/18	Okehampton Bay	24C	0.001	0.004	ND	0.044	ND	ND	0.000	ND	ND	0.093	ND	0.184
Aba 30a	Viscera	4/09/18	Okehampton Bay	24C	0.000	0.002	ND	0.015	ND	ND	0.001	0.004	ND	0.170	ND	0.220
Aba 32a	Viscera	4/10/18	Okehampton Bay	24C	0.000	0.002	ND	0.052	ND	ND	0.000	0.004	ND	0.190	ND	0.294
Aba 33a	Viscera	4/10/18	Okehampton Bay	24C	0.000	0.002	ND	0.035	ND	ND	0.000	0.003	ND	0.160	ND	0.249
Aba 34a	Viscera	4/10/18	Okehampton Bay	24C	0.000	0.003	ND	0.037	0.010	ND	0.000	0.002	ND	0.100	ND	0.180
Aba 35a	Viscera	4/10/18	Okehampton Bay	24C	0.000	0.001	ND	0.036	ND	ND	0.000	0.002	ND	0.140	ND	0.208
Aba 36a	Viscera	31/10/18	Okehampton Bay	24C	ND	ND	ND	0.012	ND	ND	0.000	0.003	ND	0.120	ND	0.135
Aba 37a	Viscera	31/10/18	Okehampton Bay	24C	ND	ND	ND	0.004	ND	ND	ND	ND	ND	0.024	ND	0.028
Aba 38a	Viscera	31/10/18	Okehampton Bay	24C	ND	ND	ND	0.018	ND	ND	0.001	0.003	ND	0.130	ND	0.161
Aba 39a	Viscera	31/10/18	Okehampton Bay	24C	0.000	ND	0.002	0.015	ND	ND	0.000	0.003	ND	0.096	ND	0.124
Aba 40a	Viscera	31/10/18	Okehampton Bay	24C	ND	ND	ND	0.008	ND	ND	ND	0.002	ND	0.078	ND	0.097
Aba 82a, 83a, 84a, 85a ,86a	Pooled viscera	7/10/19	Okehampton Bay	24C	ND	0.003	ND	0.015	0.013	ND	0.002	ND	ND	0.1	ND	0.153
Aba 1a	Viscera	5/09/18	Pirates Bay	22C	ND	ND	ND	ND	ND	ND	0.001	0.003	ND	0.052	ND	0.056
Aba 2a	Viscera	5/09/18	Pirates Bay	22C	ND	ND	ND	0.005	ND	ND	0.001	0.001	ND	0.032	ND	0.040
Aba 3a	Viscera	5/09/18	Pirates Bay	22C	ND	ND	ND	ND	ND	ND	0.001	0.003	ND	0.031	ND	0.035
Aba 4a	Viscera	5/09/18	Pirates Bay	22C	ND	ND	ND	ND	ND	ND	0.001	0.002	ND	0.025	ND	0.032
Aba 5a	Viscera	5/09/18	Pirates Bay	22C	ND	ND	ND	ND	ND	ND	0.001	0.002	ND	0.024	ND	0.026
Aba 21a	Viscera	11/10/18	Pirates Bay	22C	ND	ND	ND	ND	ND	ND	0.001	0.001	ND	0.016	ND	0.017
Aba 22a	Viscera	11/10/18	Pirates Bay	22C	ND	ND	ND	0.003	ND	ND	0.001	0.001	ND	0.055	ND	0.060
Aba 23a	Viscera	11/10/18	Pirates Bay	22C	ND	ND	ND	ND	ND	ND	0.000	ND	ND	0.014	ND	0.014

Sample	Tissue	Sampling date	Origin	Block	**C1**	**C2**	**dcGTX2**	**GTX2**	**GTX1**	**GTX4**	**doSTX**	**dcSTX**	**dcNEO**	**STX**	**NEO**	**Total PST (mg STX.2HCl eq/kg)**
Aba 24a	Viscera	11/10/18	Pirates Bay	22C	0.000	ND	ND	ND	ND	ND	0.001	0.001	ND	0.030	ND	0.032
Aba 25a	Viscera	11/10/18	Pirates Bay	22C	ND	ND	ND	ND	ND	ND	0.001	0.001	ND	0.027	ND	0.029
Aba 41a	Viscera	8/11/18	Pirates Bay	22C	ND	ND	ND	ND	ND	ND	0.000	ND	ND	0.022	ND	0.022
Aba 42a	Viscera	8/11/18	Pirates Bay	22C	ND	ND	ND	ND	ND	0.003	0.000	ND	ND	0.026	ND	0.033
Aba 43a	Viscera	8/11/18	Pirates Bay	22C	ND	ND	ND	ND	ND	ND	0.001	ND	ND	0.027	ND	0.028
Aba 44a	Viscera	8/11/18	Pirates Bay	22C	ND	ND	ND	ND	ND	ND	0.000	ND	ND	0.014	ND	0.014
Aba 45a	Viscera	8/11/18	Pirates Bay	22C	ND	ND	ND	ND	ND	ND	0.001	ND	ND	0.035	ND	0.036

Appendix 4. Industry fact sheet

Improving risk management of paralytic shellfish toxins in the Blacklip Abalone (*Haliotis rubra rubra*)

Supported by funding from the FRDC on behalf of the Australian Government

Background

Australian abalone have recently been impacted by PST producing *Alexandrium* blooms, resulting in prolonged harvest closures that are costly to monitor and manage. Until 2017, elevated levels of PST in abalone had only been reported during *Gymnodinium* blooms in southern Tasmania, with PST levels of abalone exposed to *Alexandrium* blooms on Tasmania's east coast and in NSW not exceeding 0.3 mg STX.2HCl equiv/kg. In the 2017 *Alexandrium* bloom on the Tasmanian east coast, however, abalone were found to exceed the bivalve regulatory limit at 1.2 mg STX equiv/kg.

The closure of abalone harvest zones is currently based on information arising from the bivalve shellfish monitoring program, with more than 25 Tasmanian block closures to date, some of which have been continuous for over 2 years. This conservative approach has been adopted due to both the paucity of information on PST accumulation in abalone exposed to *Alexandrium* blooms, the cost of PST testing ($500 per sample) and the high consequence of any PST detection in overseas markets.

Objectives

This project has been developed to improve the risk management of PST in abalone, particularly to reduce the economic impact on fishers by:

1. Determination of PST uptake by abalone from two routes: exposure to *A. catenella* and contaminated feed.
2. Determination of relative risk of PST accumulation in abalone compared to Southern Rock Lobster at two field sites.
3. Validation of rapid test kits for PST analysis in abalone foot and viscera tissues.

Methodology

1. Wild abalone will be exposed to *A. catenella* microalgal cultures at the South Australian Aquatic Biosecurity Centre in a tank trial run by SARDI (2018). Abalone will be fed with either non-toxic control feed or specially formulated PST containing pellets. After 4 weeks of continuous exposure, PST levels in abalone viscera, epipodium and foot tissues will be analysed to determine PST accumulation.
2. Two field sites will be selected from sites currently being monitored for PST in Southern Rock Lobster (FRDC 2017-051). This will maximise environmental data collected, provide a reference point as to the level of bloom development, and provide information on the relative risk of PST in lobster and abalone. Abalone will be sampled from 2 different blocks during 2017-2019 by IMAS staff. These blocks will have to remain closed to commercial harvest during this period to avoid any market consequences in case of PST levels within the block exceeding the regulatory limit.
3. PST contaminated samples generated during this project will be used by SARDI staff (2019) to determine if the rapid PST test kits currently being used by the oyster industry can be used as a screening test for PST in abalone foot and viscera tissues. The test kit will be challenged against the two different PST profiles found during *Alexandrium* and *Gymnodinium* blooms.

Output

- Determination of PST uptake by *H. rubra rubra* through diet and *Alexandrium* exposure.
- Field data relating PST levels in *H. rubra rubra* to *Alexandrium cell* concentrations and PST in lobster.
- Validation of rapid test kits for PST analysis in abalone foot and viscera.
- A report to regulators, outlining the research completed and results.

Outcomes

- Regulators and industry able to make an updated determination on the relative risk of accumulation of PST from *A. catenella* blooms on the east coast of Tasmania relative to algal cell numbers and toxin levels in bivalve shellfish and rock lobster, reducing harvest closure times.
- Improvements to the Tasmania biotoxin management plan for abalone with a fundamental change to the ability to monitor during bloom period.