Extending biotoxin capability and research in Australia through development of an experimental biotoxin contamination facility to target industry relevant issues

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Abbreviations

ACA	Abalone Council of Australia
ASCRC	Australian Seafood Co-operative Research Centre
ASQAAC	Australian Shellfish Quality Assurance Advisory Council
kL	Kilo-litres
OT	Oysters Tasmania
POMS	Pacific Oyster Mortality Syndrome
PST	Paralytic shellfish toxin
SAABC	South Australian Aquatic Biosecurity Centre
SRL	Southern Rocklobster Ltd
STX	Saxitoxin
DAWR	Department of Agriculture, Water and Resources

Executive Summary

What the report is about

A short-term experimental biotoxin contamination facility was set up at Roseworthy, South Australia, to examine the uptake and depuration of marine biotoxins from one of the most toxic dinoflagellates known: *Alexandrium catenella*. Over the period of one year, SARDI's Food Sciences Seafood Sub-program successfully conducted several studies aimed at underpinning public health, market access or fisheries management in three iconic Australian seafood species impacted by recurrent blooms of *A. catenella*: Southern Rock Lobster, Blacklip Abalone and Pacific Oysters. Issues specific to each industry were examined in a cost effective manner through sharing facility, staffing and bulk algal culturing costs. This report contains a brief description of the aquaculture systems developed at the SAABC, the toxic algal culturing system, and the collection of toxic tissues that occurred during the experiments. Details of each experiment are contained in the relevant project reports.

Background

In 2012, a bloom of *A. catenella* (then known as *Alexandrium tamarense* Group 1) developed for the first time along the entire length of the east coast of Tasmania, and showed extreme toxicity. This bloom caused the closure of shellfisheries (oysters, mussels, scallops, and clams), Southern Rock Lobster, Giant Crab and abalone fisheries, due to the accumulation of unprecedented levels of paralytic shellfish toxin (PST) in the edible tissues of these animals. The high level of toxicity meant that previous biotoxin risk management guidelines and strategies did not adequately address the risk posed by this organism. Despite the considerable research conducted since 2012, critical knowledge gaps still remain, and thus public health and market access risk management remains an expensive and conservative process.

In 2016 the Australian Seafood Cooperative Research Centre (ASCRC) released a call for research proposals that could benefit multiple seafood industries. A proposal was developed in consultation with the Abalone Council of Australia (ACA), Oysters Tasmania (OT) and Southern Rocklobster Limited (SRL) to develop a biotoxin contamination centre in a biosecure facility. The centre would be able to conduct research directed at resolving some of the knowledge gaps remaining around PST accumulation and depuration in the high value Southern Rock Lobster, Blacklip Abalone and Pacific Oyster industries.

Aims/objectives

The centre aimed to provide effective use of resources in this expensive field of research by sharing infrastructure costs, with each industry contributing additional funds to resolve specific issues of their choice. In addition, the centre would build research capability in this space, and encourage collaboration between current Australian researchers in this field. An additional aim was to develop a library of contaminated material for use in future research projects and for quality assurance and quality control activities. This will be particularly important for any industry run programs, such as validation of rapid test kits to indicate toxin levels.

Methodology

The South Australian Aquatic Biosecurity Centre (SAABC) at Roseworthy was identified as a suitable aquaculture facility, and half the area within was hired for one year. A custom-built system was installed to house Southern Rock Lobster and Blacklip Abalone in individual tanks (maximum 80 animals at once). Conventional tanks were used to replicate small-scale depuration tanks for the Pacific Oyster experiment. The centre was staffed with a full time aquaculture technician, supported by casual staff to provide 7 day coverage whilst animals were in the centre.

The *A. catenella* strain AT.TR/F (originally isolated from Triabunna in November 2012 by Chris Bolch) was obtained from the Institute for Marine and Antarctic Studies, Hobart. The microalga was grown in semi-

continuous batch cultures under growth conditions tailored to yield maximum biomass and PST cell quotas. A unique CO_2 delivery system was installed to increase growth rates and achieve maximum cell density.

Projects were developed with SRL, ACA, and the Tasmanian oyster industry that utilised the varied expertise of collaborating scientists involved in the project. Whilst the issues addressed in each project were different, all required the culturing of bulk material of toxic algae, modern aquaculture infrastructure, daily animal husbandry and effective biosecurity measures: shared support services covered by this overarching grant.

Results/key findings

The space hired in the SAABC was ideal for the projects conducted. The centre was occupied from February to December 2018, during which time four experiments were successfully completed.

1. Southern Rock Lobster haemolymph experiment: lobster were fed with contaminated mussels to examine the transfer of PST into Southern Rock Lobster haemolymph and to look at the impact of toxin accumulation on lobster vigour: http://www.frdc.com.au/project/2017-086.

2. Southern Rock Lobster uptake from direct algae exposure: lobster were exposed to high cell densities of *A. catenella* to determine if they could accumulate PST toxins in the hepatopancreas, and if exposure to toxic cells has any impact on behavioural immunological, physiological or feed measures: http://www.frdc.com.au/project/2017-086.

3. Pacific Oyster depuration experiment: oysters were contaminated with PST toxins and depurated to determine the experimental depuration constant and estimate the length of time required for commercial depuration.

4. Blacklip Abalone PST uptake experiment: Blacklip Abalone were maintained in the aquaculture system for four weeks, to determine whether they could accumulate PST from either consumption of toxic pellets, or through direct exposure to toxic *A. catenella* cells: http://www.frdc.com.au/project/2017-225.

The microalgal culturing system developed for the SAABC greatly refined conventional culturing techniques to yield optimal growth conditions for the Tasmanian PST producing *A. catenella* strain. Depending on the experimental requirements, the system proved capable of supplying both high volumes of algal culture (700 L at a time in the oyster project) or continuous high-density microalgal cultures through staggered culturing (up to 50,000 cells mL^{-1} in the lobster and abalone projects). Furthermore, it allowed for the growth and subsequent harvest of 350 L of algal culture between major experimental periods to prepare concentrated PST extracts for abalone feed pellet production.

During the course of the experiments, significant volumes of contaminated tissues were collected or created. These include: approximately 100kg of Blue Mussel (*Mytilus galloprovincialis)* in shell, 2.5 kg Pacific Oyster (*Crassostrea gigas*) whole tissue homogenate, 3.5kg Southern Rock Lobster (*Jasus edwardsii*) hepatopancreas and 2.7 kg of Blacklip Abalone (*Haliotis rubra rubra*) tissues. PST concentrations within these tissues are up to 9.0 mg STX.2HCl equiv kg^{-1}.

Implications for relevant stakeholders

The development of a temporary biotoxin contamination facility enabled several industries to conduct studies in this expensive field of research concurrently, providing a cost-effective use of resources. The ASCRC investment of $350,000 was leveraged by a further $295,599 from industry and Commonwealth funds, enabling a significant body of work to be conducted in a short period of time. The cost-sharing model used here could be reproduced to address other cross-sectoral issues. Potential examples where this could be useful would be:

- Investigation of the accumulation of other marine biotoxins or chemical contaminations with food safety/market access concern

- Studies on the fate of agricultural and veterinary chemicals as part of registration requirements

- Improvement of quality aspects of seafood as related to transport conditions.

Research collaborations were progressed in this work between IMAS, SARDI and Cawthron Institute. These are particularly relevant as the marine biotoxin issues impact seafood in many Australian states and New Zealand. Leveraging and building expertise in both countries allows researchers to combine resources without duplicating expertise.

The research methods developed in this work will benefit future research in this field. In particular the successful use of CO_2 to improve the growth of *A. catenella* provides opportunities to conduct research requiring large quantities of toxic cells and/or toxins.

This project has built capability in Australia in the biotoxin field, particularly in areas of direct application to the seafood industry. Every research project undertaken in the SAABC will continue following the closure of the experimental centre, with the outcomes being extended into the field.

Recommendations

The implications of the work conducted in the biosecurity centre will be discussed in the respective final reports of each individual study. A common theme across all projects is the consideration of validating experimental outcomes in the field.

Future cross-sectorial projects should give consideration to the time-frames required to develop and implement experimental and field work. This project was conducted over 18 months, however additional time would have been beneficial for the multi-stakeholder negotiations, contracting, building and dismantling of new systems.

Keywords

Cross-sector project, capability building, collaboration, marine biotoxins, paralytic shellfish toxin, *Jasus edwardsii*, *Haliotis rubra rubra*, *Crassostrea gigas*, *Alexandrium catenella, Alexandrium tamarense* Group 1

Introduction

The Australian seafood industry was first impacted by marine biotoxins in the late 1980's when blooms of *Gymnodinium catenatum* in the D'Entrecasteaux Channel and Huon River, Tasmania, caused the long-term closure of many shellfish farms due to the accumulation of paralytic shellfish toxins (PST) produced by these blooms (Hallegraeff 1992). Since then, the management of shellfish growing areas impacted by biotoxin producing algal blooms has been undertaken by state controlled shellfish quality assurance programs. These programs have followed guidance produced by the Australian Shellfish Quality Assurance Advisory Committee (ASQAAC) (ASQAP 2016).

In 2012, a bloom of *Alexandrium catenella* (then referred to as *Alexandrium tamarense* Group 1 (Litaker et al. 2018) developed to significant levels for the first time along the entire east coast of Tasmania and showed extreme toxicity. This bloom caused the closure of shellfisheries (oysters, mussels, scallops, and clams), Southern Rock Lobster, Giant Crab, and abalone fisheries, due to the accumulation of unprecedented levels of PST in these seafoods (Campbell et al. 2013). It was the first time in Australia that lobsters had been shown to contain elevated levels of biotoxins. Since 2012, there has been significant research into the cause, distribution and potential management methods of these blooms (Campbell et al. 2013, McLeod et al. 2014, Dorantes-Aranda et al. 2017, Madigan et al. 2018a, McLeod et al. 2017, Dorantes-Aranda et al. 2018, Hallegraeff et al. 2018, Madigan et al. 2018b, Turnbull et al. 2018a, Turnbull et al. 2018b, Turnbull et al. 2018c, Madigan et al. *In preparation*), as the high level of toxicity meant that previous guidelines and management strategies did not adequately address the public health and market access risks posed by this organism.

Despite the research gains made, PST accumulation from *A. catenella* blooms is an ongoing issue across a broad range of seafood species. There are critical knowledge gaps in understanding: uptake pathways for the toxins; the potential for using rapid test kits for PST analysis; and the potential for depuration or processing to reduce toxin content. There is a need for access to contaminated materials to resolve some of these issues.

In 2016 the Australian Seafood Cooperative Research Centre (ASCRC) released a call for research proposals that could benefit multiple seafood industries. A proposal was developed in consultation with the Abalone Council of Australia (ACA), Oysters Tasmania (OT), and Southern Rocklobster Limited (SRL) to develop a biotoxin contamination centre in a biosecure facility, that would be able to conduct research directed at resolving some of the knowledge gaps remaining around PST accumulation and depuration in the high value Southern Rock Lobster (*Jasus edwardsii*), Blacklip Abalone (*Haliotis rubra rubra*) and Pacific Oyster (*Crassostrea gigas*) industries. The centre aimed to provide effective use of resources in this expensive field of research by sharing infrastructure costs, with each industry contributing additional funds towards resolving the specific issue of their choice. In addition, the centre would build research capacity in this space, and encourage collaboration between current Australian researchers in this field. An additional aim was to develop a library of contaminated material for use in future research projects and for quality assurance and quality control activities in this field. This will be particularly important for any industry run programs, as proposed with the rapid test kits.

The following research questions that could be supported through this facility were identified and progressed:

- Can the cost effective Neogen rapid test kit for PST be adapted for Southern Rock Lobster hepatopancreas, significantly reducing monitoring costs?
- Are PST levels in Southern Rock Lobster haemolymph indicative of levels in the hepatopancreas, and therefore usable for non-destructive sampling?

- Can we develop a model describing depuration time of PST in Pacific Oysters in relation to temperature, salinity, flow rate and stocking densities to allow contaminated product to be depurated commercially prior to Pacific Oyster Mortality Syndrome (POMS) season?
- Is there any physiological impact of blooms on Southern Rock Lobster?
- Is there a significant risk for PST uptake from *A. catenella* for abalone?

Objectives

Objective 1. Establish key infrastructure (a biotoxin contamination facility) to be utilised concurrently by multiple industries to resolve specific and varied issues related to biotoxins.

Objective 2. Support future research and quality assurance programs through provision of a library of contaminated materials.

Objective 3. Extend capability in biotoxin research in Australia.

Objective 4. Resolve at least one biotoxin related issue for each of the oyster, abalone and Southern Rock Lobster industries.

Method

Projects enabled by this capability development grant

During the development of the project proposal, consultation occurred with each industry group to determine potential specific projects that could be conducted in the centre. Following the success in obtaining the ASCRC grant these projects were costed and presented in full to SRL, ACA, and the Tasmanian oyster industry. Projects with the SRL and ACA were progressed through the FRDC, whilst the Pacific Oyster project was contracted directly to two oyster companies (Tas Prime Oysters and ACA Aquaculture) and Oysters Tasmania.

SRL were interested in investigating a non-destructive method for sampling PST in Southern Rock Lobsters, and determining PST uptake from direct exposure to toxic *A. catenella* cells. During these experiments the PST impact on lobster health would also be determined and tissues provided for validation of a rapid test kit for PST analysis. ACA were interested in improving knowledge on the uptake pathways and rates of *A. catenella* toxins, specifically in Blacklip Abalone (*Haliotis rubra rubra*), and in a provision of toxic materials for rapid test kit validation. The oyster industry desired a pilot depuration trial to determine the commercial viability of toxin depuration from Pacific Oysters during bloom periods.

Experimental Research Facility

The South Australian Aquatic Biosecurity Centre (SAABC) at Roseworthy, South Australia, was identified as a suitable facility, and half the area was hired for one year. The infrastructure at the aquaculture facility includes saltwater tanks of 35 kL storage capacity, reticulated fresh-water, air-conditioning, air-blowers, and wastewater treatment of heating to adjustable settings followed by UV sterilisation, with 35 kL waste storage. Aquaculture tanks of 5 kL or 500L were available. The air-blowers, air-conditioning, and plumbing were controlled by an automated system with alarms. Clean saltwater was transported from SARDI Aquatic Sciences at West Beach in 24 kL tankers, and treated wastewater returned to the same facility in identical tankers, sanitised between uses.

The centre was staffed with a fulltime Aquaculture Technician who was responsible for developing and maintaining biofilters, assisting to develop the aquaculture systems used for the experiments, algal subculturing, animal husbandry, and assisting with experiments. Casual staff were employed as needed, such that two staff were usually present when animals were housed in the facility. Three scientific staff were employed on a part time basis. Laboratory and office space were provided by SARDI at the nearby JS Davies Building, and microbiological culturing facilities were provided by the University of Adelaide at the adjacent Veterinary Diagnostic Laboratory.

Details of the experimental method for each project will be provided in the respective project reports. This report contains a brief description of the aquaculture systems developed at the SAABC, the toxic algal culturing system, and the collection of toxic tissues that occurred during the experiments.

Aquaculture systems

The Southern Rock Lobster and Blacklip Abalone experiments were conducted in an aquaculture system built specifically to house animals individually in tanks in either flow-through or static systems (Figure 1), based on the system previously pioneered by the SARDI Seafood group to investigate biotoxin uptake in Southern Rock Lobster (Madigan et al. 2018a). This system consisted of rows of abalone raceways set out in pairs. Each raceway was supplied with recirculating freshwater that collected in a 150 L sump, and was chilled to approximately 13 °C. Each raceway contained 5 lobster tanks of 30 L. For the flow through system, the tanks were drip fed with salt-water from a tank

containing 3 kL of chilled salt-water, at a rate of 3-10 L hr^{-1}, and waste collected from an overflow. For the static systems the tanks were emptied each day by siphoning, and re-filled directly from the chilled salt-water sump. For the Blacklip Abalone experiment the volume of each tank was reduced to 20 L. The water temperature was controlled by adjusting the air-conditioning, fresh water and/or salt-water chiller. Each tank was supplied with air, delivered through a sponge biofilter. Biofilters were pre-conditioned in a 5 kL tank containing approximately 1 kL salt-water over 6 weeks using cold adapted marine microbes for nitrification (*Nitrosomonas* spp.) and denitrification (*Nitrobacter* spp.) supplied by Baseline, QLD. The biofilter tank was maintained through regular additions of ammonia throughout all experiments, and contained spare sponge filters in case replacements were necessary.

Figure 1: Aquaculture system used to house Southern Rock Lobster and Blacklip Abalone

The Pacific Oyster depuration work was conducted in 500 L tanks in a system set up to replicate the conditions of commercial depuration for *E. coli* (Figure 2). Diploid Pacific Oysters, were held in two baskets, each of which was divided into four subsections to allow appropriate random sampling. A corrugated plastic sheet was placed horizontally and used as a baffle between the two baskets. A vertical water manifold and an air inlet was used for water circulation and aeration. A canister filter containing biological media and activated charcoal was used to help maintain optimum conditions for the recirculating water in the housing tank. A water chiller was plumbed in-line after the canister filter to maintain water temperature at 13 °C. The flow rate was maintained at approximately 2000 L hr^{-1}.

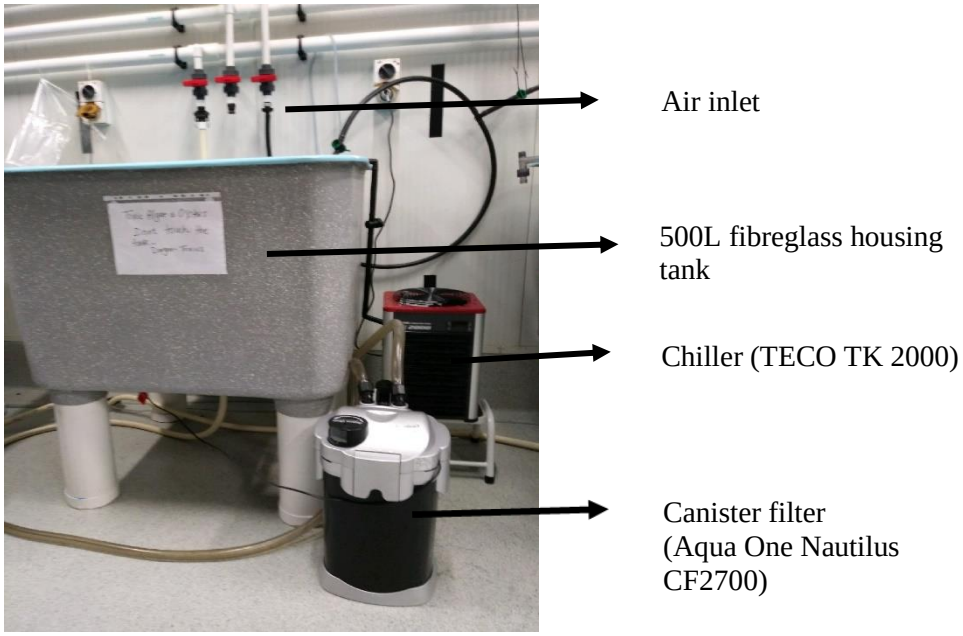

Figure 2: Holding tank, canister filter and chiller set up for Pacific Oyster depuration

Culturing of algae to use in contamination projects and determination of toxicity

The microalga *A. catenella* strain AT.TR/F was obtained from the algal culture collection at Institute for Marine and Antarctic Studies, Hobart. The strain was isolated from Triabunna, Tasmania during the 2012 bloom event by Chris Bolch (University of Tasmania).

Detailed risk assessments (Appendix 3) were conducted and extensive standard operating procedures (SOPs) developed to ensure safe handling of the PST containing algal material and guarantee containment of microalgal cultures within the SAABC. All staff were trained in the relevant procedures and protocols strictly adhered to.

The microalga was grown in batch cultures contained within 15 L carboys under growth conditions tailored to yield maximum biomass and PST cell quotas (Figure 3). The seawater growth medium was substituted with modified GSe nutrient concentrations and light supplied through a custom array of low temperature LEDs. The unique design of the carboy top allowed for sterile subsampling to monitor algal growth as well as aeration with either ambient air (low density cultures) or CO_2 (high density experimental cultures). A custom CO_2 delivery system was designed to control not only CO_2 concentration (1.5-2.5% v/v), but also timing of CO_2 addition (coupled to 12:12 h light: dark cycle to avoid acidification of cultures in the dark), as well as individual flow control to each carboy (flow was increased proportional to microalgal cell density).

Figure 3: Algal cultures growing in the SAABC

Subsamples of the microalgal cultures were routinely taken to ascertain PST concentration and composition. Samples were prepared by concentrating microalgal cultures through centrifugation to produce cell fragment free PST extracts that were analysed via liquid chromatography–mass spectrometry (LC-MS) at the Cawthron Institute, New Zealand.

Waste materials arising from the work with *Alexandrium* cultures were treated and disposed of as specified in the biosecurity SOPs that were specifically developed for the SAABC. Large volume waste (up to 24 kL/week) from experimental exposures was UV treated, stored in a 35 kL tank at the SAABC and chlorinated for 24 h before transport from the SAABC to SARDI Aquatic Sciences at West Beach, where it underwent secondary UV and chlorination treatments before discharge.

Macrophyte culture

In addition to the microalga, seven species of South Australian macroalgae were successfully maintained at the SAABC. Predominantly cultivated to wean wild caught Blacklip Abalone onto experimental feeds, six of the seven macrophyte species were opportunistically exposed to excess *Alexandrium* culture. The main aim of the experiment was to trial the feasibility of extending the application of the LC-MS PST analytical technique to seaweed extracts for assessment of PST uptake by macrophytes as a potential vector for Blacklip Abalone PST uptake.

Collection of contaminated tissues

Two experiments were conducted on the transfer of PST between Southern Rock Lobster tissues, following consumption of toxic mussels and direct exposure to algal cells. Toxic Blue Mussels (*Mytilus galloprovincialis*) were obtained from Great Oyster Bay, harvested on 07/09/2017 during an *A. catenella* bloom, and delivered to SARDI under a biosecurity permit. Two pooled samples of 12 mussels each were homogenised and sent to Cawthron Institute for PST analysis. The mussels were stored in the shell at -20 °C. Following the experiments, lobsters were euthanised in iced slurry and dissected. The hepatopancreas was collected from every animal studied, homogenised, and segregated into two samples – one for PST analysis and one for potential future use in other research projects. Haemolymph was also collected from each lobster in the first experiment (n=76) and likewise split into two samples. In addition, antennal glands, gills, foregut and hindgut samples were collected from each animal in both experiments, and the brain and heart also collected from the algal exposure experiment. All tissues were stored at -80 °C.

The study on Pacific Oysters involved a depuration experiment that included a pilot trial using 20 dozen Pacific Oysters to ensure oysters could be contaminated to appropriate toxin levels. During the pilot trial, 215 L of *A. catenella* culture, ranging from 2,250 to 10,000 cells mL^{-1}, was added to the oyster tanks. Water samples were regularly taken during and after the trial to determine PST uptake. The remaining oysters were shucked, the pooled tissue homogenised and analysed for PST. All tissues were stored at -80 °C.

The experiment examining the uptake of PST in Blacklip Abalone exposed the abalone to toxins through toxic feed pellets and direct exposure. Following the experiment the animals (n = 35) were dissected into viscera and foot samples, with the foot further separated into meat and epipodium. As with the Southern Rock Lobster tissues, each tissue sample was homogenised and segregated into a PST sample for analysis and tissue for further research. All tissues were stored at -80 °C.

Results

Experimental Research Facility

The space existing in the SAABC facility was ideal for the projects conducted. The centre was occupied from February to December 2018. Other research projects were being undertaken in the facility at the same time, but did not impinge on any operations. Additional floor space was used for the two 5 kL tanks containing chilled saltwater and the conditioning biofilters.

Significant saltwater volume was required to maintain nutrient water quality for the first Southern Rock Lobster experiment, as an early close to the lobster fishing season meant that biofilters were not adequately conditioned prior to lobsters arriving. For a short period 6 kL of water per day was used, necessitating tanker movements twice a week. This put pressure on the waste-water management system, and is likely the upper volume of saltwater usage possible at the centre.

Whilst at the centre several power failures occurred due to storms, resulting in failures in the airblowers, waste pumps and air conditioning, automated alarm systems were in place to monitor this. No impact was observed on the experiments, with the exception of one airblower failure of approximately 8 hours that resulted in the death of one lobster. At no time was any untreated waste released from the centre.

Algal culturing

The microalgal culturing system developed for the SAABC greatly refined conventional culturing techniques through the addition of controlled CO_2 flows, to yield optimal growth conditions for the Tasmanian PST producing *A. catenella* strain (Figure 3). Depending on the experimental requirements, the system proved capable of supplying both high volumes of algal culture (700 L at a time in the Pacific Oyster project) and continuous high density microalgal cultures through staggered culturing (up to 50,000 cells mL^{-1} in the Southern Rock Lobster and Blacklip Abalone projects). Furthermore, it allowed for the growth and subsequent harvest of 350 L of algal culture between major experimental periods to prepare concentrated PST extracts for abalone feed pellet production.

Under the growth conditions at the SAABC, the cultured *A. catenella* strain (AT.TR/F) yielded an average PST cell quota of 11.8 pg STX.2HCL equiv. $cell^{-1}$, in line with other estimates of toxicity of this culture, but well below that generally seen in the field (unpublished UTAS data). As expected from previous analysis of Tasmanian field samples, the typical toxin profile of this strain was dominated by gonyautoxin (GTX) 1&4 (80%) and C1&2 (12%), with some neosaxitoxin detected (4%).

Toxic materials in storage for future use

During the experiments conducted at the SAABC several contaminated tissues were either obtained or created. In some cases these materials were used in the course of the experiments, or for PST analyses. The details of the tissues remaining in storage are given in Table 1. The *M. galloprovincialis* are held at -20 ˚C whereas all other tissues are held at -80 ˚C.

Table 1: Details of toxic materials held in storage at for future work.

Species	**Tissue**	**Toxin level** **(mg STX.2HCl equiv kg^{-1})**	**Approx weight**
Mytilus galloprovincialis (Blue Mussel)	Whole animal in shell	5.6	100 kg
Jasus edwardsii (Southern Rock Lobster)	Hepatopancreas	Samples ranging from 0 – 9.0	123 pots of approx. 30 g each. Currently in use at UTAS for Neogen rapid PSP test kit validation but there will be some left over.
Jasus edwardsii (Southern Rock Lobster)	Antennal gland, hind gut, foregut	Unknown – yet to be analysed	123 pots of 5-10 g each
*Crassostrea gigas** (Pacific Oyster)	Whole animal	3.1	2 kg in bulk storage 100 pots dispensed in 5 g lots for industry QA/QC program
Haliotis rubra rubra (Blacklip Abalone)	Viscera, foot, epidermis	Unknown – yet to be analysed	1 kg viscera, 400 g epipodium and 1.3 kg of foot tissue

**C. gigas* homogenate is POMS free, and therefore suitable for all Tasmanian growing areas – a limitation of current quality assurance samples.

In addition to the tissues above, Southern Rock Lobster haemolymph samples from 30 control animals taken immediately after euthanising in the PST haemolymph experiment were provided to Johanna Mahadevan and Steven Pyecroft (Adelaide University Veterinary School) for FRDC Project No: 2016/235, in order to build capability for Australian researchers to develop indicators of Southern Rock Lobster health. The samples were analysed immediately for a variety of haemolymph parameters (essential elements, sugars, lipids, proteins, metabolites, and enzymes), to aid in development of these methods in Australia. Additional samples of the same haemolymph were provided again after storage for 6-12 weeks at -80 ˚C to enable the University of Adelaide to determine if storage has an impact on the results. Analysis results were compared with duplicate samples tested by Crustipath (Canada) for this project.

Projects enabled by this capability development grant

The projects developed and conducted in the experimental biotoxin contamination facility are detailed in Table 2. Four experiments were conducted, and two further pieces of work will be completed based on the results of these.

Table 2: Details of the projects conducted in the experimental biotoxin contamination centre.

Issue	**FRDC Project No**	**Funder**	**Time frames experiment**	**Value ($)**
The potential to use Southern Rock Lobster haemolymph to estimate PST (non-destructive sampling). Uptake of PST in Southern Rock Lobster through direct exposure to toxic algal cells The impact of PST on Southern Rock Lobster health measured during both experiments	2017-086	SRL	Mar – June 2018 Oct – Nov 2018	200,547
Experimental trial of PST depuration in Pacific Oysters Plan for commercial trials of depuration	Direct funded	ACA Aquaculture Tas Prime Oysters Oysters Tasmania	June-July 2018 Jan – July 2019	36,132
The potential for Blacklip Abalone to uptake *Alexandrium catenella* toxins Neogen validation for Blacklip Abalone tissues	2017-225	ACA	Nov – Dec 2018 Feb – June 2019	58,920
Total				**295,599**

All projects were successfully completed and results of each will be reported under the contractual agreements related to each respective project.

Discussion and Conclusion

Objective 1. Establish key infrastructure (a biotoxin contamination facility) able to be utilised concurrently by multiple industries to resolve specific and varied issues related to biotoxins.

A short-term biotoxin contamination centre was successfully operated in South Australia for 11 months. The infrastructure of a biosecure facility with aquaculture systems, toxic algal culturing in large volumes and full time staffing is expensive, and was covered by this grant ($350,000). This enabled projects to be developed with three seafood industries that are currently heavily impacted by biotoxin blooms (Southern Rock Lobster, wild Blacklip Abalone and Pacific Oysters). These industries provided an additional $295,599 in project funds to cover animal supply and feed, laboratory supplies, analysis costs, staffing and other items specific to their projects. It would have been significantly more expensive to run each project separately due to the need to duplicate facilities, algal culturing and staffing. Thus the operation of the joint facility proved an effective use of resources. The projects conducted will build a better understanding of the impact of blooms of *A. catenella,* required to improve existing management strategies.

The SAABC is designed as a biosecure facility and was easily adapted for biotoxin research without excessive change. Custom built systems for housing lobster and abalone were used during three major experiments, and components of these have been kept for potential future use. The algal culture system was built specifically for the centre, and removed afterward. Again, the expensive components have been retained for future use. Importantly, the ability to treat the waste-water to prevent live algal cells leaving the site was essential.

Objective 2. Support future research and quality assurance programs through provision of a store of contaminated materials.

A significant volume and variety of contaminated tissues has been collected and will be an invaluable resource for future research.

The Southern Rock Lobster hepatopancreas (both toxic and non-toxic) has been sent to Tasmania, and is currently being used in a study to validate the use of Neogen PST test kits for rapid screening of Southern Rock Lobster hepatopancreas (FRDC 2017-086). If the validation is successful, this rapid test kit will save time and money for the Tasmanian Southern Rock Lobster industry, reducing the number of samples that need to be sent for LCMS analysis. If accepted by regulators, a negative result could serve as a criteria to re-open fishing areas immediately on the day of sampling.

The Blacklip Abalone foot and viscera tissue will like-wise be used to validate the Neogen PST test kits for Blacklip Abalone in follow on work from the biotoxin centre. Such validation has proven successful for the oyster industry, where the kit is currently in use by industry members to control business risk, and under consideration for the regulatory risk management program. Should the abalone tissues prove to be non-toxic, they will be spiked with toxin standard solutions for validation purposes.

Approximately 500 g of the contaminated Pacific Oyster homogenate has already been dispensed into 5 g pots, ready for distribution to the oyster industry. It will be used this coming biotoxin season as quality assurance samples to ensure growers are using the Neogen kits correctly, and the kits are performing as expected. This is part of their documented quality assurance and quality control program. Stores of contaminated material for this purpose are hard to access, with biosecurity legislation around transferring POMS infected stock meaning that growing areas in the north (St. Helens to Smithton) and far south (Bruny Island to Recherche Bay) of the state have been unable to

source any contaminated material. The material also has potential for use in future Neogen training programs, or other research projects.

A significant volume of highly contaminated mussel tissues is in storage. This material will be valuable for future method development and validation projects, as feedstock for future uptake or depuration experiments, and for other research projects e.g. to improve the current sensitivity of the Neogen PST test kit for mussels. Another potential application is their use as quality assurance samples for any rapid test kits the industry adopts.

The toxicity of other Southern Rock Lobster tissues collected is yet to be tested, with the exception of the haemolymph samples, which were PST free. However, the haemolymph samples were serendipitously used in a co-occuring project at Roseworthy to develop and validate testing of lobster haemolymph for biochemistry markers of animal health. A total of 62 samples were provided for this study, which also enabled the researchers to examine the impact of storage on the blood chemistry.

Objective 3. Extend capability in biotoxin research in Australia

Throughout the project collaborations between the biotoxin researchers at SARDI, UTAS, and Cawthron have been strengthened. SARDI has employed a recent PhD postgraduate in this field (Dr. Andreas Seger) who has gained significant knowledge of the impact of PST on fisheries, and has contributed valuably to the work completed. An aquaculture technician was employed for the life of the centre and was also trained in handling and culturing toxic algae, as well as animal husbandry for Southern Rock Lobster, Blacklip Abalone and Pacific Oyster species.

New skills have been introduced to the team through the collaboration with Drs Quinn Fitzgibbon (lobster aquaculturist and physiologist), Johanna Mohavendra and Stephen Pyecroft (veterinary pathologists). This has expanded the knowledge and skills across research fields.

Several methodologies employed and developed during the work within the centre will be useful for future projects. We were able to contaminate Southern Rock Lobster to 2.4 times the level of previous work by increasing both the number and toxicity of the feed mussels. We managed to produce palatable feed pellets for Blacklip Abalone that were formulated to contain 10 times the toxicity achieved by previous researchers (Dowsett et al. 2011) by adding seaweed to improve palatability. It is yet to be shown whether or not the Blacklip Abalone accumulated the toxins to high levels, but if so, this could provide a means of sourcing contaminated Blacklip Abalone for future studies on depuration or the impact of processing on PST levels. The use of CO_2 in culturing *A. catenella* was critical to the success of the project. This is a new finding that will be used to advantage in future research as previous work with this species has been limited by low cell densities and growth rates.

Importantly the research has also forged closer relationships between industry and researchers in Australia and New Zealand. In particular, the Southern Rock Lobster research has direct application in New Zealand, and the New Zealand industry were updated on progress throughout the project alongside the Australian industry.

Objective 4. Resolve at least one biotoxin related issue for each of the oyster, abalone and Southern Rock Lobster industries

Projects were designed with industry that aimed to resolve five issues for industry:

- The potential to use Southern Rock Lobster haemolymph to estimate PST (non-destructive sampling)
- Uptake of PST in Southern Rock Lobster through direct exposure to toxic algal cells
- The impact of PST on Southern Rock Lobster health measured during both experiments
- The potential for commercial depuration of PST from Pacific Oysters
- The potential for Blacklip Abalone to uptake *Alexandrium catenella* toxins.

All experiments were successfully completed, and analysis of the results is underway. Outcomes will be reported against each project in the final reports.

In addition, contaminated tissues developed during this project will assist in the validation of the Neogen rapid test kit for Southern Rock Lobster and Blacklip Abalone tissues, and a significant volume of POMS free tissue will be supplied to the Tasmanian oyster industry for QA/QC of the Neogen on-farm.

Implications

The development of a temporary biotoxin contamination facility enabled several industries to conduct studies in this expensive field of research concurrently, providing a cost-effective use of resources. The ASCRC investment of $350,000 was leveraged by a further $295,599 from industry and Commonwealth funds, enabling a significant body of work to be conducted in a short period of time. The cost-sharing model used here could be reproduced to address other cross-sectoral issues impacting a number of fisheries. Potential examples were this could be useful would be: to look at accumulation of other marine biotoxins or chemical contaminations with food safety/market access concern; to conduct studies on the fate of agricultural and veterinary chemicals as part of registration requirements; or to improve quality aspects of seafood associated with conditions during transport.

The joint funding of projects to be run in the SAABC has also strengthened ties between the various industry groups and regulators in charge of risk management. The implications of these results will need to be considered carefully by both these stakeholder groups, and there are further synergies to be gained by taking a cross-sectorial approach to marine biotoxins.

Research collaborations were progressed in this work between IMAS, SARDI and Cawthron Institute. These are particularly relevant as the marine biotoxin issues impact seafood in many Australian states and New Zealand. Leveraging and building expertise in both countries allows researchers to combine resources without duplicating expertise.

The research methods developed in this work will benefit future research in this field. In particular the successful use of CO_2 to improve the growth of *A. catenella* provides opportunities to conduct research requiring large quantities of toxic cells and/or toxins.

This project has built capability in Australia in the biotoxin field, particularly in areas of direct application to the seafood industry. Every research project undertaken in the SAABC will continue following the closure of the experimental centre, with the outcomes being extended into the field.

Benefits to consumers will come from improved biotoxin risk management plans based on better knowledge of uptake and depuration of toxins in these seafood species. Recreational fishers will also benefit from improved risk management plans as recreational fisheries closures are triggered by the commercial fisheries closures.

Recommendations

The final reports of the specific research projects should be disseminated to relevant industry, researcher and regulatory bodies.

- Planning for commercial Pacific Oyster depuration trial is underway. There is a need to work with industry to determine who is interested and which growing areas could be involved. Currently these commercial trials are not funded, and will be expensive due to the need to provide statistical rigour. Funding sources should be explored. Regulators need to be involved in the planning stages to ensure the results will satisfy their needs.

- The library of contaminated materials should be highlighted to both industry and key national and international biotoxin researchers.

- The implications of the individual studies will be discussed in the final reports relating to those studies. In general, consideration needs to be given to validating the experimental outcomes in the field.

- Future cross-sectorial projects should give consideration to the time-frames required to develop and implement experimental and field work. This project was conducted over 18 months, however additional time would have been beneficial for the multi-stakeholder negotiations, contracting, building and dismantling of new systems.

Extension and Adoption

Communication of project results to industry and regulators is vital to obtaining uptake of the recommendations from the individual projects.

During this project the following extension activities have occurred:

- Quarterly updates to the SRL and ACA research boards
- Presentation to Tasmanian oyster industry, Hobart, 4/9/2018
- Presentation at ASQAAC 11/9/2018 (Pacific Oyster depuration).

Further extension activities planned for after the project are:

- Results presented to TransTasman SRL BMP workshop Hobart, April 2019.
- Presentation at next Abalone Congress, Hobart 29-31 July, 2019
- Presentation to next TransTasman Rock Lobster Congress, Christchurch New Zealand, 11-13th August.

Appendix 1: Staff list

SARDI Staff

Ms. Alison Turnbull (Principal Investigator, Sub-Program Leader, Seafood)

Ms. Navreet Malhi (Research Officer)

Dr. Andreas Seger (Research Scientist)

Mr. Elliot Brown (Aquaculture Technician)

Ms. Janet Lee (Aquaculture Technician)

Mr. Geoffrey Holds (Aquaculture Technician)

Mr. Grant Mann (Aquaculture Technician)

Dr. Matthew Bansemer (Research Scientist)

Assoc. Prof. David Stone (Sub-Program Leader, Aquaculture nutrition)

UTAS Staff

Prof. Gustaaf Hallegraeff (Professor Aquatic Botany)

Dr. Quinn Fitzgibbon, (Research fellow)

Cawthron Institute

Dr. Tim Harwood (Marine Toxin Chemist)

Appendix 2: References

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Appendix 3: Risk assessments

Government of South Australia
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BIOSECURITY RISK ASSESSMENT

Note: There are some checklists available for the identification of hazards associated with specific tasks such as Manual Handling, Chemicals, Plant and Equipment – refer to the *PIRSAFE* Intranet site for further information.

Business unit	**Food Safety and Innovation Group**	**Division**	**Seafood Group**	**Site/location**	**South Australian Aquatic Biosecurity Centre** **Roseworthy**
Original assessment date	**12/12/2017**	**Conducted by (name & role)**	**Navreet Malhi - Research Officer**		
Review date	**12/12/2018**	**Conducted by (name & role)**	**Alison Turnbull - Research Scientist**		
Associated job role	**Growing toxic algal species and feeding toxic mussels to lobster: *Alexandrium tamarense* Group 1 and *A. minutum***			**SOP name** **Objective document ID**	

Job Task/Step	Hazard	Risk	Inherent Risk Rating			Control Measures	Level of Control	Residual Risk Rating		
			Consequence	Likelihood	Risk Level			Consequence	Likelihood	Risk Level
1. Growing bulk cultures of paralytic	Biosecurity risk: Genotype unknown in South Australia	Accidental introduction of toxic	Major	Rare	Moderate	• All equipment that has been in touch with toxic algae will be either autoclaved or disposed of in medical waste.	Level 2 and Level 3	Insignificant	Rare	Low

104%

11/04/2019

	shellfish toxic algae: *Alexandrium tamarense* Group 1 and *Alexandrium minutum*		algal cells to SA waters				• At SAABC, all waste water will be heat treated to destroy algal cells before releasing it to the waste water tank for transportation to West Beach Aquatic Sciences for further heat treatment. A time – temperature combination to achieve maximum kill rate will be used. For *Alexandrium* cells 45 ^{0} for 3 min is adequate (Rigby et al. 2004)[1]. Heat tolerant algal resting cysts are extremely unlikely to be formed as the algal cultures are clonal and consist of single mating types only (Hallegraeff et al. 2004)[2]. Water containing algae from experimental tanks will be microscopically inspected to check for presence/absence of resting cysts. Longer time – temperature combinations for waste water treatment will be employed in the unlikely event that cysts should be present, using guidance from Hallegraeff et al. (1997)[3] to achieve the desired outcomes. Waste algal culture from routine culturing will be autoclaved at 121 psi for 30 min (recommended procedure for QC2 compliant waste treatment). • The containers will be cleaned as specified to ensure no cross contamination occurs and accidents will be reported immediately to control contamination. • Toxic substance placards will be displayed to caution and avoid any unauthorised personnel interference				
2.	Feeding mussels containing	Biosecurity risk: mussels may contain cysts of toxic algae of	Accidental introduction of toxic	Major	Rare	Moderate	• Highly unlikely that mussels contain cysts – initial cyst concentrations in blooms off east coast Tasmania have been very sparse (0.1-3 cysts per gram of sediment wet weight. Freeze thawing process would effectively kill most				

[1] Rigby, g., G. Hallegraeff & A. Taylor (2004). Ballast Water Heating offers a Superior Treatment Option. J. of Marine Env. Eng., 7, 217-230
[2] Enevoldsen, H. O. (2003). Manual on harmful marine microalgae (pp. 25-49). G. M. Hallegraeff, D. M. Anderson, & A. D. Cembella (Eds.). Unesco.
[3] Hallegraeff, G. M., Valentine, J. P., Marshall, J. A., & Bolch, C. J. (1997). Temperature tolerances of toxic dinoflagellate cysts: application to the treatment of ships' ballast water. Aquatic Ecology, 31(1), 47-52.

PST to lobster	genotype unknown in SA	algal cells to SA waters				cysts, and UV and chlorine would effectively kill anything remaining. See email from G. Hallegraeff 5/4/2018. • Heating to 45 degrees for 3 min is most effective treatment for cysts as per Rigby et al. 2004[1].				

Risk Assessment Matrix

Consequence *This is the consequence of the event occurring when performing the activity. Consider what would reasonably have happened as well as what actually happened.*		Likelihood *This is the likelihood of the consequence happening with current controls in place*	
Critical	Fatality or irreversible severe disability or impairment, where a worker is unable to return to the workplace.	**Almost Certain**	Almost certain to occur in most circumstances.
Major	Lost Time Injury (LTI) - Extensive injuries, where a worker sustains permanent partial disability or time lost from work of one day/ shift or more.	**Likely**	Is likely to occur in most circumstances.
Moderate	Medical Treatment Injury (MTI) - Significant non-permanent injury with limited period of disability, where medical treatment is required from a health practitioner.	**Possible**	Possible to occur in most circumstances.
Minor	First Aid Injury (FAI) – Insignificant non-permanent injury/ illness, where treatment is administered by a first aider.	**Unlikely**	Unlikely to occur in most circumstances.
Insignificant	Report Only – No injury/ illness	**Rare**	May occur but only in rare and exceptional circumstances.

		Consequences				
		Insignificant	**Minor**	**Moderate**	**Major**	**Critical**
Likelihood	**Almost Certain**	**Moderate**	**Moderate**	**High**	**Extreme**	**Extreme**
	Likely	**Moderate**	**Moderate**	**High**	**High**	**Extreme**
	Possible	**Low**	**Moderate**	**Moderate**	**High**	**High**
	Unlikely	**Low**	**Low**	**Moderate**	**Moderate**	**High**
	Rare	**Low**	**Low**	**Low**	**Moderate**	**High**

Risk Level	**Priority for Action Guide** *(When determining appropriate control measures apply the ALARP principle: Reduce the level of risk to As Low As Reasonably Practicable)*

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BIOSECURITY RISK ASSESSMENT

Extreme	**Act immediately:** The situation is critical. Cease task immediately until risk is eliminated/reduced from the extreme risk level.
High	**Act today**: The situation must be controlled as a matter of priority. Implement immediate temporary controls. Further permanent controls must be managed via the Control Action Plan.
Moderate	**Act soon**: The situation is important. Immediate/permanent controls must be managed via the Control Action Plan.
Low	The situation may be managed using routine procedures. Be sure that this level does not have the potential to escalate to a higher level.

PIRSA·WHS·Risk·Management¶

PIRSA·WHS·RISK·ASSESSMENT·TEMPLATE¶

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Note: There are some checklists available for the identification of hazards associated with specific tasks such as Manual Handling, Chemicals, Plant and Equipment – refer to the *PIRSAFE* Intranet site for further information.¶

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Business unit	**Food Safety and Innovation Group**	**Division**	**Seafood Group**	**Site/location**	**PRC, Level 2, Waite campus and Aquafeeds Mount Barker**
Original assessment date	**21/09/2018**	**Conducted by (name & role)**	**Andreas Seger – Senior Research Officer**		
Review date	**21/09/2019**	**Conducted by (name & role)**	**Alison Turnbull – Research Scientist**		
Associated job role	**Preparation of paralytic shellfish toxin extract for abalone pellet preparation**			**SOP name…… Objective document ID**	**……PST extract abalone pellet……**

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PIRSA-WHS-RISK-ASSESSMENT-TEMPLATE¶

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Job-Task/Step¤	Hazard¤	Risk¤	Inherent-Risk-Rating¤			Control-Measures-¤	Level-of-Control¤	Residual-Risk-Rating¤		
			Consequence¤	Likelihood¤	Risk-Level¤			Consequence¤	Likelihood¤	Risk-Level¤
1.→ Concentrating-PST-producing-*Alexandrium-tamarense*-culture-by-centrifuging-¤	Human-health·–·Accidental·Ingestion·of·paralytic·shellfish·toxin·in·large·quantities¶ (No·known·health·impact·from·skin·contact,·or·breathing-aerosols).¶ ¶ ¤	¶ Poisonous·–·neurotoxic.·Can·cause-paralysis,-may-be-fatal-if-ingested-in-high-quantities,-but-we·are·unlikely·to·be·able·to·build-cell-numbers-up-to-levels-that-will-cause-illness.¶ ¶ ¤	Minor¤	Rare¤	Low¤	•→ PPE-for-handling-the-material.¶ •→ Signs-for-toxic-materials¶ •→ Not-be-taken-signs¶ •→ Only-trained-and-authorised-personnel-allowed-to-handle-the-algae¶ •→ In-case-of-accidental-ingestion,-first-aid-help-will-be-provided-immediately,-medical-follow-up-if-any-symptoms-identified¤	Level-2-and-Level-3¤	Minor-¤	Rare¤	Low¤

PIRSA·WHS·RISK·ASSESSMENT·TEMPLATE¶

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Job·Task/Step¤	Hazard¤	Risk¤	Inherent·Risk·Rating¤			Control·Measures·¤	Level·of·Control¤	Residual·Risk·Rating¤		
			Consequence¤	Likelihood¤	Risk·Level¤			Consequence¤	Likelihood¤	Risk·Level¤
2.→ Cleaning·of·the·equipment·used¤	•→ Paralytic·shellfish·toxin¶ •→ Genotype··unknown·in·South·Australia·(Biosecurity·risk)¶ ¤	•→ Accidental·spread·of·toxic·algal·cells¶ •→ Note·extract·contains·no·viable·*Alexandrium*·cells¤	Major¤	Possible¤	High¤	•→ Growth·rooms·are·not·connected·to·any·water·disposal·source·or·drains·at·Waite·Campus¶ •→ Equipment·which·has·been·in·direct·contact·with·the·algae,·will·be·autoclaved·to·kill·the·cysts·and·then·cleaned.¶ •→ Any·algae·that·may·need·to·be·disposed·off·will·be·treated·as·medical·waste.¶ •→ Toxic·substance·placards·are·displayed·to·caution·and·avoid·any·unauthorised·personnel·interference¤	Level·2·and·Level·3¤	Major¤	Rare¤	Moderate¤

PIRSA·WHS·RISK·ASSESSMENT·TEMPLATE¶

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Job·Task/Step¤	Hazard¤	Risk¤	Inherent·Risk·Rating¤			Control·Measures·¤	Level·of·Control¤	Residual·Risk·Rating¤		
			Consequence¤	Likelihood¤	Risk·Level¤			Consequence¤	Likelihood¤	Risk·Level¤
3.→ Extracting·paralytic·shellfish·toxin·(PST)·from·centrifuged·algal·concentrate¤	¶ •→ Human·health·–·Accidental·Ingestion·of·paralytic·shellfish·toxin·¶ •→ Breathing·of·aerosols·during·hot·(85·°C)·extraction·step¶ •→ Skin·contact·with·paralytic·shellfish·toxin·extract·(no·known·cases·of·illness)¤	•→ Paralytic·shellfish·poisoning·through·ingestion·or·possibly·aerosols·or·skin·contact¶ •→ Highest·PST·concentration·=·0.05·mg/mL·Lowest·Adverse·effect·level·(2·µg/kg·body·weight)·reached·by·direct·uptake·of·2.8·mL·of·liquid·PST·extract·(based·on·70·kg·body·weight).¶ ¤	Major¤	Rare¤	Moderate¤	•→ Training·of·staff·for·risk·awareness·(includes·information·on·paralytic·shellfish·poisoning·symptoms·and·response)·and·PPE·required¶ •→ Clearly·label·all·containers·to·identify·risk·to·others¶ •→ PPE:·nitrile·gloves·and·lab·coat·to·avoid·skin·contact,·face·shield·to·protect·eyes/face·from·accidental·splashes¶ •→ Cooling·of·hot·extract·(85·°C)·on·ice·before·opening·containers·in·fume·cupboard¶ •→ Frequent·reports·(10·min)·to·designated·contact·if·working·alone·in·lab.¤	Level·1,·Level·2·and·Level·3¤	Major¤	Rare¤	Moderate¤

PIRSA·WHS·RISK·ASSESSMENT·TEMPLATE¶

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4.→ Preparation·of·paralytic·shellfish·toxin·containing·wet·abalone·pellet¤	• → Human·health·–·Accidental·Ingestion·of·paralytic·shellfish·toxin·¶ • → Skin·contact·with·paralytic·shellfish·toxin·extract·(no·confirmed·cases·of·illness)¤	• → Paralytic·shellfish·poisoning·through·ingestion·or·possibly·aerosols·or·skin·contact·(no·known·cases·for·latter·two)¶ • → Highest·PST·concentration·in·extract·=·0.05·mg/mL.·Lowest·Adverse·effect·level·(2·µg/kg·body·weight)·reached·by·direct·uptake·of·2.8·mL·of·liquid·PST·extract·(based·on·70·kg·body·weight).¶ • → Highest·PST·concentration·in·pellet·=·0.012·mg/g·Lowest·Adverse·effect·level·(2·µg/kg·body·weight)·reached·by·direct·uptake·of·11.7·g·of·pellet·(based·on·70·kg·body·weight).¤	Major¤	Rare¤	Moderate¤	• → Training·of·staff·for·risk·awareness·(includes·information·on·paralytic·shellfish·poisoning·symptoms·and·response)·and·PPE·required·¶ • → Evacuate·all·unnecessary·staff·from·the·immediate·work·area¶ • → Work·in·well-ventilated·area¶ • → Clearly·label·all·containers·to·identify·risk·to·others¶ • → PPE:·nitrile·gloves·and·lab·coat·to·avoid·skin·contact,·face·shield·to·protect·eyes/face·from·accidental·splashes¶ • → Half·face·respirator·with·combination·filter·cartridge·for·dust·+·chemical·vapour·must·be·worn·to·protect·from·dust·and·potential·aerosols¶ • → Work·in·pairs.¤	Level·2·and·Level·3¤	Major¤	Rare¤	Moderate¤
5.→ Drying·of·paralytic·shellfish·toxin·containing·abalone·pellet¤	• → Human·health·–·Accidental·Ingestion·of·paralytic·shellfish·toxin·¶	• → Paralytic·shellfish·poisoning·through·ingestion·or·adsorption·through·skin·contact·with·dust·particles·(no·known·cases·for·latter·two)¶	Major¤	Rare¤	Moderate¤	• → Training·of·staff·for·risk·awareness·(includes·information·on·paralytic·shellfish·poisoning·symptoms·and·response)·and·PPE·required¶	Level·2·and·Level·3¤	Major¤	Rare¤	Moderate¤

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	• Breathing·of·dust·particles·during·drying·of·abalone·pellet¶ • Skin·contact·with·paralytic·shellfish·toxin·extract·(no·known·cases·of·illness)[a]	• Highest·PST·concentration·in·pellet·=·0.012·mg/g·Lowest·Adverse·effect·level·(2·µg/kg·body·weight)·reached·by·direct·uptake·of·11.7·g·of·pellet·(based·on·70·kg·body·weight)[a]				• Evacuate·all·unnecessary·staff·from·the·immediate·work·area¶ • Work·in·well-ventilated·area¶ • Clearly·label·all·containers·to·identify·risk·to·others¶ • PPE:·nitrile·gloves·and·lab·coat·to·avoid·skin·contact,·face·shield·to·protect·eyes/face·¶ • Half·face·respirator·with·combination·filter·cartridge·for·dust·+·chemical·vapour must·be·worn·to·protect·from·dust·and·potential·aerosols¶ • Abalone·pellets·to·only·be·moved/broken·up·within·oven·during·drying·process¶ • When·decanting·dried·abalone·pellets·into·storage·container,·do·so·in·separate,·well-ventilated·room·to·avoid·accidental·exposure·of·others·to·dust·particles¶ • Frequent·reports·(10·min)·to·designated·contact.[a]				

PIRSA-WHS-RISK-ASSESSMENT-TEMPLATE¶

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6. Feeding of abalone with paralytic shellfish toxin containing pellet¤	• Human health – Accidental Ingestion of paralytic shellfish toxin ¶ • Breathing of dust particles during weighing of abalone pellet/feeding of abalone¶ • Skin contact with paralytic shellfish toxin extract (no known cases of illness)¤	• Paralytic shellfish poisoning through ingestion or adsorption through skin contact with dust particles (no known cases for latter two)¶ • Highest PST concentration in pellet = 0.012 mg/g Lowest Adverse effect level (2 µg/kg body weight) reached by direct uptake of 11.7 g of pellet (based on 70 kg body weight)¤	Major¤	Rare¤	Moderate¤	• Training of staff for risk awareness (includes information on paralytic shellfish poisoning symptoms and response) and PPE required¶ • Evacuate all unnecessary staff from the immediate work area¶ • Work in well-ventilated area¶ • Clearly label all containers to identify risk to others¶ • PPE: nitrile gloves and lab coat to avoid skin contact, safety glasses to protect eyes¶ • Dust mask must be worn during weighing/feeding of abalone pellet to protect from dust¶ • Report to designated contact before and after feeding.¤	Level 2 and Level 3¤	Major¤	Rare¤	Moderate¤

PIRSA-WHS-RISK-ASSESSMENT-TEMPLATE¶

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··Risk·Assessment·Matrix¶

Consequence¶ *This·is·the·consequence·of·the·event·occurring·when·performing·the·activity.·Consider·what·would·reasonably·have·happened·as·well·as·what·actually·happened.*		Likelihood¶ *This·is·the·likelihood·of·the·consequence·happening·with·current·controls·in·place*	
Critical	Fatality·or·irreversible·severe·disability·or·impairment,·where·a·worker·is·unable·to·return·to·the·workplace.	**Almost·Certain**	Almost·certain·to·occur·in·most·circumstances.
Major	Lost·Time·Injury·(LTI)·-·Extensive·injuries,·where·a·worker·sustains·permanent·partial·disability·or·time·lost·from·work·of·one·day/·shift·or·more.·	**Likely**	Is·likely·to·occur·in·most·circumstances.
Moderate	Medical·Treatment·Injury·(MTI)·–·Significant·non-permanent·injury·with·limited·period·of·disability,·where·medical·treatment·is·required·from·a·health·practitioner.··	**Possible**	Possible·to·occur·in·most·circumstances.
Minor	First·Aid·Injury·(FAI)·–·Insignificant·non-permanent·injury/·illness,·where·treatment·is·administered·by·a·first·aider.	**Unlikely**	Unlikely·to·occur·in·most·circumstances.
Insignificant	Report·Only·–·No·injury/·illness	**Rare**	May·occur·but·only·in·rare·and·exceptional·circumstances.

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		Consequences				
Likelihood		Insignificant	Minor	Moderate	Major	Critical
	Almost·Certain	Moderate	Moderate	High	Extreme	Extreme
	Likely	Moderate	Moderate	High	High	Extreme
	Possible	Low	Moderate	Moderate	High	High
	Unlikely	Low	Low	Moderate	Moderate	High
	Rare	Low	Low	Low	Moderate	High

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Risk·Level	Priority·for·Action·Guide *(When·determining·appropriate·control·measures·apply·the·ALARP·principle:·Reduce·the·level·of·risk·to·As·Low·As·Reasonably·Practicable)*
Extreme	Act·immediately:·The·situation·is·critical.·Cease·task·immediately·until·risk·is·eliminated/reduced·from·the·extreme·risk·level.·
High	Act·today:·The·situation·must·be·controlled·as·a·matter·of·priority.·Implement·immediate·temporary·controls.·Further·permanent·controls·must·be·managed·via·the·Control·Action·Plan.
Moderate	Act·soon:·The·situation·is·important.·Immediate/permanent·controls·must·be·managed·via·the·Control·Action·Plan.
Low	The·situation·may·be·managed·using·routine·procedures.·Be·sure·that·this·level·does·not·have·the·potential·to·escalate·to·a·higher·level.