# SALTWATER AND FRESHWATER TRUSTS
## Final Project Report

**Report date:** 4 April 2024

**Project title**: Ciguatera Toxins in NSW Spanish Mackerel: Where, when and how much?

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**Project start date:** 30/1/2021
**Project completion date:** 30/4/2024

**Project objectives:**

- Identify detailed information on the prevalence of finding Ciguatera toxins (CTX) in NSW Spanish Mackerel in relation to site, fish length and weight, and annual and seasonal environmental fluctuations in marine physico-chemical data.
- Develop recommendations for food safety risk management for the Spanish Mackerel incorporating information on CTX prevalence, and factors associated with CTX prevalence.
- Communicate this information to the recreational fishing community using talks and written material, in order to increase awareness of Ciguatera Fish Poisoning and the factors that impact it.

**Outcomes:** (explain how objectives were achieved, results, conclusions and completion dates, attach relevant maps, photographs, etc.)

# Ciguatera Toxins in NSW Spanish Mackerel: Where, when and how much?

# 1 Introduction

## 1.1 Ciguatera Poisoning

Ciguatera Poisoning (CP) is a significant safety concern in some Australian seafood (Sumner, 2011) and a prevalent global issue associated with fish consumption (Friedman et al., 2008). Globally, it affects 50,000 to 500,000 people annually (Friedman et al., 2017) and is caused by the ingestion of fish containing toxic levels of ciguatoxins (CTXs) (Hamilton et al. 2010).

CTXs are primarily produced by microalgae species of the *Gambierdiscus* genus (Chinain et al., 1997; Holmes, 1998; Chinain et al., 1999; Chinain et al., 2010; Rhodes et al., 2010; Fraga et al., 2011; Holland et al., 2013) and accumulate in the food chain, particularly in carnivorous reef fish (Murata et al., 1990; Lewis et al., 1991; Lewis & Holmes, 1993; Vernoux &d Lewis, 1997; Lewis et al., 1998; Yasumoto et al., 2000; Pottier et al., 2002; Pottier et al., 2003). These toxins activate sodium channels in nerve cells (Lewis et al., 1992; Mattei et al., 1999; Lewis et al., 2000, leading to various gastrointestinal and neurological symptoms in humans with severe cases even affecting the cardiovascular system (Figure 1). Diagnosing CP is challenging due to over 175 documented symptoms (Sims, 1987), which can vary based on portion size (Sims, 1987), individual susceptibility, age (Bagnis et al., 1979; Glaziou & Martin, 1993), geographical region (Lewis et al., 2000; Dickey, 2008) and potential overlap with other illnesses.

CP cases are increasing globally, with a 60% rise in the Pacific region over the past decade (Farrell et al., 2017). Regional differences in CTXs highlight the importance of characterising toxins from different areas. Understanding CTX accumulation patterns in various fish species can aid in prevention. However, accurate identification of specific CTX congeners is essential to comprehensively assess CP risks locally.

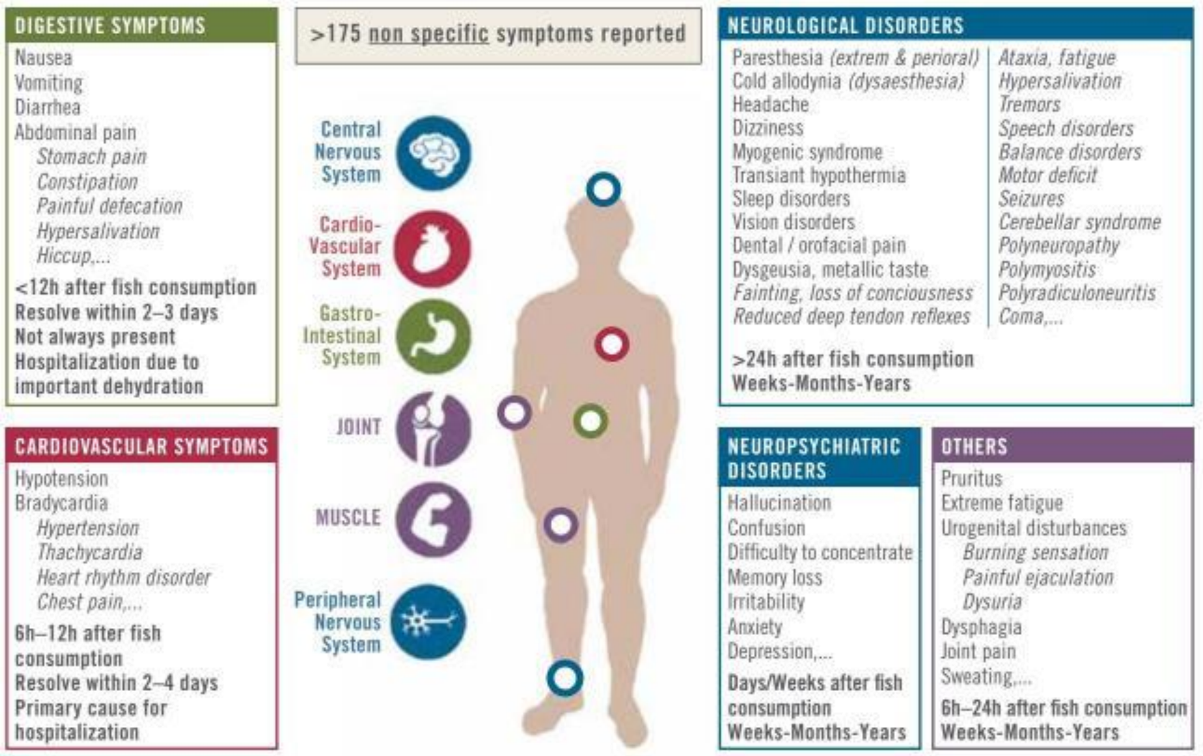

**Figure 1** Symptoms connected with ciguatera intoxication (FAO and WHO 2020).

### 1.1.1 Chemistry of CTXs

CTXs are cyclic polyether ladders with remarkable thermostability and liposolubility. They have been extracted from various fish species and different *Gambierdiscus* strains (Table A1, Appendix A). These toxins are categorised into P-CTXs (from the Pacific Ocean), C-CTXs (from the Caribbean region) and I-CTXs (from the Indian Ocean) based on their origin and structural distinctions.

Within P-CTXs, there are two main types: type I with 13 rings and 60 carbon atoms (Murata et al., 1990; Lewis et al., 1991; Lewis & Holmes, 1993; Yasumoto et al., 2000), exemplified by CTX1B (Murata et al., 1989, Murata et al., 1990; Lewis et al., 1991) and type II with similar features, represented by CTX3C (Legrand et al., 1998) (Figure 2).

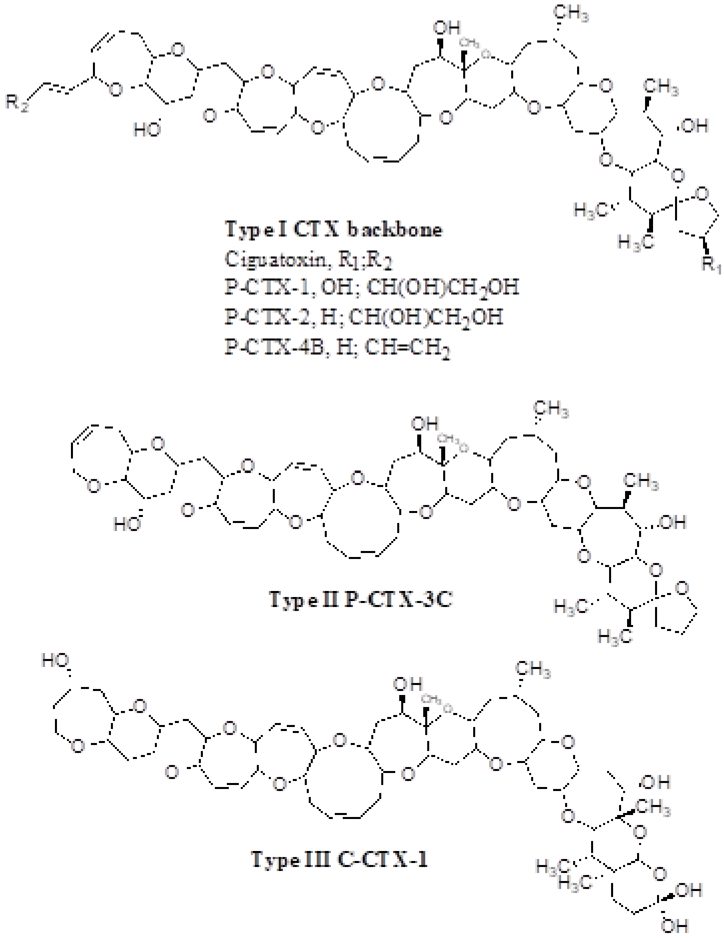

**Figure 2** Structure of Ciguatoxins (CTXs). P-CTX-1, P-52-EPI-54-DEOXY-CTX-1B (FORMERLY KNOWN AS CTX-2) and C-CTX-1 derived from fish. P-CTX-3C and P-CTX-4B derived from *Gambierdiscuss* spp. (Kohli et al., 2015).

Additionally, 52-epi-54-deoxy-CTX-1 (formerly known as CTX-2) and 54-deoxy-CTX-1B (formerly known as CTX-3), derived from dinoflagellate CTXs, CTX-4A and CTX4B (Lewis & Holmes, 1993; Yasumoto et al., 2000), have variations in their structures, affecting toxicity. Type II P-CTXs, include 49-epi-CTX-3C and M-seco-CTX-3C isolated from a *Gambierdiscus* sp. (Satake et al., 1993) and *G. polynesiensis* (Chinain et al., 2010). New variants, such as 2,3 dihydroxyCTX3C and 51-hydroxyCTX3C, have also been identified from the Moray eel (Satake et al., 1998).

Caribbean CTXs, larger than P-CTXs, have 14 rings and 62 carbon atoms (Vernoux & Lewis, 1997; Lewis et al., 1998; Pottier et al., 2002; Pottier et al., 2003). Numerous congeners of C-CTXs have been isolated from carnivorous fish (Vernoux & Lewis, 1997; Lewis et al., 1998; Pottier et al., 2002; Pottier et al., 2003). Unlike P-CTXs there have been no reports of C-CTXs originating from *Gambierdiscus* spp. However, recently *G. excentricus* has been identified as a major CTX producer in the Caribbean (Fraga et al., 2011) and CTXs from this strain are being characterised.

I-CTXs from the Indian Ocean have higher molecular ion masses than P-CTXs and C-CTXs. Four types (I- CTX-1, I- CTX-2, I-CTX-3, I-CTX-4) have been identified but await structural elucidation (Hamilton et al., 2002a; Hamilton et al., 2002b). Toxicity varies among CTX congeners as observed in mouse bioassays (MBA), but further validation is needed (Table A1, Appendix A). Importantly, understanding these structural distinctions is essential for assessing the risks posed by different CTXs.

### 1.1.2 Detection of CTXs in Seafood

CP cases primarily occur in mid-latitude tropical and sub-tropical zones, reflecting the distribution of *Gambierdiscus* (Kohli et al., 2015). However, CP has been reported in non-endemic areas due to seafood imports of susceptible species (Glaziou & Legrand, 1994; Ting & Brown, 2001). While most studies have focused on reef fish, toxin accumulation has been observed in various species, such as eels, sea cucumbers, starfish, seals and jellyfish (Kohli et al., 2015).

Local knowledge in small island nations often guides safe fish consumption. However, a study in French Polynesia found CTXs in supposedly safe-to-eat fish (Darius et al., 2007). Experimentally, CTX toxin profiles and structures have been determined using chromatographic techniques, nuclear magnetic resonance (NMR) and radio ligand binding (RLB; Murata et al., 1989; Murata et al., 1990; Lewis et al., 1991; Satake et al., 1996; Hamilton et al., 2002a; Hamilton et al., 2002b). These methods are costly and not practical for routine testing. Purified and certified CTX standards are limited, hindering accurate quantification.

Various biological assays, such as the MBA and enzyme-linked immunosorbent assay (ELISA), have been developed to detect ciguateric fish. While MBA remains widely used, it has limitations. ELISA offers higher throughput, but has produced false results (Hokama, 1990; Campora et al., 2008; Bienfang et al., 2011). In more recent years, a different approach to produce antibodies was tried, and no cross-reactivity was observed with other marine toxins (Tsumuraya et al., 2018; Tsumuraya & Hirama, 2019). These results led to the development of a new kit named “CTX-ELISA 1B” (Fujifilm Wako Corporation, Osaka, Japan) based on a fluorescent ELISA assay. Since the results obtained with this strategy were promising, the same antibodies were used to develop biosensors which have a limit of detection ten times lower than the United States Food and Drug Administration (US FDA) guidance threshold of 0.01 µg/kg (USFDA, 2011; Leonardo et al., 2020; Campàs et al., 2022). While these tools are portable and user-friendly, the protocol for CTXs detection still necessitates a lengthy extraction process from fish flesh. Other assays, such as a sodium channel binding mouse neuroblastoma cell assay (N2a) (Manager et al., 1993; Viallon et al., 2020) and receptor binding assay (RBA) (Hardison et al., 2016) have shown promise. However, they cannot quantify specific CTX congeners. LC-MS analysis is crucial for this purpose, but analytical challenges include the lack of purified standards and the presence of multiple CTX analogues in fish specimens (Endean et al., 1993; Vernoux & Lewis, 1997).

## 1.2 CP in Australia

CP is a concern in the warmer waters of Australia, primarily along the coastlines of the Northern Territory (NT), Queensland (QLD) and south to Byron Bay in NSW (~28°S). There are no confirmed reports of CP from Western Australia (WA). Most CP outbreaks are linked to fish caught in QLD and the NT, with Spanish Mackerel being the most frequently implicated species (Gillespie et al., 1986; Farrell et al., 2016a). Until 2014, cases of CP in NSW, Victoria, or other southern states were usually traced back to fish from QLD, the NT or imported fish (Farrell et al., 2016a).

Approximately 200 fish and invertebrate species may be involved in CP outbreaks, although precise figures are challenging to determine (Kohli et al., 2015; FAO and WHO, 2020). While many implicated species are carnivorous, herbivorous species have also been linked to CP outbreaks (Friedman et al., 2017). Species like Amberjack (*Seriola* spp.), Wrasse (*Cheilinus* spp.) and Trevally (*Caranx* spp.) are common vectors of CTXs in the Pacific region (Lewis, 2001; Stewart et al., 2010) (

Table **A5**, Appendix A).

In NSW, confirmed CP cases related to Spanish Mackerel consumption from NSW waters have been reported in several locations, including Brunswick Heads in 2002, Evans Head in February 2014 (4 people), Scott's Head in March 2014 (9 people), and South West Rocks in April 2015 (4 people). These cases involved classic CP symptoms and many required hospitalisation with at least one victim disabled for an extended period (Farrell et al., 2016a). P-CTX-1B was detected via LC-MS/MS in Spanish Mackerel samples during these outbreaks. Additionally, suspected CP outbreaks in 2005 and 2009 in NSW were linked to fish from Fiji and QLD respectively but lacked chemical analysis to confirm P-CTX-1B presence. The NSW CP cases from 2014–2015 marked the southern most confirmed sources of CP in Australia (Farrell et al., 2016a).

### 1.3 Management of CP

The US Food and Drug Administration (FDA) has recommended a guidance level for Pacific CTX-1B in fish flesh of less than or equal to 0.01 ppb CTX equivalent (0.01 µg kg$^{-}$1 CTX) (USFDA, 2011). Due to the absence of rapid and cost-effective screening tests for CTXs, health authorities worldwide have typically issued guidelines to prevent high-risk fish from entering the commercial market to reduce the risk of CP (Stewart et al., 2010). It is generally believed that the size or age of certain fish species may be related to the levels of CTXs found, because these toxins can accumulate over time.

Relatively few studies have directly explored the relationship between fish size and CTX presence, with variable results. In a Japanese study, a positive relationship was observed between size and toxicity in several fish species, including *Lutjanus monostigma* (Onespot Snapper, Figure B1, Appendix B), *Epinephelus fuscoguttatus* (Flowery Rockcod, Figure B2, Appendix B), *Lutjanus bohar* (Red Bass, Figure B3, Appendix B), and *Variola louti* (Yellowedge Coronation Trout, Figure B4, Appendix B) (Oshiro et al., 2010). Another study involving Great Barracuda (*Sphyraena barracuda*) found toxic samples, but no clear correlation between fish size/weight and toxicity (Dechraoui et al., 2005). These findings indicated mixed results in the few studies that have directly examined the relationship between fish size and CTX presence (Figure B5, Appendix B).

In Australia, guidelines to prevent high-risk fish from entering the market are provided by the Sydney Fish Markets (Table A3 and Table A4, Appendix A) the country's largest domestic fish distributor (Stewart et al., 2010). Queensland (QLD) and Northern Territory (NT) authorities also follow these guidelines, and CP cases are notifiable conditions in QLD (QLD Health, 2015). The guidelines are based on the observation of outbreaks and illnesses rather than studies relating CTX levels in high-risk fishes. In Queensland, QLD Health established protocols for collecting epidemiological related information (patient symptoms, suspected fish details) and samples for the quantification of P-CTX-1, 2 and 3. However, further research is needed to assess and mitigate the risk of CP in Australia.

## 2 Analysis of Spanish Mackerel samples from NSW and QLD for CTXs

### 2.1 Background

The significant number of CP cases reported since 2014 in Australia (Figure 3, **Error! Reference source not found.**) generated concern among the commercial and recreational fishing communities, highlighting the need to determine appropriate management strategies to prevent CP illnesses in Australia. In an initial NSW Recreational Fisheries Trust project in 2014, a relatively high proportion of a small sample of Spanish Mackerel caught from QLD and NSW waters were found to contain detectable CTXs. In that study, detectable P-CTX-1B was present in both muscle and liver tissues in fish from NSW (n =71, 1.4% prevalence rate, with a confidence interval of 1%–4%, and 7% prevalence, 1%–12%, in flesh and liver, respectively). In the small sample of fish from Queensland, there was a 46% prevalence (19–73%, n=13). Toxin levels found were 0.13 µg kg$^{-1}$ to<0.1 µg kg$^{-1}$ in muscle flesh, and 1.39 µg kg$^{-1}$ to<0.4 µg kg$^{-1}$ in liver, indicating that liver tissue had a significantly higher concentration (~5 fold) of P-CTX-1B. No apparent relationship was observed between the length or weight of *S. commerson* and the detection of P-CTX-1B (Kohli et al 2017). Given the need to understand the distribution and abundance of fish contaminated with CTXs in NSW and QLD, it was determined that samples from two other fishing seasons (2020/2021 and 2021/2022) would need to be collected to have more representative data coverage in order to understand prevalence rates of CTXs in Spanish Mackerel stocks in eastern Australia. Data was also sourced from independent sampling carried out annually by QLD Health on fish associated with CP cases in QLD. With several years of information on CTXs in Spanish Mackerel, it might then be possible to determine environmental, temporal and spatial trends in CTX presence, as well as trends related to fish size or other factors.

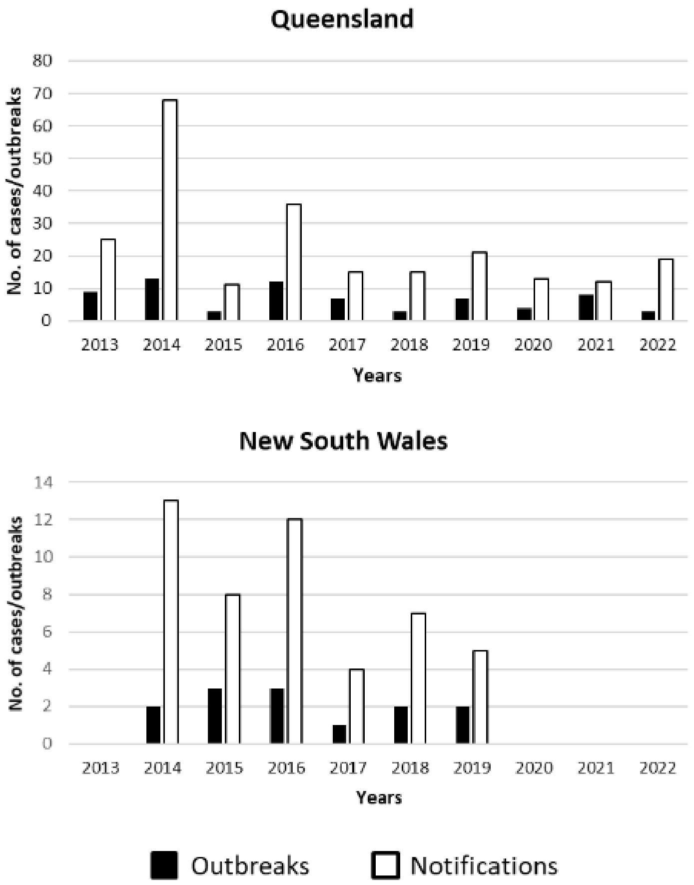

**Figure 3.** Ciguatera notifications and outbreaks, QLD and NSW, 2013 - 2022 (Farrell et al., 2016a, 2016b, Edwards et al., 2019, Szabo et al., 2022).

**Table 1** List of confirmed CP cases caused by consuming fish caught from NSW waters.

| Date | Cases | Fish Species/Origin | P-CTX-1B (µg kg$^{-1}$) |
|---|---|---|---|
| Feb. 2014 | 4 | Spanish Mackerel/Evans Head, NSW | nd, 0.6, 1 |
| Mar. 2014 | 9 | Spanish Mackerel/Scotts Head, NSW | 0.4 |
| Apr. 2015 | 4 | Spanish Mackerel/South West Rocks, NSW | n/a |
| Mar. 2016 | 3 | Spanish Mackerel/Crowdy Head, NSW | 0.93 |
| Apr. 2016 | 4 | Spanish Mackerel/Crescent Head, NSW | 0.11, 0.37 |
| Feb. 2018 | 4 | Spanish Mackerel/Coffs Harbour, NSW | n/a – no samples available |
| Apr. 2018 | 3 | Spanish Mackerel/Wooli, NSW | n/a – no samples available |

n/d; not determined; n/a: not available

## 2.2 Methods

The objectives of this project were to:

1. Identify detailed information on the prevalence of finding Ciguatera toxins (CTX) in NSW Spanish Mackerel in relation to site, fish length and weight, and annual and seasonal environmental fluctuations in marine physico-chemical data.

2. Develop recommendations for food safety risk management for the Spanish Mackerel incorporating information on CTX prevalence, and factors associated with CTX prevalence.

3. Communicate this information to the recreational fishing community using talks and written material, in order to increase awareness of Ciguatera Fish Poisoning and the factors that impact it.

### 2.2.1 Sample collection

Sampling kits were distributed to fishing clubs in Sydney, QLD and the northern NSW coast. The majority of the Spanish Mackerel catch in NSW is recreational and comes from these areas. The sample pack consisted of several labelled tubes, which could contain ~10 g samples of liver and muscle (flesh) tissue. It also contained a laminated diagram explaining the project and how to take samples, a data sheet in order to record information about the fish, and the contact details of the scientists involved. Following sample collection, samples were stored at – 20 ºC until further analysis. The date of catch, length from head to tail and weight of the specimen were recorded. The sampling kit and information sheet is shown in Figure B6, Appendix B.

Fish were collected by individuals from the: Coffs Harbour Fishing Cooperative, Ballina Fishing Cooperative, Byron Bay Deep Sea Fishing Club, Mackay Game Fishing Club, Newcastle Neptune's Spearfishing Club, Tweed-Gold Coast Freedivers Club, the Sydney Fish Market, and the NSW Department of Primary Industries Research Angler Program.

Additional information regarding CTX positive samples from QLD was sourced from the QLD Health. QLD Health provided information on location, size and CTX content (P-CTX-1B, 52-epi-54- deoxy-CTX-1B (formerly known as CTX-2) and 54-deoxy-CTX-1B (formerly known as CTX-3) of the collected Spanish Mackerel specimens. Toxins were analysed using LC-MS by QLD Health.

### 2.2.2 Fish sample extraction

Each tissue sample was chopped using a scalpel blade and 5 ± 0.1 g biomass was weighed and placed in a 50 mL centrifuge tube. To this, 15 mL of 60 % LC-MS grade Methanol (Sigma, St. Louis, MO) was added and the tissue samples were homogenised using an Ultra-Turrax (Thermo Fisher, Waltham, MA) at maximum speed for 1 min. The tissue samples were then incubated at 95 ºC for 10 min and cooled on ice for 5 min. Further, tissue samples were centrifuged at 3200 x g for 10 min to pellet insoluble debris and a 5 mL aliquot of the supernatant was transferred to a new 15 mL centrifuge tube for liquid-liquid partitioning.

### 2.2.3 Liquid-liquid partitioning

A 5 mL aliquot of liquid chromatography-mass spectrometry (LC-MS) grade dichloromethane (DCM) (Sigma, St. Louis, MO) was added to the 5 mL of sample extract and then vortexed for 15 seconds. Samples were centrifuged at 3200 x g for 1 min to ensure partitioning of the solvent layers. The volume in the top layer (aqueous methanol) was aspirated and the lower DCM layer was aspirated down to 4 mL level. The remaining 4 mL of DCM-toxin mix was dried in a 55 ºC heating block and under a nitrogen flow.

### 2.2.4 Solid phase extraction

A 200 mg/3mL solid phase extraction cartridge CUNAX123 (United Chemical Technologies, Levittown PA) was conditioned with 10 mL DCM. The dry sample-residue was dissolved in 4mL DCM and the entire volume loaded onto the cartridge. The cartridge was washed with 4 mL DCM. For elution, 4 mL of 9:1 dichloromethane:methanol was passed through the cartridge and the volume collected in 10 mL tubes. The samples were then dried at 55 ºC under a stream of nitrogen. The dry sample tubes were stored at -80 ºC until LC-MS analysis. For analysis, the dried samples were reconstituted in 200 µL of 80% methanol and transferred into a glass autosampler vial.

### 2.2.5 Liquid chromatography-mass spectrometry analysis

Analysis of the fish extracts was performed at the Cawthron Institute (New Zealand) using a triple quadrupole LC-MS/MS instrument.

A Waters® Acquity UPLC BEH Phenyl (1.7 µm, 100 x 2.1 mm column) column held at 50 ºC was used for chromatographic separation in both instruments. The mobile phases consisted of (A) Milli-Q containing 0.2% ammonia and (B) Acetonitrile containing 0.2% ammonia. Each buffer solution was prepared freshly every day. The gradient conditions are described below (**Error! Reference source not found.**).

**Table 2** Gradient conditions used during LC-MS analysis.

| Time [min] | A [%] | B [%] | Flow [µL/min] |
|---|---|---|---|
| 0.00 | 60 | 40 | 550 |
| 2.00 | 40 | 60 | 550 |
| 2.50 | 5 | 95 | 550 |
| 3.00 | 5 | 95 | 550 |
| 3.01 | 60 | 40 | 550 |
| 5.00 | 60 | 40 | 550 |

At the Cawthron Institute, the analysis was performed on a Waters Xevo TQ-S triple quadrupole mass spectrometer coupled to a Waters Acquity UPLC i-Class with flow through needle sample manager. An injection volume of 2 µL was used. The electrospray ionisation source was operated in positive-ion mode at 150 ºC, capillary 3.5 kV, cone 30–75 V, nitrogen gas desolvation 1000 L h$_{-1}$ (600 °C), cone gas 150 L h$_{-1}$, and the collision cell argon gas flow 0.15 mL min$^{-1}$. For quantitative analysis, a total ion chromatogram generated from the following multiple reaction monitoring (MRM) transitions was used: m/z 1128.6>95.0 (CE 65 eV), m/z 1128.6>109.0 (CE 55 eV) m/z 1133.6>1133.6 (CE 55 eV). A dwell time of 20 ms was used for all transitions monitored. Peak areas were integrated and sample concentrations calculated from linear calibration curves generated from standards. TargetLynx software was used for the analysis (Water-Micromass, Manchester, UK).

### 2.2.6 Spike recovery

To ensure satisfactory performance of the method, numerous flesh and liver samples were analysed in duplicate, with one of the samples spiked with a known amount of P-CTX-1B standard (11 of 168 samples). The spiking of samples with CTX was carried out for calibration purposes only, and these results were not included in the final concentrations. Mean recoveries were calculated for each matrix and applied to the toxin concentration determined in samples. The P-CTX-1B spiking solution was provided by the Cawthron Institute with a given concentration of 58.651 ng mL$^{-1}$. The Cawthron Institute also provided three standard solutions for instrument calibration: P-CTX-1B of 0.341 ng mL$^{-1}$, 1.705 ng mL$^{-1}$ and 3.41 ng mL$^{-1}$. These calibration standards were analysed at the same time as the various fish samples and were used to create a calibration curve. The concentration of P-CTX-

1B was calculated by comparing the peak areas observed in contaminated fish samples with the calibration curve generated at the time of analysis.

### 2.2.7 Spanish Mackerel identification via qPCR

To determine the identity of fish specimens, collected DNA was extracted from approximately 20 mg of flesh from fish specimens using QIAamp 96 DNA Qiacube HT Kit (Qiagen). Flesh samples were incubated in proteinase K and lysis buffer provided by the manufacturer. The lysate was then purified using wash buffers as per the manufacturer's instructions. DNA was quantified using Nanodrop ND-1000 spectrophotometer and analysed using the qPCR primers (Forward: TGGGCCGTCCTTATTACAGC, Reverse: CTCCTCCTGCTGGGTCAAAG) specific for the cytochrome oxidase subunit I (COI) gene from *S. commerson* (Ward et al., 2005).

All PCR reactions were performed in 5 µL reaction volumes containing 2.5 µL iTaq Universal SYBR Green Supermix (Biorad), 1.1 µL nuclease free water, 0.2 µL of forward and reverse primer (0.5 µM final concentration) and 1 µL of DNA template. The plate was prepared with an epMotion®5075l Automated Liquid Handling System. The qPCR assay was performed using the BIORAD CFX384 Touch™ Real-Time PCR Detection System™ using a 95 ºC holding stage for 10 min, followed by 35 cycles of 95 ºC for 15 s and 60 ºC for 1 min, followed by a melt curve analysis (Table 3, **Error! Reference source not found.**, Appendix B). Spanish Mackerel from a previous study (FRDC project 2014-035) was used as a positive control and Purple Rock Cod (*Epinephelus cyanopodus*) was used as a negative control for this analysis. All samples were verified based on having similar melt curves and amplification cycles to the positive control.

**Table 3** Cycling conditions used for qPCR identification *of S. commerson* specimens.

| Step | Temperature | Time |
|---|---|---|
| Holding stage | 95 ºC | 10 min |
| Cycles | 95 ºC<br>60 ºC | 15 s<br>1 min |
| Melt curve | 95 ºC<br>60 ºC<br>95 ºC | 15 s<br>1 min<br>30 s |

## 2.3 Results and Discussion

### 2.3.1 Spanish Mackerel from fishing seasons 2014–2015, 2020–2021 and 2021–2022

Samples of Spanish Mackerel were collected in NSW and QLD during 3 fishing seasons, 2014–15 (previously collected as part of NSW DPI L127 – Safeguarding recreational fishing in NSW from ciguatera fish poisoning), and as part of this project, during the 2020–21 and 2021–22 fishing season. All samples were verified to be Spanish Mackerel via qPCR analyses.

During the 2014–2015 fishing season, a total of 84 samples were collected and analysed for CTXs (Table A8, Appendix A). Using LC-MS analysis, P-CTX1B was detected in 5 fish specimens from NSW (Table A8, Appendix A). Among the 13 fish specimens collected in QLD, P-CTX1B was found in the liver and flesh tissues of six different fishes.

For the 2020–2021 fishing season, 101 fish were collected and analysed for CTXs. Fish ranged in weight from 2.7–21.8 kg and were collected from locations in northern NSW and QLD. P-CTX-1B was below the limit of detection (LOD) for all flesh and liver samples analysed via LC-MS (

Table A22, Appendix A).

For the 2021–2022 fishing season, 148 fish were collected and analysed for CTXs. Fish ranged in weight from 2.8–21.5 kg and were collected from locations in northern NSW and QLD. P-CTX-1B was below the limit of detection (LOD) for all flesh and liver samples analysed via LC-MS (Table A15, Appendix A).

It was determined that the ELISA test kit was more sensitive with a lower LOD than the LC-MS method for the measurement of CTX-1B. Hence, it was decided to verify the absence of CTXs in specimens by analysing them using the ELISA CTX method. The 148 specimens from the 2021–2022 fishing season were analysed as described above. P-CTX-1B amounts were detected in 18 flesh and 14 liver samples (35 fish of 148). Of the 35 fish with detectable CTXs for the ELISA test kit, most were below the range where toxin amounts were quantifiable (Table A15, Appendix A). Three samples from the fishing season 2021–2022 exceeded the recommended ≥ 0.01 µg kg$^{-1}$ P-CTX-1 B equivalents set by the U.S. Food and Drug Administration (FDA) as a guidance level for CTXs in seafood. The highest level found was 0.012 µg kg$^{-1}$ (Table A15, Appendix A).

The prevalence of CTXs in fish caught in QLD was higher than those caught in NSW over the three fishing seasons, based on data from LC-MS for the 2014–2015 samples and data from the ELISA method for the 2021–2022 samples. The ELISA method revealed that in the 2021/22 fishing season, no fish caught in NSW waters contained CTXs (0 of 32), whereas 35 of 116 fish (30%) caught in QLD contained low levels of CTXs ( Table A15, Appendix A). These CTX+ fish were collected from the vicinity of Fraser Island, Hervey Bay, Rockhampton, Wigton Islands and Coolum.

A known ciguatoxic Spanish Mackerel was extracted periodically alongside the environmental samples and showed consistent detections for P-CTX-1B, despite the low level of CTX and large variability (Table A1 and Table A2, Appendix A). Full spike results showed a comparatively low recovery of P-CTX-1B from tissue samples across both seasons, which was lower than what had been previously observed using the extraction protocol (Table A3, Appendix A). The extraction of CTXs from fish matrix tissue presents unique challenges, with extraction efficiencies observed to be comparatively low and variable in our study. This is in concordance with what has been previously

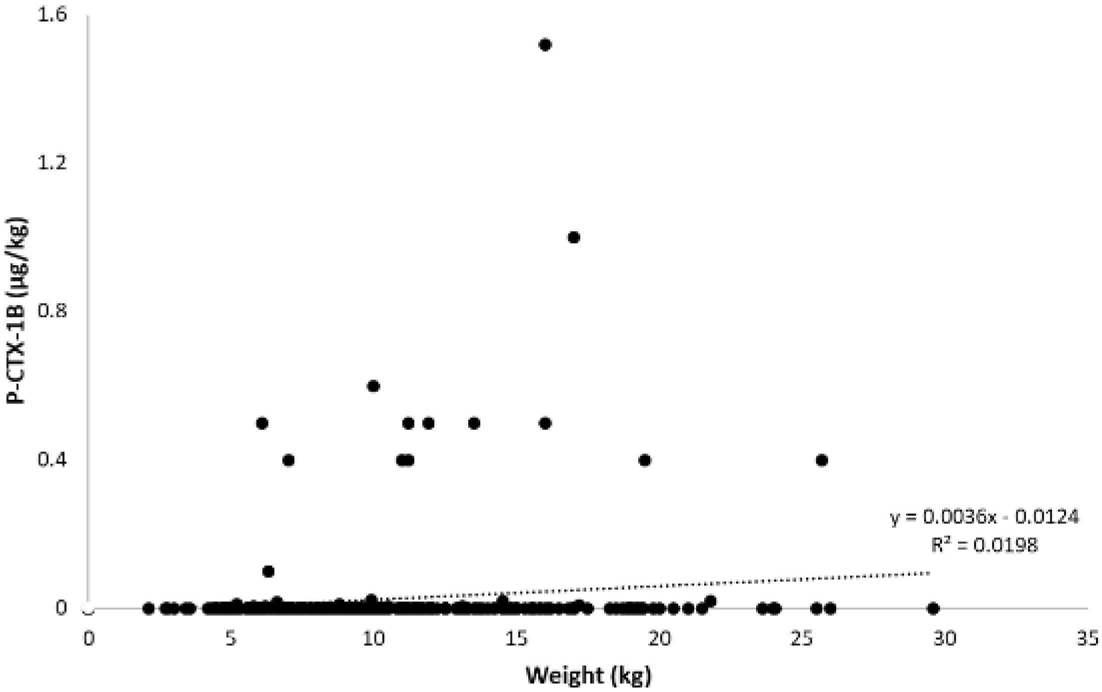

**Figure 4.** Fish weight (kg) and CTX content using LC-MS (µg/kg) of all Spanish Mackerel samples collected (2015-2022).

observed in other studies with Spanish Mackerel of general fish tissue samples spiked with P-CTX-1B prior to extraction, that reported recovery rates of 25.8% (Kohli et al. 2017), 44% (Murray et al. 2018), and 24–110% (Spielmeyer et al. 2021). Unlike other marine biotoxins and shellfish matrices, CTX extraction from fish tissue is generally less efficient. These results underscored the necessity for further research and optimisation of extraction methods to enhance detection and quantification of CTXs in fish samples.

To ensure confidence in the non-detects of the fish samples, 16 fish were selected based on their length, weight and geographical location and were re-extracted a second time at the Cawthron Institute. All samples were again blank giving confidence that the extraction protocol was not a significant factor in the ability to recover CTXs.

No significant correlation was observed between the amount of P-CTX-1B and the weight of the fish (Figure ). Despite the absence of a statistical correlation, a higher number of fish below 15 kg showed the presence of CTXs rather than the larger specimens, an observation that aligns to research conducted in French Polynesia on other fish species (Gaboriau et al., 2014).

### 2.3.2 Analysis of samples from QLD Health and statistical analyses

Nineteen outbreaks of CP were reported to QLD Health over the period 2019–2023 (Figure 3, Figure ). Of these, information on the size and weight of Spanish Mackerel associated with these outbreaks was collected and P-CTX 1B was measured using LC-MS. These data were added to our dataset from fishing season 2014–2015 to examine the relationship of fish size with CTXs.

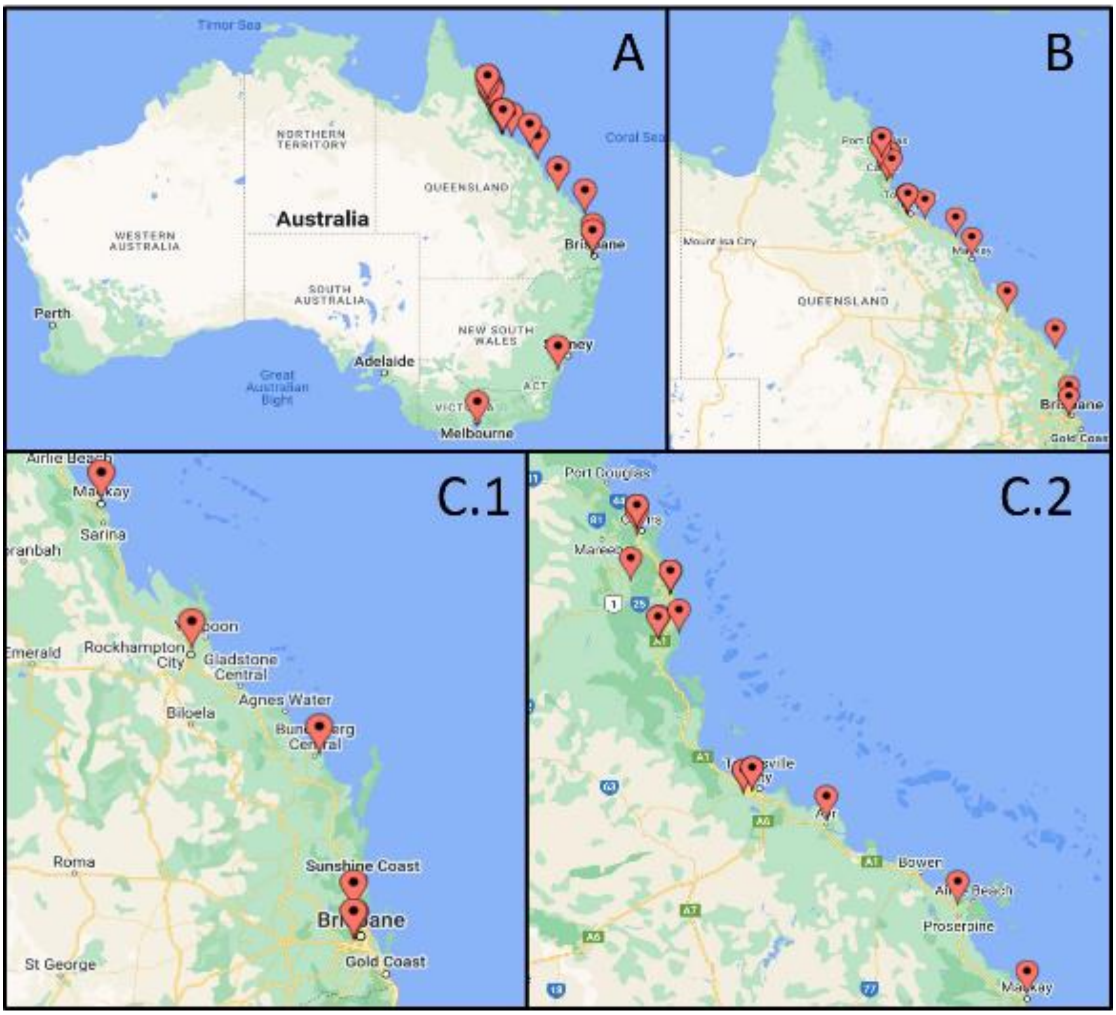

**Figure 5.** Map of the sites where CP cases occurred over the period 2019–2023 in information provided by QLD Health (A). Queensland (B). Brisbane to Mackay (C.1) and Mackay to Port Douglas (C.2).

To further explore the possible relationship between fish size and CTX contamination, physical data from fish samples that tested positive and negative for CTXs were combined and plotted together (**Error! Reference source not found.**). As the length of the fish increased, its weight also increased as would be expected ie Onespot Snapper (Figure B1, Appendix B), Flowery Rockcod (Figure B2, Appendix B), Red Bass (Figure B3, Appendix B) and Yellowedge Coronation Trout (Figure B4, Appendix B). However, there is no direct evidence to suggest that fish below a certain weight are more likely to contain CTXs, as observed by Oshiro et al., (2010) (Figures B1–B4, Appendix B). Among the 25 positive samples of our study, 14 fish had a weight below 15 kg and 7 fish had a weight below 10 kg. No statistical correlation was observed between fish weight/length and likelihood of containing CTXs (Figure 7).

Fish caught in QLD, particularly in the Fraser inshore region and Hervey Bay, have been linked to CP. These areas are within the Great Sandy Marine Park and include Platypus Bay where CP has been well-documented since the late 1970s and 1980s. The region boasts extensive seagrass meadows, and Spanish Mackerel, Barracuda and Blotched-javelin caught here have all been associated with CP.

Spanish Mackerel are the largest mackerel species in Australian waters, known for their size, taste, and the excitement of catching them. While they can reach lengths of up to 2.4 m and weights of up to 70 kg, such large specimens are now rarely caught. The largest recorded catch in recent years was a 54 kg fish off Fraser Island in 2015. Interestingly, data from the three fish responsible for CP intoxication revealed that fish of varying weights can carry different amounts of CTXs (0.6, 1 and 0.4 µg/kg, as shown in **Error! Reference source not found.**). These specific fish weighed 10, 17, and 25 kg (Table A8, Appendix A), with the largest fish having the lowest level of CTXs. These findings again suggested that there is no clear correlation between fish weight and CTX concentration.

Historically, most CP cases along the east coast of Australia have been associated with Spanish Mackerel caught south of approximately Mackay (around 21°S latitude). However, there have been no new reports of CP in NSW since 2018. This information parallels our finding of comparatively little or no CTXs in the Spanish Mackerel collected in our 2020–2021 and 2021–2022 fishing seasons with LC-MS, which was notably lower than was found in 2014-–2016. Potential environmental factors associated with CTXs in QLD and NSW are reviewed in the following section.

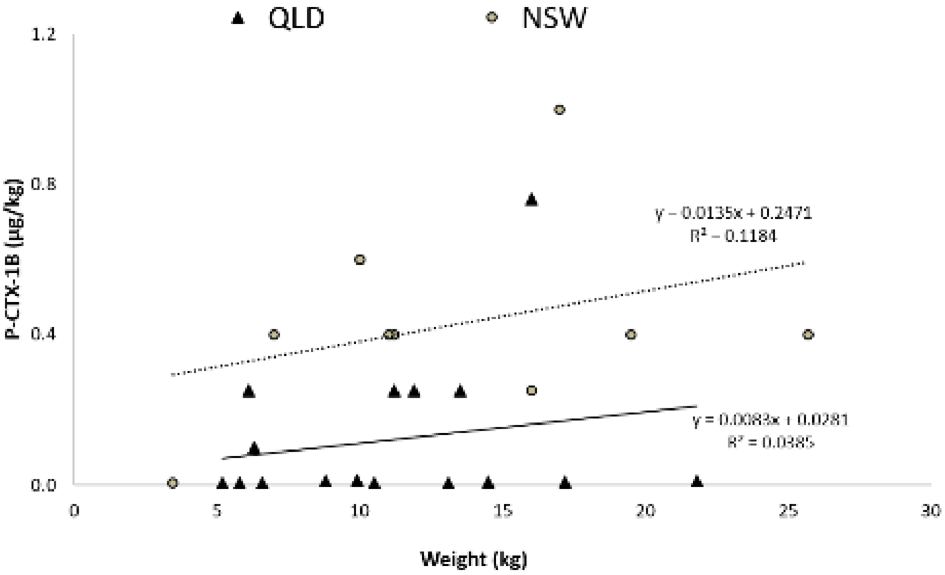

**Figure 4.** CTX content in Spanish Mackerel according to the weight and geographical location in which they were collected. Sample collected between 2015–2022. Dotted line represents NSW trend, black line represents QLD trend.

### 2.3.3 Effects of natural disturbances on Spanish Mackerel CTX content

Several studies have connected natural disturbances, such as cyclones with increased cases of CP, as reported in Rongo and van Woesik, (2013). In the same study, the authors noticed a relationship between the increase of CP cases and the increase of severity of disturbance. This correlation coincided also with the inter-annual cycle of El Niño Southern Oscillation (ENSO).

It appears that the substantial waves generated by cyclones have the effect of resetting the pattern of algal succession (Rongo and van Woesik 2013). This, in turn, creates favourable conditions for the establishment of ciguatoxic dinoflagellates, Consequently, this phenomenon raises the likelihood of CP. For instance, cyclones can mix and upwell ocean waters, bringing nutrients from deeper layers to the surface. This increased nutrient availability can promote the growth of phytoplankton, including *Gambierdiscus*, and lead ultimately to an increase of algal blooms. Moreover, previous studies have proposed that early-successional, opportunistic turf algae (such as *Gambierdiscus* spp.), in comparison to late-successional algae, are characterised by higher nutrient content and enhanced palatability (as observed in Littler & Littler, 1980, and Steneck & Dethier, 1994). In the Cook Islands, after the cyclones of 2003–2005, there was a notable increase in the prevalence of these opportunistic turf algae, which play a significant role as hosts for ciguatoxic dinoflagellates, as documented in Cruz-Rivera and Villareal, 2006. This increase heightened the potential for the transfer of CTXs into the food web through herbivorous fish.

The 2014–2015 cyclone season in northern Australia was below average, but unusually intense: only seven cyclones affected the Australian region during the season (November–April), but almost all belonged to category three, four or five (Table 4). In the Australian region, this was the first season in the last 35 years where every cyclone, regardless of whether they made landfall or not, attained the status of severe tropical cyclones, according to the BOM climatologist Joel Lisbonbee (https://www.9news.com.au/national/australia-s-strange-2014-15-cyclone-season/05b40d95-a193-

4ca9-8533-7953bdfee6af, Figure 8). On the other hand, in the 2021–2022 cyclone season only two out of ten were categorised as severe BOM reports, http://www.bom.gov.au/, Figure 8). These climatic events could be associated with the higher proportion of CTXs and greater number of CP cases observed in the 2016 peak of CP cases. However, it is worth noting that the low disturbance frequency observed in the 2021–2022 season could potentially increase the probability of CP events. These changes in cyclone patterns could trigger a series of societal and ecological consequences. A fear of CP could lead to a decline in fishing activities (Rongo and van Woesik, 2013; Chinain et al., 2023), an increase in fish populations and a decrease in reported CP cases. This, paradoxically, fosters the belief that reef fish are safe to consume, potentially leading to overfishing and can elevate the risk of CP.

**Table 4** Locations impacted by cyclonic disturbances and the number of such disturbances during the years 2012–2015 (http://www.bom.gov.au).

| Place affected | Cyclonic Storm | Severe Cyclonic Storm | Very Severe Cyclonic Storm | Extremely Severe Cyclonic Storm |
|---|---|---|---|---|
| Cape York Peninsula | 1 | - | - | - |
| East Timor | - | - | 1 | 1 |
| Indonesia | 1 | - | - | 1 |
| New Caledonia | - | 1 | - | - |
| New Zealand | - | - | - | 1 |
| Northern Territory | 1 | - | - | 1 |
| Papua New Guinea | - | - | - | 1 |
| Queensland | 1 | 1 | 2 | 3 |
| Solomon Islands | - | 1 | - | 1 |
| South Australia | - | - | 1 | - |
| Tonga | 1 | - | - | - |
| Western Australia | 2 | 4 | 3 | - |

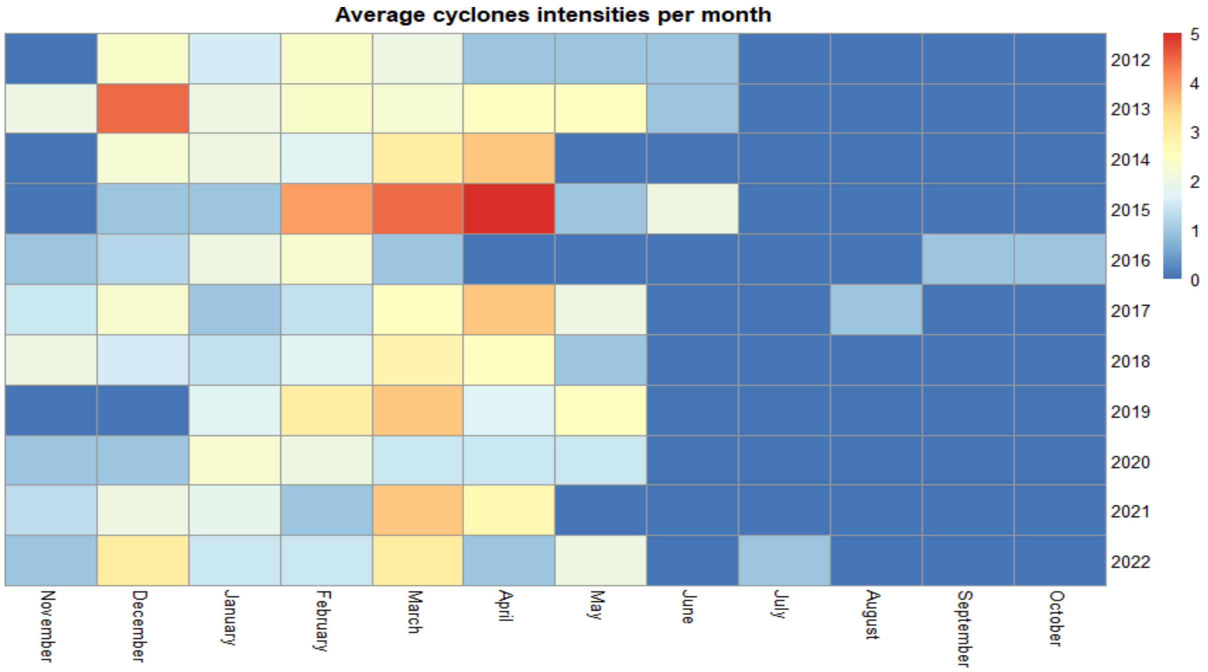

**Figure 5.** Average cyclone intensities per month from 2012–2022. Intensity 0 corresponds to undetected activity, 0–1 to Depression (wind between 31–50 km/h*), 1–2 to Deep Depression (wind between 51–62 km/h*), 2–3 to Cyclonic Storm (wind between 63–88 km/h), 3–4 to Severe Cyclonic Storm (wind between 89–117 km/h*), 4–5 to Very Severe Cyclonic storms (wind between 118–165 km/h*), 5 to Extremely Severe Cyclonic Storm (wind between 166–220 km/h*), above 5 to Super Cyclonic Storm (wind more than 220 km/h*). *3 min average measurements.

A positive correlation between SOI (southern oscillation index), as well as El Niño or La Niña events and CPUE (catch-per-unit-effort) for Spanish Mackerel has been previously observed, with higher catches during La Niña events and lower during El Niño (Welch et al., 2014). Over the past three years, Spanish Mackerel total catch has declined, accompanied by a decrease in CP reports and CTX levels in the individual fish caught. An increase in CTX content in fish and the potential for CP outbreaks remain significant concerns. Therefore, sampling not only Spanish Mackerel, but also *Gambierdiscus* species in known CP hotspots is likely to yield positive material for CTXs to validate the use of different strategies to detect them. A more extensive sampling approach will provide insights that contribute to a better understanding of CP, knowledge that can be used to define monitoring strategies.

# 3 Discussion

Food safety risks in Australia and New Zealand are managed under a joint food regulatory system. Core elements of that system are described as “model food provisions” and food production and labelling standards named by the “Australia New Zealand Food Standards Code” (Code). The model food provisions and the Code have been adopted by each Australian state and territory as the basis for their respective food legislation (Australian Food Regulation Secretariat).

Food Standards Australia New Zealand (FSANZ), a statutory authority in the Australian Government health portfolio, maintains the Code subject to policy set by the Australia and New Zealand Ministerial Forum on Food Regulation to ensure that food is safe and suitable for human consumption. In Australia, the model food provisions and the Code are enforced domestically by state and territory departments, agencies and local councils. In addition, the Australian Federal Government Department of Agriculture, Fisheries and Forestry (DAFF) enforces imported food compliance with the Code. Within NSW, the NSW Food Authority is the relevant domestic regulator. The relevant NSW legislation is the *Food Act 2003* (NSW), the Food Regulation 2015 (NSW) and the Code. This includes a general requirement under the Food Act to ensure food supplied is both safe and suitable (ss 16 and 17) and specific requirements for managing seafood safety risks through a Seafood Safety Scheme under Part 11 of the Food Regulation 2015 (NSW).

CP risk is highly complex and management of CP requires a multifaceted approach that traverses environmental, food safety and health variables. A flow diagram (Figure 9) that summarises current CP responses and needs (WHO, 2020) highlights the many intricate subjects involved in understanding and managing CP. The current status of CP management and regulation in NSW, and the rest of Australia, reflects the limitations and knowledge gaps of this syndrome. Within the Food Standards Code, Schedule 19 *Maximum levels of contaminants and natural toxicants*, provides maximum limits for algal toxins, such as paralytic shellfish toxins, diarrhetic shellfish toxins and amnesic shellfish toxins (FSANZ, 2023). There is no equivalent maximum concentration limit for CTXs in seafood in the Food Standards Code. This is primarily due to testing limitations and limited reference standard availability. In addition, in Australia the position has been that risk is dependent on the size and type of fish consumed. As a result, in lieu of testing, management approaches to CP are precautionary with fishing bans and restrictions on locations and fish sizes for known ‘hot spots’. The 2006 Guide to the Australian Primary Production and Processing Standard for Seafood developed by FSANZ (FSANZ, 2006), notes that CTXs are a potential hazard and provides similar advice to skippers to avoid fishing in areas that are known to be linked to CP outbreak and/or be aware of size restrictions on certain fish species. This aligns with the general principle that food contaminants should be as low as reasonably achievable regardless of whether maximum limits are established (FSANZ, 2006).

**Figure 6.** Flow diagram showing ciguatera poisoning responses and needs (from FAO and WHO, 2020).

Such measures and guidelines are in place at the Sydney Fish Market (Sydney Fish Market, 2015) to safeguard consumers against CP. For example, Platypus Bay, QLD is a prohibited supply region for Spanish Mackerel and size restrictions (10 kgs whole or 8 kg for headed and gutted fish) are in place for Spanish Mackerel caught from other QLD locations and NSW waters.

Current advice for consumers is published on the NSW Food Authority website: https://www.foodauthority.nsw.gov.au/consumer/food-poisoning/fish-ciguatera-poisoning

## 3.1 Risk assessment based on project data

Risk assessments for food contamination consists of four formal science-based steps: hazard identification, hazard characterisation, exposure assessment and risk characterisation (FAO and WHO, 2023). Table 5 discusses these steps in the context of the available information and the results of this project.

**Table 5** Summary of risk assessment process (FAO and WHO, 2023) within the context of the current project.

| Risk Assessment Process | Process Definition | Status |
|---|---|---|
| 1. Hazard identification | The identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods. | • CTXs are highly potent neurotoxins that can bioaccumulate and biotransform in the marine food chain. Human illness occurs when contaminated seafood is consumed.<br>• Some of the highest risk fish are predatory species from warm water, tropical areas.<br>• Currently there is no valid method of establishing whether a specific ‘catch’ from a high-risk area does or does not pose a CP risk. |
| 2. Hazard characterisation | The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical, and physical agents which may be present in food. | • CTXs cause a range of gastrointestinal, neurological and cardiovascular symptoms, with a complex array of clinical manifestations.<br>• In humans, the individual response to ciguatoxin exposure can vary, with potential for chronic and recurring issues. This is also related to portion size (dose) and previous exposure to ciguatoxins.<br>• P-CTX-1 is the most potent of known ciguatoxins, but information is limited, and we do not yet understand how the other (more than 30) analogues contribute to illness.<br>• CP cases linked to Spanish Mackerel caught in NSW waters appeared to spike between 2014 and 2018, with no previous reports since 2002. Since 2018, there have been no confirmed cases of CP linked to Spanish Mackerel caught in NSW waters. The reason for this is not clear, and may be related to environmental variables, fisher awareness or a combination of both.<br>• The nature and extent of patient reporting and clinical diagnosis of cases of CP is unknown but is believed to be poor. |

**Table 6** (continued)

| Risk Assessment Process | Process Definition | Status |
|---|---|---|
| 3. Exposure assessment | The qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food, as well as exposures from other sources if relevant. | • CTX levels can vary between individual fish, and tend to be more concentrated in the head, roe, liver or other viscera. The metabolic processes of ciguatoxins are complex. Different fish may metabolise toxins differently (Ikehara et al., 2017).<br>• Spanish Mackerel determined levels of the ciguatoxin analogue P-CTX-1 (also known as P-CTX-1B) via LC-MS, ELISA and N2a assays in Spanish Mackerel. These baseline data are some of the most extensive Australian data collected in terms of the number of Spanish Mackerel tested and in terms of the timeframes over which the studies occurred (2015 and 2021–2022). P-CTX-1B results were reported between 0.005–0.43 ng/ml (ELISA), 0.02–0.14 ng/g (N2a) and 0.023-0.063 ng/ml (LC-MS).<br>Samples of a cooked meal or associated fish are not always available during illness investigations. Spanish Mackerel samples linked to CP in NSW reported between ‘not detected’ and 1 µg/kg P-CTX-1B (**Error! Reference source not found.**). This is up to two orders of magnitude higher than the USFDA guidance level of 0.01 µg/kg P-CTX-1B, which is the same level that the European Food Safety Authority’s panel on Contaminants in the Food Chain estimated should not have any negative health impacts.<br>• Routine testing of seafood for ciguatoxins has been limited by reference standard availability. A concentration of 0.02 ug/kg CTX1B-equiv is the lowest reported level of ciguatoxins in fish associated with symptoms in humans, but the insufficient amount of animal and human exposure data has limited the establishment of an acute reference dose (FAO and WHO, 2020).<br>• In NSW the food consumed by one reported CP case was analysed and found not to contain P-CTX-1, despite strong clinical symptoms, indicating there are limitations in using current analysis methods to quantify exposure to CP. |

**Table 7** (continued)

| Risk Assessment Process | Process Definition | Status |
|---|---|---|

| | | |
|---|---|---|
| 4. Risk characterisation | The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterisation and exposure assessment. | • Risk characterisation is limited beyond current guidelines.<br>• As the level of CTXs can be highly variable between each fish, and fish of different species, it is difficult to extrapolate beyond the specific ciguatoxin analogue tested in each individual fish.<br>• The size of Spanish Mackerel does not seem to be linked to toxin level, but most reported illness cases in NSW were linked to larger (>10 kg) Spanish Mackerel.<br>• Modelling of environmental data may provide insight over longer term studies (e.g. temperature, cyclones, southern EAC intensification). While environmental data may indicate 'hot spot' reefs, Spanish Mackerel are a migratory species, their origin is not easily distinguished, and they can travel several 100 kms.<br>• CP cases are largely underreported (Figure 3. Ciguatera notifications and outbreaks, QLD and NSW, 2013 - 2022 (Farrell et al., 2016a, 2016b, Edwards et al., 2019, Szabo et al., 2022).), and this has limited our understanding of illness prevalence. Nationally consistent collection and reporting of epidemiological data and linking to toxicological data/case information was identified as a critical issue by the National Ciguatera Strategy (Beatty et al., 2019). |

From the literature and our own data, we have compiled information on the P-CTX-1B levels in any fish known to be associated with CP illnesses in Australia (**Error! Reference source not found.**) and overseas (Table 9). This shows that levels above ~0.1 µg kg$^{-1}$ have been known to be associated with illness, with mean levels found in implicated fish flesh of 1.2 µg kg$^{-1}$ (from 6 Australian samples) and 1.3 µg kg$^{-1}$ (from 16 overseas samples) (Table 8, Table 9). This compares to the US FDA 'guidance level' of 0.01 µg kg$^{-1}$, which was established due to the consideration that levels above 0.1 µg kg$^{-1}$ may cause illness, based on the results of the mouse bioassay (Lewis et al., 1991). There are several other factors aside from the levels of P-CTX-1B that may lead to differences in toxicity among samples. These are the fact that other CTX analogs likely exist in these fish alongside P-CTX-1B, which we currently cannot measure accurately using LC-MS, as we lack standards for these analogs. The presence of these additional analogs may increase the overall toxicity at low levels of P-CTX-1B. As several of the fish in this study were found to contain P-CTX-1B at very low levels, it appears that further research is required to determine the appropriate safe level of P-CTX-1B in fish in Australia. In any study such as this, it would be necessary to compare fish using several methods, such as toxicity assays (bioassays, or other assays such as the receptor binding assay) and LC-MS/MS.

CTX remains a significant risk for the fishing industry and Australian seafood consumers (Table A6, Appendix A). The work conducted under this project has opened several lines of enquiry that show promise for future advancements, particularly with rapid test kits. Unfortunately, none of the analytical methods currently available are suitable for real-time risk management as they are expensive, require laborious extraction of toxins prior to analysis, and this can only be done in a laboratory setting.

**Table 8** P-CTX-1B levels in fish known to be associated with illness with CP symptoms in Australia.

| Location | Fish species | P-CTX-1B in flesh (µg $kg^{-1}$) | Reference |
|---|---|---|---|
| Capel Bank, Coral Sea | Purple rock cod | 0.100 | SIMs Unpublished data |
| Scotts Head, NSW | Spanish Mackerel | 0.400 | Farrell et al., 2016a |
| Evans Head, NSW | Spanish Mackerel | 0.600–1.000 | Farrell et al., 2016a |
| Capel Bank Seamount | Redthroat Emperor | 0.023 | Farrell et al., 2017 |
| Capel Bank Seamount | Purple rock cod | 0.069 | Farrell et al., 2017 |
| Capel Bank Seamount | Green Jobfish | 0.006–0.036 | Farrell et al., 2017 |
| Crowdy Head, NSW | Spanish Mackerel | 0.93 | Farrell et al., 2016b |
| Crescent Head, NSW | Spanish Mackerel | 0.11–0.37 | Farrell et al., 2016b |
| Gove, Arnhem Land, NT | Coral Cod | 3.900 | Lucas et al., 1997 |
| Queensland | Sawtooth Barracuda | 1.100 | Hamilton et al., 2010 |

**Table 9** Toxicity and level of P-CTX1B in leftover meals from CP incidents in Japan (Oshiro et al., 2010). 1 MU toxicity equals 7 ng of P-CTX-1B in fish flesh (Yasumoto, 2005).

| Number of CP cases associated with this outbreak (in Japan) | Common name | Scientific name | Test sample | Mouse bioassay toxicity (MU/g) | P-CTX-1B (µg $kg^{-1}$) |
|---|---|---|---|---|---|
| 2 | Snapper | *Lutjanus sp.* | Cooked flesh | 0.290 | 2.030 |
| 4 | Yellow-edged Coronation Trout | *Variola louti* | Raw flesh | 0.100 | 0.700 |
| 13 | Flowery Rockcod | *Epinephelus fuscoguttatus* | Cooked flesh<br>Soup[1] | 0.050<br><0.025 | 0.250<br>0.175 |
| 17 | Onespot Snapper | *Lutjanus monostigma* | Cooked flesh | >0.200 | 1.400 |

**Table 10 (continued)**

| Number of CP cases associated with this outbreak (in Japan) | Common name | Scientific name | Test sample | Mouse bioassay toxicity (MU/g) | P-CTX-1B (µg kg$^{-1}$) |
|---|---|---|---|---|---|
| 20 | Onespot Snapper | *Lutjanus monostigma* | Cooked flesh | >0.800 | 5.600 |
| 22 | Onespot Snapper | *Lutjanus monostigma* | Raw flesh<br>Mixed soup2 | >0.200<br>0.025 | 1.400<br>0.175 |
| 23 | Onespot Snapper | *Lutjanus monostigma* | Mixed soup2 | >0.20 | 1.400 |
| 24 | Yellowedge Coronation Trout | *Variola louti* | Raw flesh<br>Mixed soup2 | 0.400<br>0.100 | 2.800<br>0.700 |
| 26 | Yellowedge Coronation Trout | *Variola louti* | Flesh3 | >0.200 | 1.400 |
| 26 | Yellowedge Coronation Trout | *Variola louti* | Flesh3 | 0.100 | 0.700 |
| 28 | Yellowedge Coronation Trout | *Variola louti* | Raw flesh | 0.100 | 0.700 |
| 31 | Red Bass | *Lutjanus bohar* | Cooked flesh | 0.100 | 0.700 |
| 32 | Yellowedge Coronation Trout | *Variola louti* | Raw flesh | 0.050 | 0.350 |

[1] Assay was performed after removing flesh and bones present in the soup.
[2] Assay was performed after removing bones present in the soup.
[3] The flesh had been lightly rinsed with hot water.

# 4 Recommendations

## 4.1 Public health

- New evidence from this project does not support a change to current CP risk management for Spanish Mackerel in Australia. Risk management should continue to include size restrictions and prohibitions on sale of fish caught in known CP ‘hot spots’.

- Maintenance of education for consumers and fishers is important to promote awareness on the potential risks of CP. This education should cover the entire QLD and NSW coastline because of the high likelihood of Spanish Mackerel ranging further into southern NSW waters as sea temperatures increase and the EAC pushes further southwards.

- As CTXs have been found to be higher in liver and viscera than fillets, recommendations that Spanish Mackerel be gutted prior to sale may be considered.

- Consumer education should include advice on avoiding cooking and eating the head, roe, liver or other viscera as CTXs are concentrated in these parts and may increase exposure.

- Engagement with health agencies to improve data collection on CP illnesses, involving GPs and health organisations would provide valuable data needed to improve risk assessment.
- Review of current CP monitoring and response to ensure case data (food consumption, fish size, etc) is collected and samples submitted for CTX analysis where possible.
- Investigation to support the development of a market for frozen product could lead to a 'test and release' approach. Results obtained in this process would lead to valuable data to better assess and manage this risk.
- Australian food safety management should take note of recommendations of the Codex Committee on Contaminants in Foods (CCCF16) 'Code of practice for the prevention or reduction of Ciguatera Poisoning' when they are released later in 2024.

## 4.2 Analytical

- Future research on CTX detection needs to focus on the sample extraction procedure, as it currently requires a well-equipped chemical laboratory, takes 6+ hours and can show relatively low toxin recovery rates. A faster extraction protocol would enable all CTX detection methods: LC-MS, ELISA and cell bioassays to be conducted in a more timely and cost effective manner, thereby improving toxin recovery rates.
- The ELISA test kits showed considerable promise to detect CTX, especially at low concentrations. However, they are not currently useful to those without access to a laboratory or in the field, as they require a fully equipped chemical analysis laboratory to undertake the complex sample extraction process. Further research should address the challenges of baseline drift, validate the kit for use with P-CTX-1B in key fish species and determine the LOD for this method.
- The CTX ELISA kit can be used as a pre-screening tool in future research as it is sensitive and more cost-effective than LC-MS. Other CTX detection technologies, including biosensors need to be considered in the scope of future detection approaches.

## 4.3 Environmental and biological studies

- The approach taken in this project to understand fish biology and migration, as well as environmental parameters, was useful to better understand the complex issue of CTX distribution along the Australian coastline. We recommend similar approaches in future work.
- Further fish sampling is recommended to better underpin risk management. Initially this should focus on known risk species and hot spots to increase the prevalence of CTX detection and therefore maximise information collected.
- While Spanish Mackerel is a known hazard, other fish species, such as Coral Trout are leading causes of CP, particularly in QLD. The risk of CP may be simpler to mitigate in a fish with a more localised home range, rather than one that migrates long distances. Future research on other leading CP vectors is important.
- On-going fundamental research on Spanish Mackerel stocks using population genetic approaches in combination with CTX analyses would be useful in understanding risk in relation to population biological factors, migratory patterns and potential feeding areas where CTX uptake may occur.

- Further research analysing environmental correlates of CP and CTXs is needed to understand the proximate causes of changes in CP frequency. Internationally, climate change is expected to lead to increases in CP due to increasing cyclones, storms, coral damage and marine heatwaves. The impact of these factors in Australia is not known and needs to be investigated.

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# Appendix A – Tables

**Table A1** Average CTX content in CTX+ samples from 2021 fishing season.

| 2021 Season | | |
|---|---|---|
| n=12 | | |
| Average (ng/mL) | 0.440 | 0.09 µg/kg |
| Standard deviation | 0.180 | |
| Relative standard deviation | 42% | |
| Highest (ng/mL) | 0.800 | |
| Lowest (ng/mL) | 0.200 | |

**Table A2** Average CTX content in CTX+ samples from 2022 fishing season.

| 2022 Season | | |
|---|---|---|
| n=8 | | |
| Average (ng/mL) | 0.300 | 0.06 µg/kg |
| Standard deviation | 0.140 | |
| Relative standard deviation | 46% | |
| Highest (ng/mL) | 0.532 | |
| Lowest (ng/mL) | 0.161 | |

**Table A3** Recovery values (%) of samples spiked with P-CTX-1B

| Fish ID | Recovery of P-CTX-1B (%) |
|---|---|
| UTS 17F | 22 |
| UTS 17F #2 | 18 |
| UTS 17F #3 | 19 |
| UTS 114 | 17 |
| UTS 146 | 16 |
| UTS178 | 10 |
| UTS 201 | 9 |
| FRDC229F | 18 |
| MAC117F | 16 |

**Table A4** The known congeners of CTXs and the source they were originally described from.

| Origin | Toxin Name | Molecular Ion [M +H]+ | Source | | Toxicity[1] | References |
|---|---|---|---|---|---|---|
| | | | Common name | Scientific name | | |
| Pacific (Type I) | CTX1B, CTX-1 | 1111.6 | Giant Moray eel | *Gymnothorax javanicus* | 0.35 µg/kg (CTX1B) | Murata et al., 1990<br>Lewis et al., 1991 |
| | | | Giant Moray eel | Gymnothorax javanicus | 0.25 µg/kg (CTX-1) | Lewis et al., 1991 |
| | 52-epi-54-deoxy-CTX-1 (CTX-2) | 1095.5 | Giant Moray eel | *Gymnothorax javanicus* | 2.30 µg/kg | Lewis et al., 1991 |
| | 54-deoxy-CTX-1B (CTX-3) | 1095.5 | Giant Moray eel | *Gymnothorax javanicus* | 0.90 µg/kg | Lewis et al., 1991 |
| | CTX4A | 1061.6 | Dinogflagellate | *Gambierdiscus* sp.<br>*G. polynesiensis* | 12.00 µg/kg | Chinain et al., 2010<br>Yasumoto et al., 2000 |
| | CTX4B | 1061.6 | Dinogflagellate | *Gambierdiscus* sp.<br>*G. polynesiensis* | 20.00 µg/kg | Chinain et al., 2010<br>Yasumoto et al., 2000 |
| Pacific (Type II) | CTX3C | 1023.6 | Dinogflagellate | *Gambierdiscus* sp.<br>*G. polynesiensis* | 2.50 µg/kg | Satake et al., 1993<br>Chinain et al., 2010 |
| | 49-epi-CTX-3C | 1023.6 | Dinogflagellate | *Gambierdiscus* sp.<br>*G. polynesiensis* | 8.00 µg/kg | Satake et al., 1993<br>Chinain et al., 2010 |
| | M-seco-CTX- 3C | 1041.6 | Dinogflagellate | *Gambierdiscus* sp.<br>*G. polynesiensi* | 10.00 µg/kg | Satake et al., 1993<br>Chinain et al., 2010 |
| Caribbean | C-CTX-1 | 1141.6 | Horse-eye jack | *Caranx latus* | 3.60 µg/kg | Vernoux & Lewis, 1997<br>Pottier et al., 2002 |
| | C-CTX-2 | 1141.6 | Horse-eye jack | *Caranx latus* | Toxic | Vernoux & Lewis, 1997<br>Pottier et al., 2002 |
| Indian | I-CTX-1 | 1141.6 | Red Bass | (Lutjanus bohar) | Toxic | Hamilton et al., 2002b |
| | | | Red Emperor | (Lutjanus sebae) | Toxic | Hamilton et al., 2002b |

[1] $LD_{50}$ doses calculated via i.p. injection in mice.

**Table A5** CTXs detected in seafood in Australia and the method of detection.

| Common name | Species | Scientific name | Source | CTX | Method of detection[1] | References |
|---|---|---|---|---|---|---|
| Barracuda | Pickhandle Barracuda | *Sphyraena jello* | Hervey Bay, QLD, Australia | CTX – positive | TLC & MBA | Lewis & Endean, 1984 |
| Eel | Giant Moray Eel | *Gymnothorax javanicus* | QLD, Australia | CTX-1, CTX-4B, CTX-2 CTX-3, P-CTX-1, P-CTX-2, P-CTX-3; analogues of CTX 3C: 2,3-dihydroxyCTX3C and 51-hydroxyCTX3C | HPLC/MS<br>HPLC/HNMR<br>TLC<br>DLBA<br>MBA | Scheuer et al., 1967<br>Labrousse & Matile, 1996<br>Legrand et al., 1989<br>Murata et al., 1990<br>Lewis et al., 1991<br>Lewis & Jones, 1997<br>Satake et al., 1998 |
| Coral Trout | Coral Trout | *Plectropomus* spp. | Great Barrier Reef, Australia | CTX-1, CTX-2, CTX-3 | HPLC/MS<br>MBA | Lewis & Sellin, 1992 |
| Grunt | Blotched Javelin | *Pomadasys maculatus* | Platypus Bay, QLD, Australia | CTX-1, CTX-2, CTX-3 | HPLC/MS<br>MBA | Lewis & Sellin, 1992 |
| Mackerel | Spanish Mackerel | *Scomberomorus commerson* | Hervey Bay, QLD, Australia | CTX-1 CTX-2 CTX-3 |  | Lewis & Endean, 1984<br>Lewis & Sellin, 1992<br>Endean et al., 1993 |

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[1] TLC: thin layer chromatography; MBA: mouse bioassay; HPLC/MS: High-performance liquid chromatography-mass spectrometry; HPLC/HNMR: High-Performance Liquid Chromatography Nuclear Magnetic Resonance; DLBA: Diptera Larvae Bio Assay.

**Table A6** Schedule of Ciguatera High Risk Areas provided by Sydney Fish Market (2015).

| Prohibited species – To be rejected |
|---|
| Chinamanfish (*Symphorus nematophorus*) |
| Tripletail Maori Wrasse (*Cheilinus trilobatus*) |
| Humphead Maori Wrasse (*Cheilinus undulatus*) |
| Red Bass (*Lutjanus bohar*) |
| Paddletail (*Lutjanus gibbus*) |
| Giant Moray (*Gymnothorax javanicus*) |

Prohibited supply regions- reject consignments of listed species caught in these regions

| Region | Species |
|---|---|
| Kiribati | All warm water ocean fish |
| The following Queensland waters: | All warm water ocean fish |
| - Platypus Bay on Fraser Island, bounded by the co-ordinates: GPS South 25 – 01 – 991; North 153 – 11 – 761 | Spanish Mackerel (*Scomberomrous commerson*)<br>Mackerel (*Scomberomrous* spp.) – excluding Spotted and School Mackerel under 6 kg. |
| Marshall Islands | All warm water ocean fish |
| New Caledonia and Capel Bank | All warm water ocean fish |
| The following Northern Territory waters:<br>- Bremer Island<br>- Bonner Rocks<br>- Miles Island<br>- Immediate vicinity of Cape Arnhem<br>- North East Island and Connexion Island (both near Groote Eylandt Gove Peninsula, in the immediate vicinity of Nhulunbuy) | The following species:<br>- Pickhandle Barracuda (*Sphyraena jello*)<br>Bluespotted Rockcod (*Cephalopholis cyanostigmata*)<br>- Coral Trout (*Plectropomus* spp. & *Variola* spp.)<br>Red Emperor (*Lutjanus sebae*)<br>- Queensland Groper (*Epinephelus lanceolatus*)<br>Trevally (*Caranx* spp.) |
| Fijian waters | Coral Trout (*Plectropomus* spp. & *Variola* spp.) |

**Table A7** Maximum size limit for high-risk fish species (Sydney Fish Market, 2015).

| Common name | Scientific name | Size Limit (Maximum whole size in Kg) | | | | |
|---|---|---|---|---|---|---|
| | | NSW | QLD | NT | WA | Pacific countries |
| Pickhandle Barracuda | *Sphyraena jello* | | 10 | | | 10 |
| Coral Rockcod | *Cephalopholis* spp. and *Cephalopholis miniata* | | 3 | | | 3 |
| Coral Trout | *Plectropomus* spp. and *Variola* spp. | 6 | 6 | 6 | 6 | Reject |
| Yellowtail Kingfish & Samsonfish | *Seriola* spp. | | 10 | | | 10 |
| Mackerel (various), except Spanish Mackerel | *Scomberomorus* spp. | 10 | 10 | | | 10 |
| Giant Queenfish | *Scomberoides commersonianus* | | 10 | | | 10 |
| Red Emperor | *Lutjanus sebae* | | 6 | | | 6 |
| Goldspotted Rockcod** | *Epinephelus coioides* | | 10 | | | 10 |
| Flowery Rockcod** | *Epinephelus fuscoguttatus* | | 10 | | | 10 |
| Queensland Grouper** | *Epinephelus lanciolatus* | | 10 | | | 10 |
| Greasy Rockcod** | *Epinephelus tauvina* | | 10 | | | 10 |
| Surgeonfish | *Acanthuridae* spp.[#] | | 10 | | | Reject |
| Spangled Emperor | *Lethrinus nebulosus* | | 6 | | | 6 |
| Spanish Mackerel | *Scomberomorus commerson* | 10* | 8* | | | 10 |
| Trevally | *Caranx* spp. | | 6 | | | 6 |
| Tuskfish | *Choerodon* spp. | | 6 | | | 6 |

*10 kg whole or 8 kg gutted & headed; **reef cods; [#]all family members

**Table A8** LC-MS analysis of P-CTX-1B in samples of *S. commerson* flesh and liver collected in 2015, and from an analysis of fish implicated in CP events in NSW in 2014 (at end of Table).

| Sample Code | Location | Date of Catch | Length (cm) | Weight (kg) | P -CTX-1B in flesh (µg kg-1)[1] | P-CTX-1B in liver (µg kg-1)[1] |
|---|---|---|---|---|---|---|
| AIMS-1 | Davies Reef, QLD | 2/01/15 | 149 | 21 | ND | ND |
| AIMS-2 | Davies Reef, QLD | 2/01/15 | 105 | 6 | ND | ND |
| AIMS-4 | Port Douglas, QLD<br>(14°.47.88S 149°.25.18E) | 12/01/15 | 134 | 13.5 | <0.1 | <0.4 |
| AIMS-5 | Port Douglas, QLD<br>(14°.47.88S 149°.25.18E) | -- | 136 | 16 | 0.13 | 1.39 |
| AIMS-6 | Great Barrier Reef, Rockhampton, QLD<br>(22°.00.48S 152°.38.85E) | 23/01/15 | 110 | 6.3 | <0.1 | ND |
| AIMS-10 | Whitsundays, QLD<br>(Reef No: 19-138) | 12/01/15 | 106 | 6.1 | <0.1 | <0.4 |
| AIMS-11 | Whitsundays, QLD<br>(Reef No: 19-138) | 13/01/15 | 120 | 11.9 | <0.1 | <0.4 |
| AIMS-12 | Townsville, QLD<br>(19°.47.88S 144°.25.18E) | 12/01/15 | 117 | 11.2 | <0.1 | <0.4 |
| AIMS-13 | Whitsundays, QLD<br>(20°.01.45S- 149°.41.02E) | 13/01/15 | 103 | 5.8 | ND | ND |
| SFM-3 | Brunswick Heads, NSW | 2/02/15 | 120 | 8 | ND | ND |
| SFM-16 | Mooloolaba, QLD | 6/01/15 | 96 | 6 | ND | ND |
| SFM-19 | Port Bundaberg, QLD | 18/12/14 | 120 | 9.4 | ND | ND |
| SFM-33 | Mooloolaba, QLD | 14/01/15 | 149 | 24 | ND | ND |

**Table A9** (continued)

| Sample Code | Location | Date of Catch | Length (cm) | Weight (kg) | P -CTX-1B in flesh (µg kg-1)¹ | P-CTX-1B in liver (µg kg-1)¹ |
|---|---|---|---|---|---|---|
| SFM-34 | Mooloolaba, QLD | 16/01/15 | 133 | 17 | ND | ND |
| CF-B-1 | Coffs Harbour, NSW | 12/02/15 | 110 | 12 | ND | ND |
| CF-B-2 | Split island, Coffs Harbour, NSW | 19/02/15 | 125 | 12.2 | ND | ND |
| CF-B-8 | Lighthouse, Coffs Harbour, NSW | 10/02/15 | 130 | 13.6 | ND | ND |
| CF-B-16 | Patch, Coffs Harbour, NSW | 2/03/15 | 131 | 13.3 | ND | ND |
| CF-B-19 | Patch, Coffs Harbour, NSW | 2/03/15 | 130 | 12.5 | ND | ND |
| CF-B-22 | Lighthouse, Coffs, Harbour, NSW | 12/02/15 | 120 | 11.1 | ND | ND |
| CF-B-25 | Coffs Harbour, NSW | 23/01/15 | 110 | 12 | ND | ND |
| CF-B-26 | South Solitary island, Coffs Harbour, NSW | 26/02/15 | 128 | 15.8 | ND | ND |
| CF-B-27 | Patch, Coffs Harbour, NSW | 2/03/15 | 124 | 11.2 | ND | ND |
| CF-B-28 | South Solitary island, Coffs Harbour, NSW | 26/02/15 | 143 | 20.5 | ND | ND |
| CF-B-30 | Patch, Coffs Harbour, NSW | 28/02/15 | 125 | 11.2 | ND | ND |
| CF-D-3 | Evans Head, NSW | 5/03/15 | 150 | 23.6 | ND | ND |

**Table A10** (continued)

| Sample Code | Location | Date of Catch | Length (cm) | Weight (kg) | P -CTX-1B in flesh (µg kg-1)[1] | P-CTX-1B in liver (µg kg-1)[1] |
|---|---|---|---|---|---|---|
| CF-C-2 | Evans Head, NSW | 28/04/15 | 129 | 13.5 | ND | ND |
| CF-C-5 | Black Head, NSW | 26/03/15 | 129 | 13.1 | ND | ND |
| CF-C-10 | Evans Head, NSW | 28/04/15 | 127 | 12.5 | ND | ND |
| CF-C-11 | Ballina, NSW | 12/03/15 | 128 | 11.2 | ND | <0.4 |
| CF-C-13 | Evans Head, NSW | 28/04/15 | 124 | 12.5 | ND | ND |
| CF-C-22 | Ballina, NSW | 12/03/15 | 142 | 19.5 | ND | <0.4 |
| CF-E-5 | Brunswick Head, NSW | 26/03/15 | 110 | 10.5 | ND | ND |
| CF-E-12 | Brunswick Head, NSW | 21/03/15 | 120 | 13 | ND | ND |
| CF-E-16 | Brunswick Head, NSW | 9/04/15 | 110 | 11 | ND | ND |
| CF-E-21 | Brunswick Head, NSW | 27/03/15 | 120 | 12 | ND | ND |
| CF-E-22 | Brunswick Head, NSW | 5/04/15 | 90 | 9 | ND | ND |
| CF-E-24 | Brunswick Head, NSW | 21/01/15 | 90 | 9 | ND | ND |
| CF-E-27 | Brunswick Head, NSW | 14/02/15 | 100 | 10 | ND | ND |
| CF-E-28 | Brunswick Head, NSW | 26/01/15 | 95 | 9 | ND | ND |

**Table A11** (continued)

| Sample Code | Location | Date of Catch | Length (cm) | Weight (kg) | P -CTX-1B in flesh (µg kg-1)[1] | P-CTX-1B in liver (µg kg-1)[1] |
|---|---|---|---|---|---|---|
| CF-E-30 | Brunswick Head, NSW | 29/03/15 | 110 | 8 | ND | ND |
| RF-Q-2 | Byron Bay, NSW | 19/04/15 | 80 | 4.5 | ND | ND |
| RF-X-5 | Byron Bay, NSW | 19/04/15 | 90 | 6 | ND | ND |
| RF-X-6 | Byron Bay, NSW | 4/03/15 | 120 | 12 | ND | ND |
| RF-T-1 | Byron Bay, NSW | 4/03/15 | 95 | 7 | ND | ND |
| RF-F-1 | Coffs Harbour, NSW | 18/04/15 | 124 | 15 | ND | ND |
| RF-H-1 | Coffs Harbour, NSW | 20/03/15 | 95 | 10 | ND | ND |
| RF-H-2 | Coffs Harbour, NSW | 20/03/15 | 98.5 | 7 | ND | <0.4 |
| RF-H-3 | Coffs Harbour, NSW | 20/03/15 | 100 | 12 | ND | ND |
| RF-H-4 | Coffs Harbour, NSW | 23/03/15 | 95 | 9 | ND | ND |
| RF-H-5 | Coffs Harbour, NSW | 26/03/15 | 90 | 8 | ND | ND |
| RF-H-6 | Coffs Harbour, NSW | 26/03/15 | 100 | 12 | ND | ND |
| RF-J-1 | Solitary island, Coffs Harbour, NSW | 2/04/15 | 135 | 12 | ND | ND |
| RF-J-2 | Coffs Harbour, NSW | 23/04/15 | 110 | 11.5 | ND | ND |

**Table A12** (continued)

| Sample Code | Location | Date of Catch | Length (cm) | Weight (kg) | P -CTX-1B in flesh (µg kg-1)[1] | P-CTX-1B in liver (µg kg-1)[1] |
|---|---|---|---|---|---|---|
| RF-J-3 | Split Solitary, Coffs Harbour, NSW | 19/04/15 | 145 | 17.5 | ND | ND |
| RF-M-1 | Coffs Harbour, NSW<br>(30°.17S 153°. 10E) | 15/03/15 | 110 | 11 | ND | <0.4 |
| RF-M-2 | Coffs Harbour, NSW<br>(30°.22S 153°. 50E) | 31/03/15 | 120 | 12 | ND | ND |
| RF-M-3 | Coffs Harbour, NSW<br>(30°.75S 153°. 10E) | 15/03/15 | 115 | 11.5 | ND | ND |
| RF-M-4 | Coffs Harbour, NSW<br>(30°.22S 153°. 50E) | 31/03/15 | 130 | 19 | ND | ND |
| RF-M-5 | Macqualies, Coffs Harbour, NSW | 1/04/15 | 120 | 14.5 | ND | ND |
| RF-M-6 | Coffs Harbour, NSW | 2/04/15 | 129 | 18.7 | ND | ND |
| RF-N-1 | Coffs Harbour, NSW | 7/03/15 | 123 | 11 | ND | ND |
| RF-N-2 | Coffs Harbour, NSW | 29/03/15 | 140 | 14.7 | ND | ND |
| RF-N-3 | Coffs Harbour, NSW | 26/04/15 | 120 | 17 | ND | ND |
| RF-N-4 | Coffs Harbour, NSW | 30/05/15 | 110 | 11 | ND | ND |
| RF-Y-1 | Coffs Harbour, NSW | 5/04/15 | 118 | 14.8 | ND | ND |
| RF-Y-2 | Coffs Harbour, NSW | 5/04/15 | 127 | 19.8 | ND | ND |
| RF-Y-3 | Coffs Harbour, NSW | 5/04/15 | 134 | 19.2 | ND | ND |

**Table A13** (continued)

| Sample Code | Location | Date of Catch | Length (cm) | Weight (kg) | P -CTX-1B in flesh (µg kg-1)[1] | P-CTX-1B in liver (µg kg-1)[1] |
|---|---|---|---|---|---|---|
| RF-Y-4 | Coffs Harbour, NSW | 19/04/15 | 131.5 | 16.2 | ND | ND |
| RF-Y-5 | Coffs Harbour, NSW | 7/04/15 | 135 | 19.4 | ND | ND |
| RF-Z-1 | Coffs Harbour, NSW | 3/04/15 | 132 | 18.9 | ND | ND |
| RF-Z-2 | Coffs Harbour, NSW | 3/04/15 | 134.5 | 19 | ND | ND |
| RF-Z-3 | Coffs Harbour, NSW | 3/04/15 | 117 | 14.2 | ND | ND |
| RF-Z-4 | Coffs Harbour, NSW | 3/04/15 | 135 | 19.4 | ND | ND |
| RF-Z-5 | Coffs Harbour, NSW | 4/04/15 | 120 | 14.5 | ND | ND |
| RF-AA-1 | Coffs Harbour, NSW | 6/04/15 | 130.4 | 16 | ND | ND |
| RF-AA-2 | Coffs Harbour, NSW | 10/04/15 | 117 | 14 | ND | ND |
| RF-AA-3 | Coffs Harbour, NSW | 14/04/15 | 134.5 | 19.2 | ND | ND |
| RF-AA-5 | Coffs Harbour, NSW | 12/04/15 | 133 | 18.9 | ND | ND |
| RF-AP-1 | South Solitary island, Coffs Harbour, NSW | 30/05/15 | 142 | 16 | <0.1 | <0.4 |
| RF-AP-2 | North Solitary island, Coffs Harbour, NSW | 30/05/15 | 145 | 17 | ND | ND |
| RF-AB-1 | Forster, NSW | 6/04/15 | 125 | 13 | ND | ND |

**Table A14** (continued)

| Sample Code | Location | Date of Catch | Length (cm) | Weight (kg) | P -CTX-1B in flesh (µg kg-1)[1] | P-CTX-1B in liver (µg kg-1)[1] |
|---|---|---|---|---|---|---|
| RF-AC-1 | Forster, NSW | 6/04/15 | 120 | 12 | ND | ND |
| RF-AD-1 | Coffs Harbour, NSW | 31/03/15 | 134 | 14.6 | ND | ND |
| V1207-A | Scott's Head, NSW[2] | 2/3/14 | -- | 25.7 | 0.4 | NT |
| V1207-B | Evans Head, NSW[2,3] | 13/2/14 | -- | 10 | 0.6 | NT |
| V1207-C3 | Evans Head, NSW[2,3] | 13/2/14 | -- | 17 | 1.0 | NT |
| V1207-D4 | Evans Head, NSW[2] | 13/2/14 | -- | 3.40 | ND | NT |

ND: Not detected; NT: Not tested

[1] LC-MS analysis was performed at the Cawthron Institute, Nelson, New Zealand

[2] Results related to CFP in NSW in 2014, obtained from the NSW Food Authority (Farrell et al., 2016a) [3] Three flesh fillets were tested from 2 specimens of Spanish Mackerel from Evans Head in 2014, which were 10 and 17 kg. Unfortunately, the NSW Food Authority was not able to verify exactly which of the three fillets came from which fish.

**Table A15** LC–MS/MS and ELISA analyses of P-CTX-1B in samples of S. commerson flesh and liver collected during 2021-22 fishing season.

| Sample no. | Sample code | Date of collection | Tail length (mm) | fork length (mm) | Weight (kgs) | Location | P-CTX-1B in flesh (µg/kg) | P-CTX-1B in liver (µg/kg) | P-CTX-1B in flesh ELISA (µg/kg) | P-CTX-1B in liver ELISA (µg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | FRDC 1 | 8/12/2021 | 1080.0 | 980.0 | 7.3* | Fraser Island inshore | <LOD | <LOD | <LOQ | |
| 2 | FRDC 2 | 8/12/2021 | 1039.0* | 940.0 | 6.4* | Fraser Island inshore | <LOD | <LOD | | |
| 3 | FRDC 3 | 8/12/2021 | 970.0 | 860.0 | 4.9* | Fraser Island inshore | <LOD | <LOD | | <LOQ |
| 4 | FRDC 4 | 8/12/2021 | 960.0 | 850.0 | 4.7* | Fraser Island inshore | <LOD | <LOD | <LOQ | |
| 5 | FRDC 5 | 8/12/2021 | 1080.0 | 970.0 | 7.1* | Fraser Island inshore | <LOD | <LOD | | 5 |
| 6 | FRDC 6 | 8/12/2021 | 1200.0 | 1080.0 | 9.9* | Fraser Island inshore | <LOD | <LOD | | 6 |
| 7 | FRDC 7 | 8/12/2021 | 990.0 | 910.0 | 5.8* | Fraser Island inshore | <LOD | <LOD | | 7 |
| 8 | FRDC 8 | 8/12/2021 | 1000.0 | 920.0 | 6.0* | Fraser Island inshore | <LOD | <LOD | | 8 |
| 9 | FRDC 9 | 8/12/2021 | 950.0 | 850.0 | 4.7* | Fraser Island inshore | <LOD | <LOD | | 9 |
| 10 | FRDC 10 | 8/12/2021 | 980.0 | 860.0 | 4.9* | Fraser Island inshore | <LOD | <LOD | | 10 |
| 11 | FRDC 12 | 8/12/2021 | 1010.0 | 910.0 | 5.8* | Fraser Island inshore | <LOD | <LOD | <LOQ | 11 |
| 12 | FRDC 13 | 8/12/2021 | 912.0* | 820.0 | 4.2* | Fraser Island inshore | <LOD | <LOD | <LOQ | 12 |
| 13 | FRDC 14 | 27/08/2021 | 1410.0 | 1330.0 | 19.0 | Fraser Island inshore | <LOD | <LOD | | 13 |
| 14 | FRDC 15 | 14/11/2021 | 1007.0* | 910.0 | 5.8* | Rockhampton offshore | <LOD | <LOD | | |
| 15 | FRDC 16 | 27/08/2021 | 1240.0 | 1140.0 | 11.7* | Fraser Island inshore | <LOD | <LOD | | <LOQ |
| 16 | FRDC 17 | 7/12/2021 | 1040.0 | 920.0 | 6.0* | Fraser Island inshore | <LOD | <LOD | | |
| 17 | FRDC 18 | 7/12/2021 | 1010.0 | 870.0 | 5.0* | Fraser Island inshore | <LOD | <LOD | | <LOQ |
| 18 | FRDC 19 | 7/12/2021 | 990.0 | 910.0 | 5.8* | Fraser Island inshore | <LOD | <LOD | 0.005 | |
| 19 | FRDC 20 | 9/12/2021 | 1040.0 | 940.0 | 6.4* | Fraser Island inshore | <LOD | <LOD | <LOQ | <LOQ |
| 20 | FRDC 21 | 9/12/2021 | 810.0 | 710.0 | 2.7* | Fraser Island inshore | <LOD | <LOD | <LOQ | <LOQ |
| 21 | FRDC 22 | 9/12/2021 | 950.0 | 850.0 | 4.7* | Fraser Island inshore | <LOD | <LOD | | |
| 22 | FRDC 23 | 9/12/2021 | 950.0 | 860.0 | 4.9* | Fraser Island inshore | <LOD | <LOD | | |
| 23 | FRDC 24 | 9/12/2021 | 933.0* | 840.0 | 4.5* | Fraser Island inshore | <LOD | <LOD | | |
| 24 | FRDC 26 | 9/12/2021 | 950.0 | 840.0 | 4.5* | Fraser Island inshore | <LOD | <LOD | | |
| 25 | FRDC 27 | 9/12/2021 | 950.0 | 840.0 | 4.5* | Fraser Island inshore | <LOD | <LOD | | |
| 26 | FRDC 29 | 9/12/2021 | 950.0 | 855.9* | 4.8* | Fraser Island inshore | <LOD | <LOD | | |

**Table A16** (continued)

| Sample no. | Sample code | Date of collection | Tail length (mm) | fork length (mm) | Weight (kgs) | Location | P-CTX-1B in flesh (µg/kg) | P-CTX-1B in liver (µg/kg) | P-CTX-1B in flesh ELISA (µg/kg) | P-CTX-1B in liver ELISA (µg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| 27 | FRDC 30 | 16/11/2021 | 1100.0 | 1000.0 | 7.8* | Fraser Island inshore | <LOD | <LOD | | |
| 28 | FRDC 31 | 16/11/2021 | 1367.0* | 1249.3 | 15.6* | Fraser Island inshore | <LOD | <LOD | | |
| 29 | FRDC 32 | 16/11/2021 | 950.0 | 850.0 | 4.7* | Fraser Island inshore | <LOD | <LOD | | |
| 30 | FRDC 33 | 16/11/2021 | 950.0 | 840.0 | 4.5* | Fraser Island inshore | <LOD | <LOD | | |
| 31 | FRDC 34 | 16/11/2021 | 1010.0 | 910.0 | 5.8* | Fraser Island inshore | <LOD | <LOD | | |
| 32 | FRDC 35 | 16/11/2021 | 990.0 | 900.0 | 5.6* | Fraser Island inshore | <LOD | <LOD | | <LOQ |
| 33 | FRDC 39 | 26/09/2021 | 1293.5* | 1180.0 | 13.1* | Fraser Island inshore | <LOD | <LOD | | |
| 34 | FRDC 40 | 26/09/2021 | 1510.0 | 1390.0 | 21.8* | Fraser Island inshore | <LOD | <LOD | 0.010 | 0.010 |
| 35 | FRDC 41 | 26/08/2021 | 1208.7* | 1100.0 | 10.5* | Fraser Island inshore | <LOD | <LOD | 0.006 | |
| 36 | FRDC 43 | 26/08/2021 | 1460.0 | 1320.0 | 18.5* | Fraser Island inshore | <LOD | <LOD | | |
| 37 | FRDC 44 | 26/08/2021 | 1360.0 | 1290.0 | 17.2* | Fraser Island inshore | <LOD | <LOD | 0.008 | <LOQ |
| 38 | FRDC 45 | 26/08/2021 | 1198.0* | 1090.0 | 10.2* | Fraser Island inshore | <LOD | <LOD | <LOQ | <LOQ |
| 39 | FRDC 48 | 20/12/2021 | 1145.0* | 1040.0 | 8.8* | Fraser Island inshore | <LOD | <LOD | 0.010 | <LOQ |
| 40 | FRDC 58 | 25/03/2022 | 1220.0 | 1110.6* | 11.0 | Teewah | <LOD | <LOD | | |
| 41 | FRDC 59 | 1/02/2022 | 1410.0 | 1289.9* | 17.0 | Sunshine reef | <LOD | <LOD | | |
| 42 | FRDC 77 | 24/12/2021 | 980.0 | 884.2* | 5.3* | Jew Shoal, Laguna Bay | <LOD | <LOD | | |
| 43 | FRDC 78 | 15/01/2022 | 1210.0 | 1101.2* | 11.0 | Laguna Bay, Noosa | <LOD | <LOD | | |
| 44 | FRDC 79 | 26/01/2022 | 990.0 | 893.6* | 5.6 | Sunshine reef (off Noosa heads) | <LOD | <LOD | | |
| 45 | FRDC 80 | 26/01/2022 | 1010.0 | 912.5* | 6.1 | Sunshine reef (off Noosa heads) | <LOD | <LOD | | |
| 46 | FRDC 81 | 25/03/2022 | 1310.0 | 1195.5* | 20.0 | Fraser Waddy | <LOD | <LOD | | |
| 47 | FRDC 82 | 25/03/2022 | 1050.0 | 950.2* | 8.0 | Fraser Waddy Point | <LOD | <LOD | | |
| 48 | FRDC 83 | 25/03/2022 | 1130.0 | 1025.7* | 8.0 | Fraser Waddy | <LOD | <LOD | | |
| 49 | FRDC 91 | 23/03/2022 | 1230.0 | 1120.1* | 10.0 | Fraser Waddy | <LOD | <LOD | | <LOQ |
| 50 | FRDC 92 | 23/03/2022 | 1100.0 | 997.4* | 7.5 | Fraser Waddy | <LOD | <LOD | <LOQ | <LOQ |

**Table A17** (continued)

| Sample no. | Sample code | Date of collection | Tail length (mm) | fork length (mm) | Weight (kgs) | Location | P-CTX-1B in flesh (µg/kg) | P-CTX-1B in liver (µg/kg) | P-CTX-1B in flesh ELISA (µg/kg) | P-CTX-1B in liver ELISA (µg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| 51 | FRDC 93 | 23/03/2022 | 1210.0 | 1101.2* | 9.5 | Fraser Waddy | <LOD | <LOD | | |
| 52 | FRDC 94 | 21/03/2022 | 1330.0 | 1214.4* | 14.5 | Fraser Waddy Point | <LOD | <LOD | | |
| 53 | FRDC 101 | 17/01/2022 | 1020.0 | 920.0 | 6.0* | Fraser Island inshore | <LOD | <LOD | | |
| 54 | FRDC 102 | 17/01/2022 | 1050.0 | 940.0 | 6.4* | Fraser Island inshore | <LOD | <LOD | | |
| 55 | FRDC 103 | 17/01/2022 | 1060.0 | 970.0 | 7.1* | Fraser Island inshore | <LOD | <LOD | | |
| 56 | FRDC 104 | 17/01/2022 | 1040.0 | 960.0 | 6.9* | Fraser Island inshore | <LOD | <LOD | | |
| 57 | FRDC 105 | 17/01/2022 | 1060.0 | 960.0 | 6.9* | Fraser Island inshore | <LOD | <LOD | | |
| 58 | FRDC 106 | 17/01/2022 | 1100.0 | 1010.0 | 8.0* | Fraser Island inshore | <LOD | <LOD | | |
| 59 | FRDC 107 | 17/01/2022 | 1160.0 | 1040.0 | 8.8* | Fraser Island inshore | <LOD | <LOD | | |
| 60 | FRDC 109 | 17/01/2022 | 1270.0 | 1180.0 | 13.1* | Fraser Island inshore | <LOD | <LOD | 0.006 | <LOQ |
| 61 | FRDC 110 | 17/01/2022 | 970.0 | 870.0 | 5.0* | Fraser Island inshore | <LOD | <LOD | | |
| 62 | FRDC 112 | 17/01/2022 | 1160.0 | 1080.0 | 9.9* | Fraser Island inshore | <LOD | <LOD | 0.012 | 0.009 |
| 63 | FRDC 113 | 17/01/2022 | 980.0 | 870.0 | 5.0* | Fraser Island inshore | <LOD | <LOD | | |
| 64 | FRDC 114 | 25/01/2022 | 960.0 | 850.0 | 4.7* | Hervey Bay | <LOD | <LOD | | |
| 65 | FRDC 115 | 25/01/2022 | 770.0 | 660.0 | 2.1* | Hervey Bay | <LOD | <LOD | | |
| 66 | FRDC 116 | 25/01/2022 | 930.0 | 830.0 | 4.4* | Hervey Bay | <LOD | <LOD | | |
| 67 | FRDC 117 | 25/01/2022 | 1170.0 | 1050.0 | 9.1* | Hervey Bay | <LOD | <LOD | | |
| 68 | FRDC 118 | 25/01/2022 | 1000.0 | 920.0 | 6.0* | Hervey Bay | <LOD | <LOD | | |
| 69 | FRDC 119 | 25/01/2022 | 1040.0 | 910.0 | 5.8* | Hervey Bay | <LOD | <LOD | | <LOQ |
| 70 | FRDC 120 | 25/01/2022 | 970.0 | 860.0 | 4.9* | Hervey Bay | <LOD | <LOD | | |
| 71 | FRDC 121 | 25/01/2021 | 980.0 | 900.0 | 5.6* | Hervey Bay | <LOD | <LOD | <LOQ | |
| 72 | FRDC 122 | 25/01/2022 | 996.7* | 900.0 | 5.6* | Hervey Bay | <LOD | <LOD | | |
| 73 | FRDC 123 | 25/01/2022 | 1110.0 | 970.0 | 7.1* | Hervey Bay | <LOD | <LOD | | |
| 74 | FRDC 124 | 25/01/2022 | 990.0 | 870.0 | 5.0* | Hervey Bay | <LOD | <LOD | | |
| 75 | FRDC 125 | 25/01/2022 | 1010.0 | 880.0 | 5.2* | Hervey Bay | <LOD | <LOD | 0.005 | 0.006 |
| 76 | FRDC 126 | 26/01/2022 | 1250.0 | 1120.0 | 11.1* | Rockhampton offshore | <LOD | <LOD | | |

**Table A18** (continued)

| Sample no. | Sample code | Date of collection | Tail length (mm) | fork length (mm) | Weight (kgs) | Location | P-CTX-1B in flesh (µg/kg) | P-CTX-1B in liver (µg/kg) | P-CTX-1B in flesh ELISA (µg/kg) | P-CTX-1B in liver ELISA (µg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| 77 | FRDC 127 | 26/01/2022 | 1120.0 | 990.0 | 7.5* | Rockhampton offshore | <LOD | <LOD | | |
| 78 | FRDC 128 | 26/01/2022 | 1030.0 | 910.0 | 5.8* | Rockhampton offshore | <LOD | <LOD | | |
| 79 | FRDC 130 | 26/01/2022 | 1010.0 | 880.0 | 5.2* | Rockhampton offshore | <LOD | <LOD | | |
| 80 | FRDC 131 | 26/01/2022 | 1070.0 | 970.0 | 7.1* | Rockhampton offshore | <LOD | <LOD | | |
| 81 | FRDC 132 | 26/01/2022 | 1130.0 | 1000.0 | 7.8* | Rockhampton offshore | <LOD | <LOD | | |
| 82 | FRDC 133 | 26/01/2022 | 970.0 | 870.0 | 5.0* | Rockhampton offshore | <LOD | <LOD | <LOQ | |
| 83 | FRDC 134 | 26/01/2022 | 1030.0 | 910.0 | 5.8* | Rockhampton offshore | <LOD | <LOD | <LOQ | 83 |
| 84 | FRDC 135 | 26/01/2022 | 975.5* | 880.0 | 5.2* | Rockhampton offshore | <LOD | <LOD | | 84 |
| 85 | FRDC 136 | 26/01/2022 | 1120.0 | 990.0 | 7.5* | Rockhampton offshore | <LOD | <LOD | <LOQ | 85 |
| 86 | FRDC 137 | 26/01/2022 | 1070.0 | 930.0 | 6.2* | Rockhampton offshore | <LOD | <LOD | | 86 |
| 87 | FRDC 138 | 26/01/2022 | 1200.0 | 1060.0 | 9.3* | Rockhampton offshore | <LOD | <LOD | <LOQ | 87 |
| 88 | FRDC 139 | 24/01/2022 | 830.0 | 740.0 | 3.0* | Rockhampton offshore | <LOD | <LOD | <LOQ | 88 |
| 89 | FRDC 140 | 24/01/2022 | 996.7* | 900.0 | 5.6* | Rockhampton offshore | <LOD | <LOD | | 89 |
| 90 | FRDC 141 | 24/01/2022 | 1090.0 | 990.0 | 7.5* | Rockhampton offshore | <LOD | <LOD | | 90 |
| 91 | FRDC 143 | 24/01/2022 | 1060.0 | 960.0 | 6.9* | Rockhampton offshore | <LOD | <LOD | | 91 |

**Table A19** (continued)

| Sample no. | Sample code | Date of collection | Tail length (mm) | fork length (mm) | Weight (kgs) | Location | P-CTX-1B in flesh (µg/kg) | P-CTX-1B in liver (µg/kg) | P-CTX-1B in flesh ELISA (µg/kg) | P-CTX-1B in liver ELISA (µg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| 92 | FRDC 144 | 24/01/2022 | 990.0 | 920.0 | 6.0* | Rockhampton offshore | <LOD | <LOD | | 92 |
| 93 | FRDC 145 | 24/01/2022 | 1050.0 | 960.0 | 6.9* | Rockhampton offshore | <LOD | <LOD | | 93 |
| 94 | FRDC 146 | 24/01/2022 | 1018.0* | 920.0 | 6.0* | Rockhampton offshore | <LOD | <LOD | | 94 |
| 95 | FRDC 147 | 24/01/2022 | 1070.0 | 970.0 | 7.1* | Rockhampton offshore | <LOD | <LOD | | 95 |
| 96 | FRDC 148 | 24/01/2022 | 1071.0* | 970.0 | 7.1* | Rockhampton offshore | <LOD | <LOD | <LOQ | 96 |
| 97 | FRDC 149 | 1/02/2022 | 1081.5* | 980.0 | 7.3* | Rockhampton offshore | <LOD | <LOD | <LOQ | 97 |
| 98 | FRDC 150 | 1/02/2022 | 1124.0* | 1020.0 | 8.3* | Rockhampton offshore | <LOD | <LOD | | 98 |
| 99 | REC 107 | 11/12/2021 | 1200.0 | 1091.8* | 9.5 | Sunshine Reef | <LOD | <LOD | | 99 |
| 100 | REC 108 | 16/12/2021 | 1550.0 | 1421.9* | 26.0 | Sunshine Reef | <LOD | <LOD | | 100 |
| 101 | REC 109 | 11/12/2021 | 1150.0 | 1044.6* | 8.0 | Sunshine Reef | <LOD | <LOD | | 101 |
| 102 | REC 110 | 19/11/2021 | 1200.0 | 1091.8* | 11.0 | Coolum | <LOD | <LOD | <LOQ | 102 |
| 103 | REC 111 | 25/03/2022 | 1050.0 | 950.2* | 7.0 | Double Island | <LOD | <LOD | | 103 |
| 104 | REC 113 | 21/02/2022 | 1570.0 | 1440.8* | 25.5 | Sunshine Reef | <LOD | <LOD | | 104 |
| 105 | REC 115 | 25/03/2022 | 1260.0 | 1148.4* | 15.0 | Double Island | <LOD | <LOD | | 105 |
| 106 | REC 145 | 8/06/2021 | 1300.0 | 1186.1* | 13.3* | Coolum | <LOD | <LOD | | 106 |
| 107 | REC 148 | 27/11/2021 | 1000.0 | 903.1* | 5.7* | Jew Shoal, Laguna Bay, Noosa | <LOD | <LOD | | 107 |
| 108 | FRDC 162 | 18/03/2022 | 1126.0 | 1021.9* | 8.3* | Coffs Harbour | <LOD | <LOD | | 108 |
| 109 | FRDC 161 | 18/03/2022 | 1358.0 | 1240.8* | 15.3* | Coffs Harbour | <LOD | <LOD | | 109 |
| 110 | FRDC 164 | 18/03/2022 | 1368.0 | 1250.2* | 15.6* | Coffs Harbour | <LOD | <LOD | | 110 |
| 111 | FRDC 173 | 25/03/2022 | 1075.0 | 938.0 | 6.4* | Wooli | <LOD | <LOD | | 111 |

**Table A20** (continued)

| Sample no. | Sample code | Date of collection | Tail length (mm) | fork length (mm) | Weight (kgs) | Location | P-CTX-1B in flesh (µg/kg) | P-CTX-1B in liver (µg/kg) | P-CTX-1B in flesh ELISA (µg/kg) | P-CTX-1B in liver ELISA (µg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| 112 | FRDC 184 | 7/05/2022 | 1013.0 | 948.0 | 6.6* | South West Rocks, Grassy Head | <LOD | <LOD | | 112 |
| 113 | FRDC 175 | 25/03/2022 | 1179.0 | 1038.0 | 8.8* | Wooli | <LOD | <LOD | 113 | FRDC 175 |
| 114 | FRDC 182 | 25/03/2022 | 1233.0 | 1098.0 | 10.4* | Wooli | <LOD | <LOD | 114 | FRDC 182 |
| 115 | FRDC 168 | 25/01/2022 | 1119.0 | 999.0 | 7.8* | The Wash, South Solitary | <LOD | <LOD | 115 | FRDC 168 |
| 116 | FRDC 181 | 25/03/2022 | 1089.0 | 960.0 | 6.9* | Wooli | <LOD | <LOD | 116 | FRDC 181 |
| 117 | FRDC 172 | 25/03/2022 | 1620.0 | 1488.0* | 29.6 | Wooli | <LOD | <LOD | 117 | FRDC 172 |
| 118 | FRDC 174 | 25/03/2022 | 1042.0 | 917.0 | 5.9* | Wooli | <LOD | <LOD | 118 | FRDC 174 |
| 119 | FRDC 171 | 25/03/2022 | 1084.0 | 955.0 | 6.7* | Wooli | <LOD | <LOD | 119 | FRDC 171 |
| 120 | FRDC 186 | 3/02/2022 | 1461.0 | 1338* | 19.3* | North Solitary Island | <LOD | <LOD | 120 | FRDC 186 |
| 121 | RF AT 5 | 10/04/2022 | *n/a* | *n/a* | 10.2 | Coffs Harbour | <LOD | <LOD | 121 | RF AT 5 |
| 122 | RF AS 6 | 16/04/2022 | *n/a* | *n/a* | 8.4 | Coffs Harbour | <LOD | <LOD | 122 | RF AS 6 |
| 123 | RF AS 3 | 20/04/2022 | *n/a* | *n/a* | 8.0 | Coffs Harbour | <LOD | <LOD | 123 | RF AS 3 |
| 124 | RF AS 4 | 15/04/2022 | *n/a* | *n/a* | 12.0 | Coffs Harbour | <LOD | <LOD | 124 | RF AS 4 |
| 125 | RF AS 5 | 15/04/2022 | *n/a* | *n/a* | 8.0 | Coffs Harbour | <LOD | <LOD | 125 | RF AS 5 |
| 126 | RF AT 3 | 10/04/2022 | *n/a* | *n/a* | 9.3 | Coffs Harbour | <LOD | <LOD | 126 | RF AT 3 |
| 127 | RF AT 6 | 27/04/2022 | *n/a* | *n/a* | 8.6 | Coffs Harbour | <LOD | <LOD | 127 | RF AT 6 |
| 128 | CH 7 | 28/04/2022 | *n/a* | *n/a* | 9.7 | Coffs Harbour | <LOD | <LOD | 128 | CH 7 |
| 129 | CH 17 | 30/04/2022 | *n/a* | *n/a* | 11.5 | Coffs Harbour | <LOD | <LOD | | |
| 130 | CH 13 | 27/04/2022 | *n/a* | *n/a* | 9.0 | Coffs Harbour | <LOD | <LOD | | |
| 131 | CH 20 | 30/04/2022 | *n/a* | *n/a* | 8.0 | Coffs Harbour | <LOD | <LOD | | |
| 132 | CH 24 | 27/04/2022 | *n/a* | *n/a* | 11.0 | Coffs Harbour | <LOD | <LOD | | |
| 133 | CH 9 | 29/04/2022 | *n/a* | *n/a* | 12.0 | Coffs Harbour | <LOD | <LOD | | |
| 134 | CH 12 | 28/04/2022 | *n/a* | *n/a* | 6.9 | Coffs Harbour | <LOD | <LOD | | |
| 135 | CH 2 | 30/04/2022 | *n/a* | *n/a* | 7.7 | Coffs Harbour | <LOD | <LOD | | |
| 136 | CH 30 | 29/04/2022 | *n/a* | *n/a* | 7.8 | Coffs Harbour | <LOD | <LOD | | |
| 137 | CH 8 | 28/04/2022 | *n/a* | *n/a* | 11.5 | Coffs Harbour | <LOD | <LOD | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 138 | CH 11 | 28/04/2022 | *n/a* | *n/a* | 8.4 | Coffs Harbour | <LOD | <LOD | | |

**Table A21** (continued)

| Sample no. | Sample code | Date of collection | Tail length (mm) | fork length (mm) | Weight (kgs) | Location | P-CTX-1B in flesh (µg/kg) | P-CTX-1B in liver (µg/kg) | P-CTX-1B in flesh ELISA (µg/kg) | P-CTX-1B in liver ELISA (µg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| 139 | CH 15 | 28/04/2022 | *n/a* | *n/a* | 8.3 | Coffs Harbour | <LOD | <LOD | | |
| 140 | MAC 126 | 16/08/2022 | 1280.0 | 1167.0* | 14.5 | Wigton Islands | <LOD | <LOD | 0.007 | 0.012 |
| 141 | FRDC 227 | 20/04/2022 | 1150.0 | 1010.0 | 8.0* | Rockhampton offshore | <LOD | <LOD | | |
| 142 | FRDC 251 | 18/07/2022 | 1310.0 | 1200.0 | 13.8* | Fraser inshore | <LOD | <LOD | | |
| 143 | MAC 117 | 7/08/2022 | 1200.0 | 1091.8* | 10.4 | Northern overfalls | <LOD | <LOD | <LOQ | |
| 144 | FRDC 226 | 20/04/2022 | 1090.0 | 950.0 | 6.6* | Rockhampton offshore | <LOD | <LOD | 0.005 | 0.012 |
| 145 | FRDC 229 | 20/04/2022 | 1060.0 | 930.0 | 6.2* | Rockhampton offshore | <LOD | <LOD | <LOQ | 0.005 |
| 146 | REC 434 | 6/05/2022 | 900.0 | 808.0* | 4.3 | Shipping channel | <LOD | <LOD | | |
| 147 | REC 144 | 21/02/2022 | 1150.0 | 1044.6* | 10.0 | Maroola Beach | <LOD | <LOD | | |
| 148 | FRDC 221 | 20/04/2022 | 1080.0 | 960.0 | 6.9* | Rockhampton offshore | <LOD | <LOD | | |

*n/a*: data not available; * refers to values determined from equations as stated in Mackie et al. (2003); <LOD: below the limit of detection; <LOQ: below the limit of quantification.

**Table A22** LC–MS/MS and ELISA analyses of P-CTX-1B in samples of *S. commerson* flesh and liver collected during 2020-21 fishing season.

| Sample no. | Sample code | Date of collection | Tail length (mm) | Fork length (mm) | Weight (kgs) | Location | P-CTX-1B in flesh (µg/kg) | P-CTX-1B in liver (µg/kg) |
|---|---|---|---|---|---|---|---|---|
| 1 | BB bag 5 | 10/03/2021 | 1000 | 903 | *n/a* | Byron | <LOD | <LOD |
| 2 | BB bag 3 | 10/03/2021 | 1300 | 1186 | *n/a* | Byron | <LOD | <LOD |
| 3 | RF box AQ bag 3 | 16/02/2021 | 1050 | 950 | *n/a* | Brunswick Heads | <LOD | <LOD |
| 4 | Byron 95 | 12/02/2021 | 950 | 856 | *n/a* | Ballina | <LOD | <LOD |
| 5 | Byron 124 | 12/02/2021 | 1240 | 1129 | *n/a* | Ballina | <LOD | <LOD |
| 6 | CH bag 1 | 4/05/2021 | 1290 | 1177 | 16.50 | Coffs Harbour | <LOD | <LOD |
| 7 | RF box AR bag 4 | 29/04/2021 | 1300 | 1186 | 10.00 | Coffs Harbour | <LOD | <LOD |
| 8 | CH bag 5 | 4/05/2021 | 1250 | 1139 | 15.50 | Coffs Harbour | <LOD | <LOD |
| 9 | RF box AR bag 2 | 29/04/2021 | 1100 | 997 | 7.50 | Coffs Harbour | <LOD | <LOD |
| 10 | RF box AR bag 5 | 29/04/2021 | 1100 | 997 | 8.00 | Coffs Harbour | <LOD | <LOD |
| 11 | CH bag 4 | 4/05/2021 | 1150 | 1045 | 12.50 | Coffs Harbour | <LOD | <LOD |
| 12 | CH bag 21 | 13/05/2021 | 1440 | 1318 | 15.50 | Coffs Harbour | <LOD | <LOD |
| 13 | REC bag 356 | 15/05/2021 | 1560 | 1431 | *n/a* | Coffs Harbour | <LOD | <LOD |
| 14 | Fish 1 | 19/11/2020 | 1060 | 960 | 7.00 | Bustard Head | <LOD | <LOD |
| 15 | Fish 2 | 19/11/2020 | 1310 | 1196 | 15.00 | Bustard Head | <LOD | <LOD |
| 16 | Fish 3 | 19/11/2020 | 1510 | 1384 | 21.50 | Bustard Head | <LOD | <LOD |
| 17 | Fish 4 | 19/11/2020 | 980 | 884 | 6.60 | Bustard Head | <LOD | <LOD |
| 18 | AG1 | 29/04/2021 | 850 | 762 | 3.55 | Coffs Harbour | <LOD | <LOD |
| 19 | AG2 | 29/04/2021 | 1120 | 1016 | 8.95 | Coffs Harbour | <LOD | <LOD |
| 20 | AG3 | 28/02/2021 | 1130 | 1026 | 8.00 | Fingal Island | <LOD | <LOD |
| 21 | AG4 | 29/04/2021 | 1300 | 1186 | 18.25 | Coffs Harbour | <LOD | <LOD |
| 22 | RF 31 | 11/02/2021 | 1100 | 997 | 7.10 | Arrawarra | <LOD | <LOD |
| 23 | RF 32 | 12/02/2021 | 1230 | 1120 | 13.09 | Arrawarra | <LOD | <LOD |
| 24 | RF 33 | 11/02/2021 | 1030 | 931 | 7.00 | Arrawarra | <LOD | <LOD |
| 25 | RF 34 | 29/04/2021 | 1200 | 1092 | 10.10 | Coffs Harbour | <LOD | <LOD |
| 26 | RF 35 | 29/04/2021 | 1080 | 979 | 8.00 | Coffs Harbour | <LOD | <LOD |
| 27 | RF 51 | 29/04/2021 | 1100 | 997 | 8.15 | Coffs Harbour | <LOD | <LOD |
| 28 | RF 52 | 29/04/2021 | 1150 | 1045 | 10.30 | Coffs Harbour | <LOD | <LOD |

**Table A23** (continued)

| Sample no. | Sample code | Date of collection | Tail length (mm) | Fork length (mm) | Weight (kgs) | Location | P-CTX-1B in flesh (µg/kg) | P-CTX-1B in Liver (µg/kg) |
|---|---|---|---|---|---|---|---|---|
| 29 | RF 53 | 29/04/2021 | 1120 | 1016 | 10.25 | Coffs Harbour | <LOD | <LOD |
| 30 | RF 54 | 29/04/2021 | 1150 | 1045 | 9.40 | Coffs Harbour | <LOD | <LOD |
| 31 | RF 55 | 29/04/2021 | 1150 | 1045 | 10.20 | Coffs Harbour | <LOD | <LOD |
| 32 | Fish 6 | *na* | 780 | 696 | 2.80 | Sandon Shoals | <LOD | <LOD |
| 33 | AG5 | 29/04/2021 | 1100 | 997 | 8.20 | Coffs Harbour | <LOD | <LOD |
| 34 | MAC 9 | 16/06/2021 | 1240 | 1129 | 11.44 | Hyde Rock Reef | <LOD | <LOD |
| 35 | MAC14 | 16/06/2021 | 1200 | 1092 | 9.24 | Hyde Rock Reef | <LOD | <LOD |
| 36 | MAC13 | 16/06/2021 | 1150 | 1045 | 8.86 | Hyde Rock Reef | <LOD | <LOD |
| 37 | MAC10 | 16/06/2021 | 1240 | 1129 | 13.14 | Wigton Island | <LOD | <LOD |
| 38 | MAC11 | 16/06/2021 | 1050 | 950 | 5.76 | Wigton Island | <LOD | <LOD |
| 39 | MAC12 | 16/06/2021 | 1050 | 950 | 5.52 | Calder Island | <LOD | <LOD |
| 40 | MAC46 | 17/06/2021 | 1120 | 1016 | 9.80 | Wigton Island | <LOD | <LOD |
| 41 | MAC43 | 17/06/2021 | 1200 | 1092 | 10.50 | Wigton Island | <LOD | <LOD |
| 42 | MAC44 | 17/06/2021 | 1260 | 1148 | 12.94 | Wigton Island | <LOD | <LOD |
| 43 | MAC48 | 17/06/2021 | 1220 | 1111 | 9.24 | Wigton Island | <LOD | <LOD |
| 44 | MAC41 | 17/06/2021 | 1150 | 1045 | 8.96 | Hyde Rock reef | <LOD | <LOD |
| 45 | MAC24 | 17/06/2021 | 1210 | 1101 | 10.89 | Wigton Island | <LOD | <LOD |
| 46 | MAC22 | 17/06/2021 | 1210 | 1101 | 10.44 | Wigton Island | <LOD | <LOD |
| 47 | MAC47 | 20/06/2021 | 1180 | 1073 | 8.26 | Wigton Island | <LOD | <LOD |
| 48 | MAC45 | 2/07/2021 | 1560 | 1431 | 24.06 | Derwent Island | <LOD | <LOD |
| 49 | MAC42 | 2/07/2021 | 1400 | 1280 | 15.82 | Derwent Island | <LOD | <LOD |
| 50 | MAC15 | 2/07/2021 | 1200 | 1092 | 11.00 | Derwent Island | <LOD | <LOD |
| 51 | MAC16 | 2/07/2021 | 1260 | 1148 | 11.88 | Derwent Island | <LOD | <LOD |
| 52 | BAG A | 2/07/2021 | 1220 | 1111 | 11.14 | Derwent Island | <LOD | <LOD |
| 53 | BAG B | 2/07/2021 | 1100 | 997 | 9.18 | Derwent Island | <LOD | <LOD |
| 54 | BAG C | 2/07/2021 | 1200 | 1092 | 10.40 | Derwent Island | <LOD | <LOD |
| 55 | REC 470 | 17/07/2021 | 1150 | 1045 | 8.52 | Hyde Rock Reef | <LOD | <LOD |
| 56 | REC466 | 17/07/2021 | 1160 | 1054 | 9.02 | Hyde Rock Reef | <LOD | <LOD |
| 57 | REC 407 | 17/07/2021 | 1330 | 1214 | 14.82 | Singapore Rock Reef | <LOD | <LOD |

**Table A24** (continued)

| Sample no. | Sample code | Date of collection | Tail length (mm) | Fork length (mm) | Weight (kgs) | Location | P-CTX-1B in flesh (µg/kg) | P-CTX-1B in liver (µg/kg) |
|---|---|---|---|---|---|---|---|---|
| 58 | REC 406 | 17/07/2021 | 1260 | 1148 | 13.20 | Heskett Rock Reef | <LOD | <LOD |
| 59 | REC 408 | 17/07/2021 | 1260 | 1148 | 12.04 | Derwent Island | <LOD | <LOD |
| 60 | REC 468 | 17/07/2021 | 990 | 894 | 4.86 | Noel Island | <LOD | <LOD |
| 61 | REC 467 | 17/07/2021 | 1050 | 950 | 6.82 | Bailey Island | <LOD | <LOD |
| 62 | REC 469 | 17/07/2021 | 1170 | 1063 | 8.46 | Bailey Island | <LOD | <LOD |
| 63 | REC 452 | 17/07/2021 | 1350 | 1233 | 16.80 | Overfall Reef | <LOD | <LOD |
| 64 | REC 453 | 17/07/2021 | 1230 | 1120 | 9.82 | Overfall Reef | <LOD | <LOD |
| 65 | REC 455 | 17/07/2021 | 1320 | 1205 | 13.7 | Prudhoe Island | <LOD | <LOD |
| 66 | REC 454 | 17/07/2021 | 1220 | 1111 | 11.52 | Cockermouth Island | <LOD | <LOD |
| 67 | REC 451 | 17/07/2021 | 1190 | 1082 | 9.54 | Cockermouth Island | <LOD | <LOD |
| 68 | REC 464 | 17/07/2021 | 1290 | 1177 | 12.88 | Skull Rock Reef | <LOD | <LOD |
| 69 | REC 463 | 17/07/2021 | 1150 | 1045 | 10.26 | Skull Rock Reef | <LOD | <LOD |
| 70 | REC 461 | 17/07/2021 | 1200 | 1092 | 11.36 | Rattray Island | <LOD | <LOD |
| 71 | REC 462 | 17/07/2021 | 1260 | 1148 | 10.36 | Overfall Reef | <LOD | <LOD |
| 72 | REC 465 | 17/07/2021 | 1140 | 1035 | 8.76 | Bailey Island | <LOD | <LOD |
| 73 | REC 425 | 17/07/2021 | 1130 | 1026 | 8.36 | Bailey Island | <LOD | <LOD |
| 74 | REC424 | 17/07/2021 | 1440 | 1318 | 16.10 | Hyde Rock | <LOD | <LOD |
| 75 | REC 423 | 17/07/2021 | 1230 | 1120 | 10.06 | Singapore Rock Reef | <LOD | <LOD |
| 76 | REC 422 | 17/07/2021 | 1200 | 1092 | 9.82 | Rattray Island | <LOD | <LOD |
| 77 | REC 421 | 17/07/2021 | 1200 | 1092 | 9.34 | Rattray Island | <LOD | <LOD |
| 78 | REC 410 | 19/07/2021 | 1220 | 1111 | 12.02 | Wigton Island | <LOD | <LOD |
| 79 | REC 449 | 29/07/2021 | 1250 | 1139 | 11.38 | Hyde Rock | <LOD | <LOD |
| 80 | REC 450 | 29/07/2021 | 1220 | 1111 | 10.46 | Wigton Island | <LOD | <LOD |
| 81 | REC 445 | 24/08/2021 | 1100 | 997 | 7.10 | Payne Shoal | <LOD | <LOD |
| 82 | REC 442 | 29/08/2021 | 1000 | 903 | 6.00 | Payne Shoal | <LOD | <LOD |
| 83 | REC 441 | 29/08/2021 | 1180 | 1073 | 11.90 | Payne Shoal | <LOD | <LOD |
| 84 | REC 459 | 12/09/2021 | 1000 | 903 | 6.00 | Payne Shoal | <LOD | <LOD |
| 85 | REC 460 | 12/09/2021 | 1000 | 903 | 6.00 | Payne Shoal | <LOD | <LOD |

**Table A25** (continued)

| Sample no. | Sample code | Date of collection | Tail length (mm) | Fork length (mm) | Weight (kgs) | Location | P-CTX-1B in flesh (µg/kg) | P-CTX-1B in liver (µg/kg) |
|---|---|---|---|---|---|---|---|---|
| 86 | BAL bag 1 | 10/02/2021 | 1140 | 1035 | 9.60 | Brunswick Heads | <LOD | <LOD |
| 87 | CF box C bag 1 | 3/02/2021 | 1120 | 1016 | 12.00 | Ballina | <LOD | <LOD |
| 88 | CF box C bag 3 | 12/01/2021 | 1180 | 1073 | 11.70 | Ballina | <LOD | <LOD |
| 89 | CF box C bag 6 | 17/02/2021 | 1300 | 1186 | 14.20 | Ballina | <LOD | <LOD |
| 90 | CF box C bag 15 | 20/01/2021 | 1200 | 1092 | 10.20 | Ballina | <LOD | <LOD |
| 91 | CF box C bag 16 | 4/01/2021 | 1150 | 1045 | 11.70 | Ballina | <LOD | <LOD |
| 92 | CF box C bag 20 | 12/01/2021 | 1150 | 1045 | 11.00 | Ballina | <LOD | <LOD |
| 93 | CF box C bag 25 | 17/01/2021 | 1190 | 1082 | 10.80 | Ballina | <LOD | <LOD |
| 94 | REC 511 | 16/07/2021 | 1510 | 1384 | *n/a* | Fraser inshore | <LOD | <LOD |
| 95 | REC 536 | 20/07/2021 | 1310 | 1196 | *n/a* | Fraser inshore | <LOD | <LOD |
| 96 | REC 537 | 8/08/2021 | 1560 | 1431 | *n/a* | Fraser inshore | <LOD | <LOD |
| 97 | REC 538 | 8/08/2021 | 1200 | 1092 | *n/a* | Fraser inshore | <LOD | <LOD |
| 98 | REC 539 | 8/08/2021 | 1280 | 1167 | *n/a* | Fraser inshore | <LOD | <LOD |
| 99 | REC 544 | 8/08/2021 | 1180 | 1073 | *n/a* | Fraser inshore | <LOD | <LOD |
| 100 | REC 545 | 8/08/2021 | 1250 | 1139 | *n/a* | Fraser inshore | <LOD | <LOD |
| 101 | REC 546 | 8/08/2021 | 1420 | 1299 | *n/a* | Fraser inshore | <LOD | <LOD |

*n/a*: data not available; * refers to values determined from equations as stated in Mackie et al. (2003); <LOD: below the limit of detection; <LOQ: below the limit of quantification.

# Appendix B – Figures

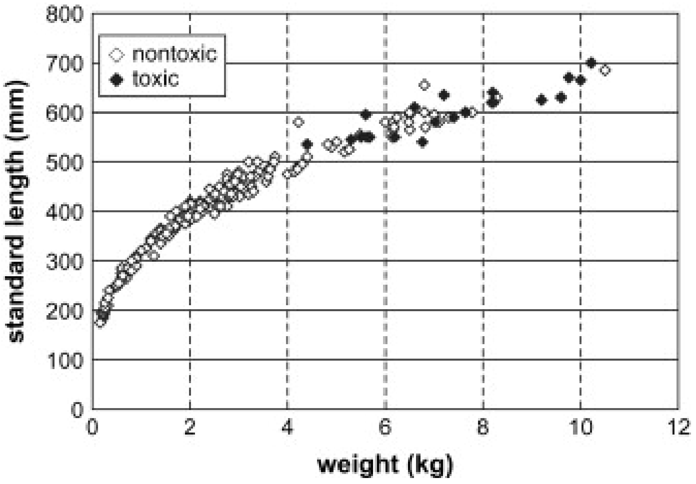


**Figure B1.** Size of toxic specimens of *L. monostigma* (Onespot Snapper) (Oshiro et al., 2010).

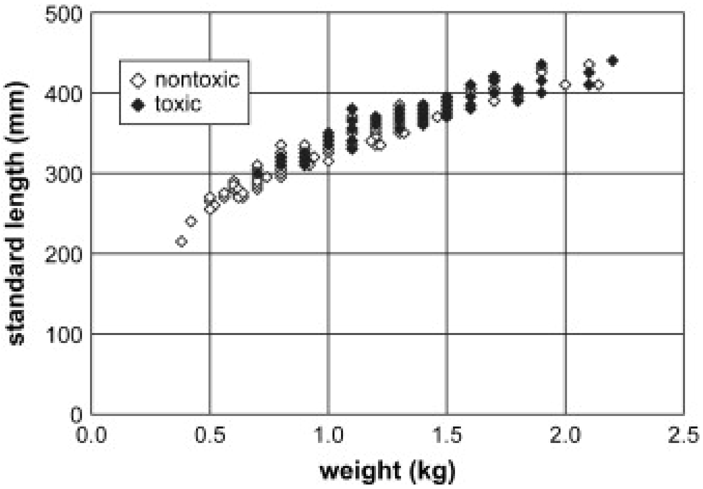


**Figure B2** Size of toxic specimens of *E. fuscoguttatus* (Flowery Rockcod, Oshiro et al., 2010).

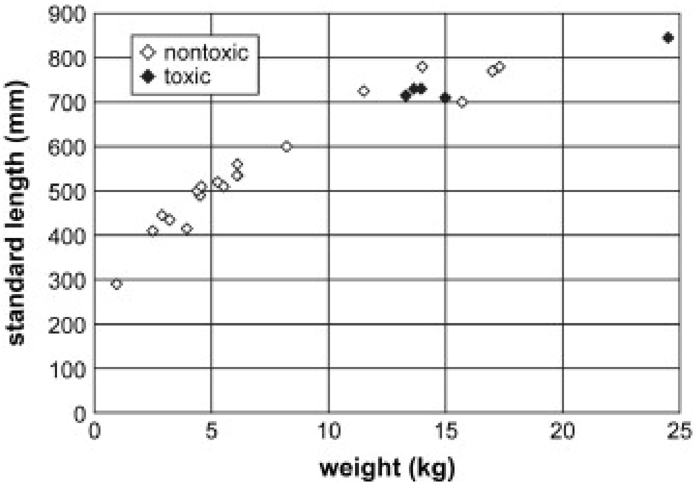


**Figure B3** Size dependency of toxic specimens of *L. bohar* (Red Bass, Oshiro et al., 2010).

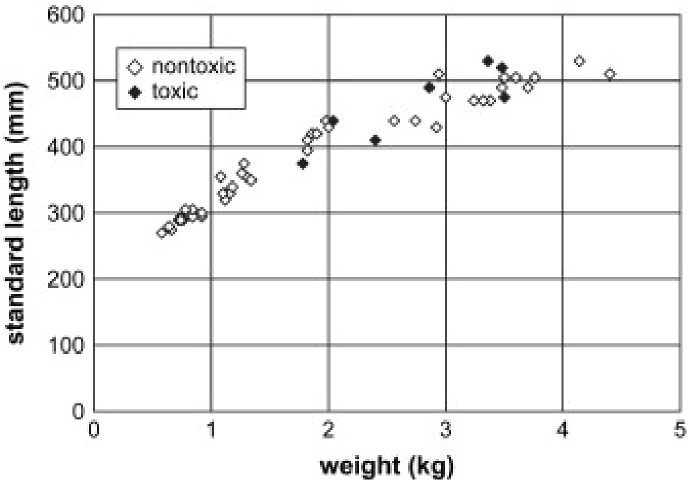


**Figure B4** Size dependency of toxic specimens of *V. louti* (Yellowedge Coronation Trout, Oshiro et al., 2010).

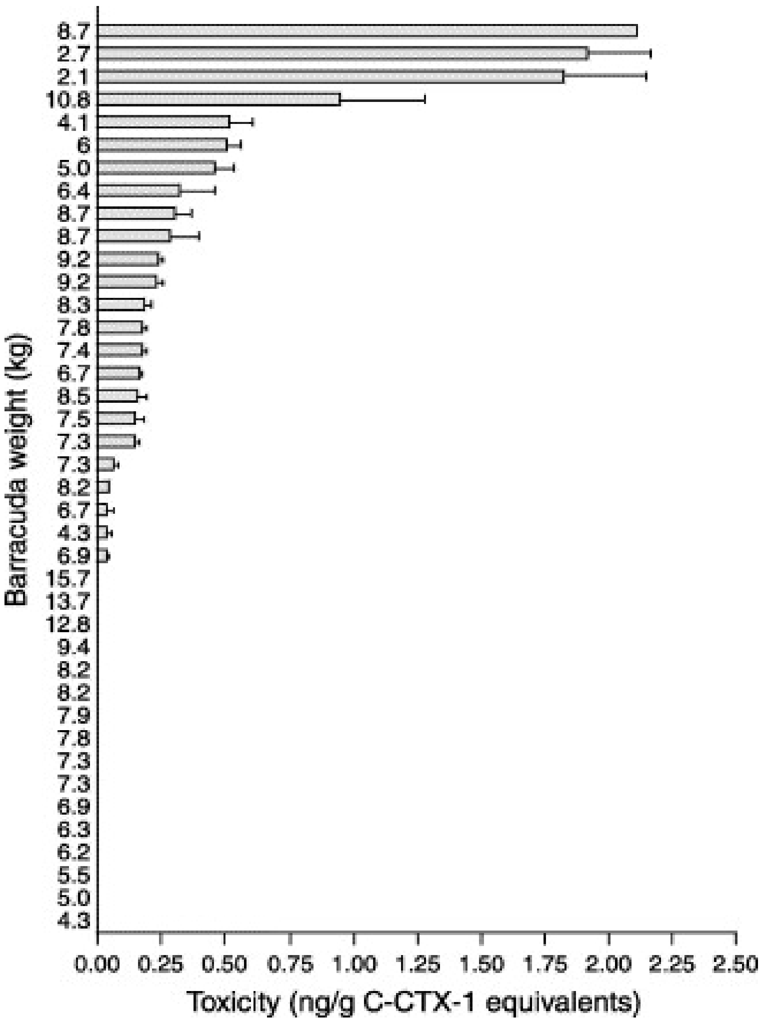
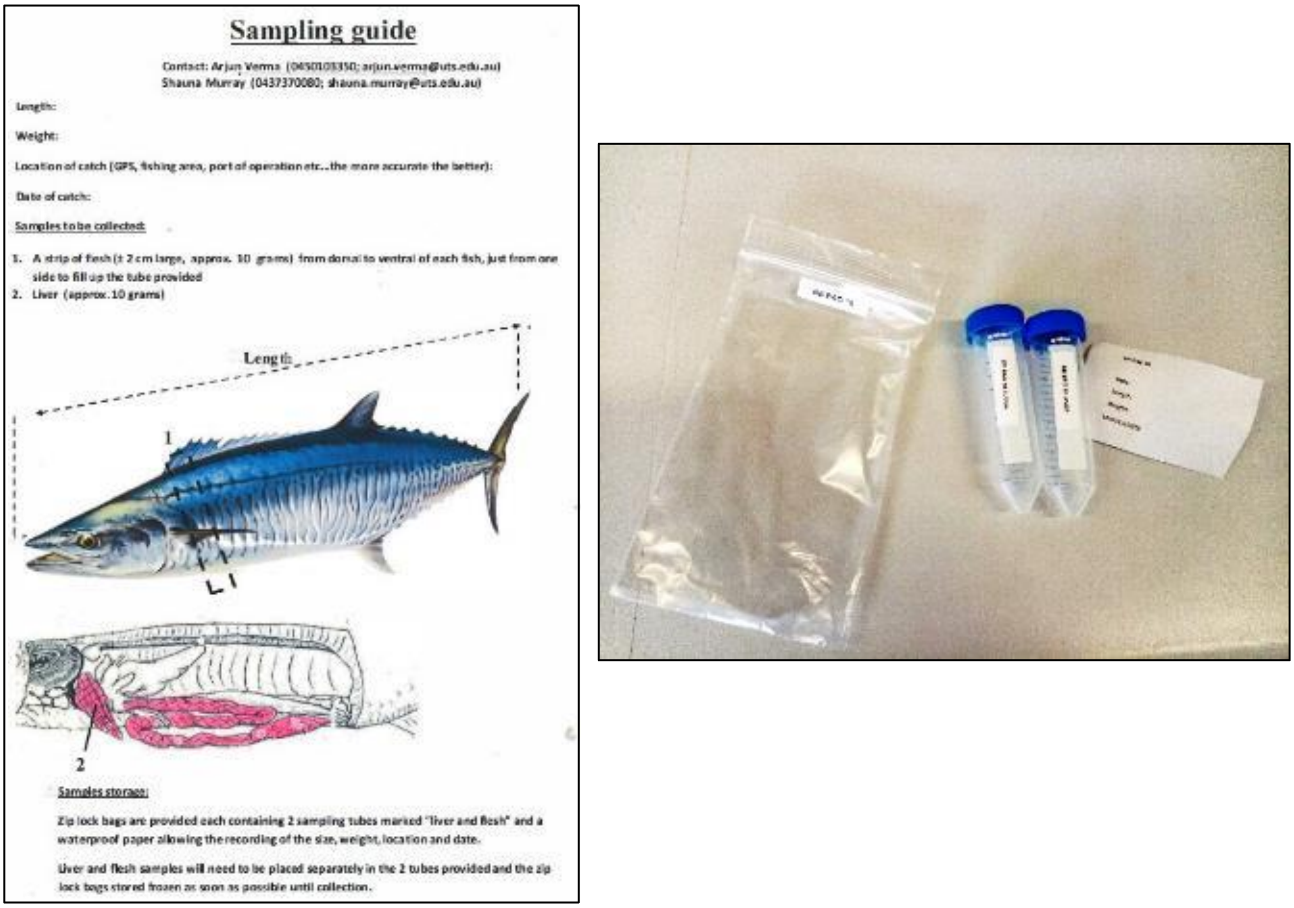


**Figure B5** Caribbean ciguatoxin C-CTX-1 equivalents measured in liver specimens of 40 *Sphyraena barracuda* (Barracuda) caught off the coast of Marathon Key, FL, USA by cytotoxicity assay. Each column, assigned with the weight of each fish, represents the mean±SEM (*n*=3 except for the fish weighing 8.7 kg) (Dechraoui et al., 2005).



Sampling guide

Contact: Arjun Verma (0450103350; arjun.verma@uts.edu.au)
Shauna Murray (0437370080; shauna.murray@uts.edu.au)

Length:

Weight:

Location of catch (GPS, fishing area, port of operation etc…the more accurate the better):

Date of catch:

Samples to be collected:

1. A strip of flesh (± 2 cm large, approx. 10 grams) from dorsal to ventral of each fish, just from one side to fill up the tube provided
2. Liver (approx. 10 grams)

Samples storage:

Zip lock bags are provided each containing 2 sampling tubes marked "liver and flesh" and a waterproof paper allowing the recording of the size, weight, location and date.

Liver and flesh samples will need to be placed separately in the 2 tubes provided and the zip lock bags stored frozen as soon as possible until collection.

**Figure B6** Sampling Guide and kit given out to recreational and commercial fishing groups.

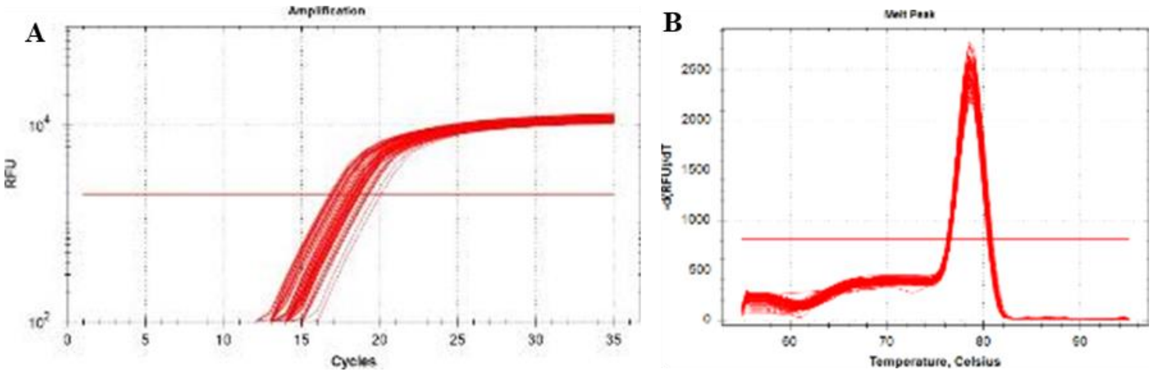


**Figure B7** qPCR amplification curve displaying Ct values and showing that the identity of all specimens was *S. commerson.* B. Melt curve analysis, for fish specimens collected during 2021–2022 fishing season.

**Certification of Recreational Fishing Trust Fund Expenditure**

☐ I certify that:

○ **all** Trust funds have been expended in accordance with the Funding Agreement; and

○ the attachments are an accurate record of that expenditure.

*OR*

☐ I certify that:

○ Trust funds of $_______ have been expended in accordance with the Funding Agreement;

○ the balance of the Trust funds being $______ will be returned to NSW DPI in accordance with the Funding Agreement; and

○ the attachments are an accurate record of that expenditure.

Signature: _________________________

Name: _________________________

Date: _________________________

*Attachments*

Grantees must attach:

○ a detailed expenditure statement; *OR*

○ an itemised list of expenses; *OR*

○ copies of invoices/receipts.