New Zealand Food Safety
Haumaru Kai Aotearoa

Risk Profile: Ciguatoxins in seafood

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Scientific Interpretative Summary

This SIS is prepared by New Zealand Food Safety (NZFS) risk assessors to provide context to the following report for MPI risk managers and external readers.

FW18009 – Risk Profile: Ciguatoxins in seafood.

Ciguatoxins (CTXs) are produced by the dinoflagellate species *Gambierdiscus toxicus* and potentially can cause ciguatera fish poisoning (CFP). Due to the increased incidence of CFP internationally, New Zealand Food Safety (NZFS), has contracted Environmental Science and Research Limited (ESR) to conduct a risk profile considering the risk of ciguatoxins in seafood, and to assess if new data have become available since the previous published risk profile in 2007. It is important to assess the current available information on ciguatoxins in seafood to determine if more risk management activities are needed.

CFP can lead to neurological, gastrointestinal, and cardiovascular disorders. Although CFP has been reported infrequently in New Zealand in the last decade, with around one outbreak a year, *Gambierdiscus spp* have been reported in new geographical areas including New Zealand. Climate change effects on sea surface temperature (SST) could impact the distribution and proliferation of CTXs. To date, however, the primary risk for CFP is likely to be private importation of reef fish into New Zealand.

The risk profile identified the lack of certified testing standards, plus the lack of validated analytical methods as important data gaps. Furthermore, data on private reef fish importation into New Zealand would be useful to be able to assess the CFP risk for seafood consumption in New Zealand.

In New Zealand no regulatory limits have been established for CTXs, as there is no evidence that ciguatoxic fish is being caught for consumption in New Zealand. Internationally, FAO is working on a full evaluation of known CTXs and congeners, including geographic distribution, rate of illness, and methods of detection.

Science for Communities

RISK PROFILE: CIGUATOXINS IN SEAFOOD

September 2019

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EXECUTIVE SUMMARY

The purpose of a Risk Profile is to provide contextual and background information relevant to a food/hazard combination so that risk managers can make decisions and, if necessary, take further action. Risk Profiles include elements of a qualitative risk assessment, as well as providing information relevant to risk management. Risk profiling may result in a range of activities, such as immediate risk management action, a decision to conduct a quantitative risk assessment, or a programme to gather more data. Risk Profiles also provide information for ranking of food safety issues.

This Risk Profile addresses risks associated with ciguatoxins in seafood and is an update of an earlier risk profile published in 2007.

Ciguatera fish poisoning (CFP) has historically been associated with the consumption of fish containing ciguatoxins (CTXs). CTXs are produced by the dinoflagellate species *Gambierdiscus toxicus* and potentially other species within this genus. Dinoflagellates are part of the polyphyletic group known as algae and are sometimes referred to as microalgae. They usually live in association with seaweed (macroalgae). There is less evidence for CTX production by species outside this genus. Dinoflagellates of the genus *Ostreopsis* have occasionally been implicated in CFP, usually due to their presence in waters in CFP-endemic regions. CTXs are generally considered to be primary mediators of CFP. While it cannot be ruled out that other dinoflagellate toxins such as maitotoxins and gambierol may contribute to CFP, there is no direct evidence to substantiate their involvement.

Increased incidence of CFP has been attributed to the expansion of fishing in CFP-endemic regions and the export of those fish to non-endemic regions. In addition, *Gambierdiscus* spp. and *Ostreopsis* spp. were also reported to have expanded into a range of new areas.

While sea surface temperature (SST) is likely to be a major determining factor for the growth of *Gambierdiscus* spp., other factors such as water salinity, light intensity, nutrient availability and availability of macroalgal substrates have also been identified as important factors. While New Zealand SSTs are projected to rise by 2.5°C by 2100, there is little evidence of SST increases to date.

CTXs are large polyether molecules, containing multiple 5, 6, 7, 8 and 9-membered rings. The molecules are arranged in a distinctive ‘ladder’ structure. The genetic basis for CTX production is currently unknown. They appear to be post-translationally biosynthesised by extension of the skeleton ladder structure. Biosynthesis is believed to involve a range of polyketide synthase enzymes. No information is available on the function of CTXs for the dinoflagellates that produce them.

There are geographical differences in the structure of CTXs found and the names given to congeners are usually prefaced by P-, C- or I- to indicate Pacific, Caribbean or Indian variants. The gambiertoxins, such as CTX-4A and CTX-4B, found in dinoflagellates undergo oxidative metabolism in fish to produce the more toxic CTXs associated with CFP, such as P-CTX-1, P-CTX-2, P-CTX-3, C-CTX-1 and C-CTX-2. Analytical methods for CTXs are specific to a point in the biological chain (dinoflagellate-fish-human) and may be effect-based or structure-based.

CFP exhibits as a range of gastrointestinal and neurological symptoms in humans. CTXs act on voltage-sensitive sodium channels in excitable membranes. The binding affinity of CTXs for the voltage sensitive sodium channels depends on the particular congener, with CTX-1 binding with approximately 30 times higher affinity than the microalgal analogue CTX-4B.

While samples of fish implicated in CFP cases in New Zealand have occasionally been tested for CTX activity, no analyses of CTXs in fish from New Zealand coastal water or exclusive economic zone (EEZ) have been reported. In Australia, CTXs have been detected in Spanish mackerel (*Scomberomorus commerson*) collected from temperate waters off New South Wales and it has been suggested that this represents a southward spread in the endemic occurrence of CFP.

No estimates of dietary CTX exposure have been determined in New Zealand or elsewhere. Currently, no biomarkers of exposure have been validated for CTXs. CFP is infrequently reported in New Zealand, with no more than one outbreak per year over the last decade. The highest number of cases per outbreak was six.

The risk of New Zealanders suffering CFP following consumption of ciguatoxic fish includes two potential routes of exposure; incursion of ciguatoxic dinoflagellates into New Zealand's EEZ and coastal water, resulting in ciguatoxic fish being caught for consumption in New Zealand, or importation of ciguatoxic fish into New Zealand.

While *Gambierdiscus* species have been found in waters around the far north of New Zealand and a known ciguatoxic species (*G. polynesiensis*) has been found in the Kermadec Islands, these isolates did not produce CTXs. To date, there is no evidence of CTX-producing dinoflagellates in waters near (within 1000 km) to New Zealand. However, the occurrence of CFP cases in Australia associated with fish caught in temperate waters suggests that the possibility cannot be discounted.

Private importation of fish from Pacific islands into New Zealand is still a permitted activity and it appears likely that this activity will be the primary risk factor for CFP in the near future. Intoxications occurring shortly before return to New Zealand may also contribute to notification in New Zealand.

Assessment of CFP risks for New Zealanders is beset by data gaps. However, some of these data gaps will be more critical than others. In particular:

- Information on the frequency of personal importation of reef fish into New Zealand and the species involved would provide a useful denominator for the notified cases of CFP. At present it is unknown whether the CFP cases that come to the attention of the public health system in New Zealand are a small or large proportion of people privately importing reef fish.
- Testing for ciguatoxicity in New Zealand and worldwide suffers from a lack of certified standards and a lack of validated methods. This means that the results of analytical testing are often difficult to interpret.
- The lack of standardised, validated methods also impacts on other areas where data are sparse. While information on the dose-response relationship for CFP is improving, definition of the dose and in particular the concentration of CTXs in the implicated fish is still method dependent. It is uncertain how comparable doses derived through different methods are.

1. INTRODUCTION

The purpose of a Risk Profile is to provide information relevant to a food/hazard combination(s) so that risk managers can make informed decisions and, if necessary, take further action. Risk Profiles are part of the Risk Management Framework (RMF)[1] approach taken by New Zealand Food Safety (NZFS). The Framework consists of a 4-step process:

- Preliminary risk management activities
- Identification and selection of risk management options
- Implementation of control measures, and
- Monitoring and review

This initial step in the RMF, Preliminary Risk Management Activities, includes a number of tasks:

- Identification of food safety issues
- Establishing broad risk management goals
- Gathering data including, if required, commissioning risk profiling and/or other scientific evaluation
- Deciding on the need for a risk assessment and, if required, the form this should take
- If needed, setting risk assessment policy and commissioning of the risk assessment
- Considering the results of the risk assessment
- Ranking and prioritisation of the food safety issue for risk management consideration.

Risk profiling may be used directly by risk managers to guide identification and selection of risk management options, for example where:

- Rapid action is needed;
- There is sufficient scientific information for action;
- Embarking on a risk assessment is impractical.

1.1 HAZARDS AND RISK MANAGEMENT QUESTIONS

This Risk Profile addresses risks associated with ciguatoxins (CTXs) in seafood.

The current Risk Profile does not address issues related to CTXs in animal feed or associated animal health issues, except where these are relevant to human health.

The sections in this Risk Profile are organised as much as possible as they would be for a conventional qualitative risk assessment.

Hazard identification, including:

- A description of the chemical(s).

Hazard characterisation, including:

- A description of the adverse health effects caused by the chemical.
- Dose-response information for the chemical in humans, where available.

[1] http://www.foodsafety.govt.nz/elibrary/industry/RMF_full_document_-_11604_NZFSA_Risk_Management_Framework_3.1.pdf Accessed 26 July 2017

Exposure assessment, including:

- Data on the occurrence of the hazard in the New Zealand food supply.
- Data on the consumption of relevant foods by New Zealanders, where appropriate.
- Qualitative estimate of exposure to the chemical (if possible).
- Overseas data relevant to dietary exposure to the chemical.

Risk characterisation:

- Information on the number of cases of adverse health effects resulting from exposure to the chemical with particular reference to the identified food (based on surveillance data) or the risk associated with exposure (based on comparison of the estimated exposure with exposure standards).
- Qualitative estimate of risk, including categorisation of the level of risk associated with the chemical in the food.

Risk management information

- A description of relevant food safety controls.
- Information about risk management options.

Conclusions and recommendations for further action

1.2 MAIN INFORMATION SOURCES

Information on the toxicology of and/or exposure to CTXs has not been widely reviewed or otherwise considered. An assessment carried out by EFSA (European Food Safety Authority) was a resource for the current project (EFSA, 2010).

Additional information was located by general searching of the World Wide Web (internet) and use of specific citation databases, including:

- PubMed. Accessed at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?DB=pubmed
- Web of Science. Accessed at: http://apps.webofknowledge.com/

As this document is an update of an earlier Risk Profile (Cressey *et al.*, 2007) searches of the scientific literature were restricted to the period since the completion of the previous Risk Profile.

2. HAZARD IDENTIFICATION -CIGUATOXINS

2.1 CAUSATIVE ORGANISM

Ciguatera fish poisoning (CFP) has historically been associated with the consumption of fish containing CTXs. CTXs are produced by the dinoflagellate species *Gambierdiscus toxicus* and potentially other species within this genus. Dinoflagellates are unicellular protists and are very diverse organisms. Approximately 90% of dinoflagellates can be found in the ocean, with the remaining living in freshwater such as rivers and lakes (Simmel, 2010). Dinoflagellates can live in pelagic, benthic, symbiotic and parasitical environments. Dinoflagellates are often referred to as microalgae. Certain species of *Gambierdiscus* are the source of several marine toxins: CTXs, maitotoxins (MTXs), gambierol and several gambieric acids. While MTXs are cytotoxic and are believed to have a role in CFP, this has yet to be confirmed (Ajani *et al.*, 2017).

The genus *Gambierdiscus* is still in the process of being elucidated. While species identification may be based on morphological characteristics, DNA analysis is increasingly being used (Litaker et al., 2010; Lyu et al., 2017). This has aided in the reclassification of some *Gambierdiscus* species to a separate genus, *Fukuyoa. Fukuyoa* spp. are characterised by a globular shape, as opposed to the lenticular shape of *Gambierdiscus* spp. (Shmukler and Nikishin, 2017). It is unclear whether *Fukuyoa* spp. have a role in CFP with different studies showing disparate evidence on the ability of this genus to produce CTXs (Laza-Martinez *et al.*, 2016; Litaker *et al.*, 2017; Munday *et al.*, 2017)

There is less evidence for CTX production by species outside this genus. Dinoflagellates of the genus *Ostreopsis* have occasionally been implicated in CFP, usually due to their presence in waters in CFP-endemic regions (Shears and Ross, 2009). While these organisms appear to produce toxins, they have not been reported as producing CTXs (Parsons *et al.*, 2012).

2.1.1 *Gambierdiscus toxicus*

Genetic analysis of *G. toxicus* strains (*n* = 28) from the Pacific and Atlantic/Caribbean identified four distinct lineages, termed Clades A-D (Richlen *et al.*, 2008). Clade A contained two Atlantic/Caribbean isolates, Clade C contained exclusively Pacific isolates and Clades B and D contained mixtures of Pacific and Atlantic/Caribbean isolates. Morphological analysis resulted in grouping of isolates into three groups (1, 2 and 3). Group 3 contained only isolates from Clade B and corresponded to the species description of *G. belizeanus*. No other concordance between Clades and morphological groups was noted. The authors concluded that *G. toxicus* should be considered as a wide-ranging species complex, rather than a single cosmopolitan species.

2.1.2 Other toxic dinoflagellates

The species definition of *Gambierdiscus* was previously based on morphology, but is more commonly being replaced by genetic analysis. It is still uncertain whether CTX production is species-specific. CTXs have been detected in isolates of *G. toxicus, G. polynesiensis* and *G. excentricus.* Reports of CTX production by other species are inconsistent.

The genus *Gambierdiscus* currently contains 15 characterised species; *G. australes, G. balechii, G. belizeanus, G. caribaeus, G. carolinianus, G. carpenteri, G. cheloniae, G. excentricus (*newly described*), G. honu, G. lapillus, G. pacificus, G. polynesiensis* and *G.*

toxicus (Guiry and Guiry, 2018; Shmukler and Nikishin, 2017). Six ribotypes[2] have also been described (*Gambierdiscus* ribotype 2; *G.* sp. type 2; *G.* sp. type 3; *G.*sp. types 4, 5 and 6) (Smith *et al.*, 2017a).

G. cf. *yasumotoi* has been found in Northland, New Zealand and shown to produce putative maitotoxin MTX-3[3], but not MTX-1 or CTX (Rhodes *et al.*, 2014a).

Growth and toxin production in a clonal strain of *G. polynesiensis* from French Polynesia was examined in batch culture (Chinain *et al.*, 2010a). Analysis of dichloromethane and methanol extracts by mouse bioassay (MBA) and a receptor-binding assay (RBA) was carried out. Mice showed symptoms specific to CTX and MTX activity, with the two toxin groups contributing equally to total toxicity, except in aged cells, where CTX toxicity was predominant. Ciguatoxicity was confirmed by RBA. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis identified CTX-3C, -3B, -4A, -4B, M-seco-CTX-3C and novel CTX congeners. Toxin composition appears to be consistent between clones.

Isolates (*n* = 12) of *Gambierdiscus* species from the Caribbean (*n* = 7), Pacific (*n*= 4) and Atlantic (*n* = 1) were extracted and separated by HPLC, with separated fractions tested for maitotoxicity and ciguatoxicity by a modified mouse neuroblastoma (N2a) assay (Lewis *et al.*, 2016). While 11 isolates exhibited maitotoxicity, only two Caribbean isolates produced detectable concentration of CTXs; an isolate of *G. carolinianus* and one of *Fukuyoa ruetzleri* (formerly *G. ruetzleri*).

Isolates (*n* = 13) of *Gambierdiscus* were collected from Atlantic, Pacific and Mediterranean sources and tested for maitotoxicity and ciguatoxicity by N2a and erythrocyte lysis assays (Pisapia *et al.*, 2017). All strains showed MTX toxicity. All but one strain showed very low concentrations of CTX toxicity (0.6-50 fg/cell of CTX-3C equivalents). This is of interest, as these strains were of species usually considered to be non-ciguatoxic (*G. australes, G. balechii, G. caribaeus, G. carpenteri, G. pacificus, G. scabrosus, G. carolinianus* and *G. silvae*). Substantially higher ciguatoxicity (1426 fg/cell CTX-3C equivalents) was detected in a strain of *G. excentricus*, a species associated with CFP in the Canary Islands.

In a further study by the same research group, 33 strains from seven *Gambierdiscus* and one *Fukuyoa* species from the Caribbean/Gulf of Mexico were assessed for ciguatoxicity by N2a (Litaker *et al.*, 2017). All but one isolate (*F. ruetzleri, G. belizeanus, G. caribaeus, G. carolinianus, G, carpenteri, G. silvae and Gambierdiscus* ribotype 2) exhibited low toxicity (<25 fg/cell P-CTX-3C equivalents). The sole isolate of *G. excentricus* contained CTX-like toxicity of 469 fg/cell P-CTX-3B equivalents. There was a general negative correlation between ciguatoxicity and growth rate in media. This suggests an evolutionary trade-off between growth and production of defence compounds.

A further *Gambierdiscus* species (*G. lapillus* sp. nov.) has recently been characterised amongst strains collected from Heron Island, Australia (Kretzschmar *et al.*, 2017). While clones of this species were found to be toxic they did not contain algal-associated CTX congeners (CTX-3B, CTX-3C, CTX-4A and CTX-4B). A putative MTX (MTX-3) was identified.

Molecular analysis was used to assign a *Gambierdiscus* ribotype from Marakei Island, Kiribati as *G. balechii* (Dai *et al.*, 2017). Central Pacific strains were ciguatoxic by N2a (1.1-

[2] In this context, ribotype refers to a group of isolates with similar ribosomal DNA (rDNA) sequences, that are distinct from currently described species. It is likely that these ribotypes represent as yet undescribed species

[3] While this compound is believed to be a maitotoxin, its structure has not yet been determined

19.9 fg/cell P-CTX-1 equivalents), while strains from the western Pacific were non-ciguatoxic.

Similarly, a novel *Gambierdiscus* species (*G. honu* sp. nov.) was isolated from macroalgae from Rarotonga and the northern Kermadec Islands (Rhodes *et al.*, 2017a). While extracts of the isolates were highly toxic to mice by intraperitoneal injection, they did not produce CTXs.

Toxicity testing of isolates of marine pelagic cyanobacteria of the genus *Trichodesmium* from New Caledonia was reported to have identified 'ciguatoxin-like' toxins (Kerbrat *et al.*, 2010). Lipid soluble extracts of cyanobacterial samples were positive in the mouse bioassay (MBA) and the N2a and RBA assays. However, no structural elucidation was carried out and it is unknown whether the toxins were polyether CTXs.

2.1.3 Ecology of ciguatoxic dinoflagellates

While sea surface temperature (SST) is likely to be a major determining factor for the growth of *Gambierdiscus* spp., other factors such as water salinity, light intensity, nutrient availability and availability of macroalgal substrates have also been identified as important factors.

Substrate

Gambierdiscus is considered to be predominantly epiphytic (Parsons *et al.*, 2012). While *Gambierdiscus* have been found on more than 50 macroalgal species, it has been suggested that they may have host preferences (Cruz-Rivera and Villareal, 2006). While *Gambierdiscus* may attach to algal hosts by envelopment in a surface mucous membrane, mobile cells that can attach and detach under the influence of various cues have been observed (Parsons *et al.*, 2012).

A laboratory study examined the epiphytic behaviour of *G. toxicus* cells in the presence of various macroalgal species (Parsons *et al.*, 2011). The studies suggest that *G. toxicus* is not an obligate epiphyte and undergoes a range of macroalgal species-specific interactions. Macroalgae were grouped into six groups (A-F). Interactions with group A were characterised by mainly unattached *G. toxicus* cells and high mortality rates, group D exhibited high attachment rates with mortality occurring late in the experimental timeframe, all other groups exhibited low attachment rates and a range of mortality rates. Interactions between macroalgae and *G. toxicus* appear to be at least partially moderated by exudates from the macroalgae.

A study in the north-western Gulf of Mexico found that, although the generally soft, muddy sea bottom was not conducive to establishment of *G. toxicus*, the many petroleum production platforms and artificial reefs in the Gulf provide hard surfaces for the establishment of corals and associated ecosystems (Villareal *et al.*, 2007). Six petroleum production platforms were examined, with *G. toxicus* found to be present as an epiphyte on the platform fouling community.

Temperature, salinity and light

The impact of temperature, salinity and irradiance on growth of eight *Gambierdiscus* species, not including *G. toxicus*, was examined (Kibler *et al.*, 2012). The species examined were *G. australes, G. belizeanus, G. caribaeus, G. carolinianus, G. carpenteri, G. pacificus, G. ruetzleri* and *G. ribotype* 2. Minimum growth temperatures ranged from 15.0°C (*G. carolinianus*) to 19.8°C (ribotype 2), with optimum growth temperatures in the range 23.2-32.4°C. Optimum salinity for growth was about 25-30‰. The maximum salinity tested was 41‰ . This salinity was only lethal to ribotype 2, but reduced the growth rate of all species except *G. pacificus*. All species were adapted to growth in low light conditions.

Using these data on growth temperature optima, data on SST and CFP incidence in the Caribbean and West Indies were examined (Tester *et al.*, 2010). Data from the southern Gulf of Mexico showed that the number of days with SST ≥29°C had nearly doubled (44 to 86

days) in the previous three decades. CFP incidence rates were highest in the eastern Caribbean, where the SST is warmest and least variable. All CFP cases occurred in areas where the annual average SST was ≥25°C.

Observations of *Gambierdiscus* spp. cell densities and associated nutrient and physicochemical data collected during 3.5 years in Hawaii were used to develop a simulation model for the population dynamics of these organisms (Parsons *et al.*, 2010). The model developed was shown to have acceptable predictive capability. *Gambierdiscus* cell abundance was most sensitive to water temperature and salinity. The authors noted that their model did not predict toxin production and all field measurements of ciguatoxicity were below the analytical limit of detection (LOD). The model suggested that, at a sea temperature increase of 0.028°C/year, *G. caribaeus* abundance would increase 9-fold within 10 years, but in the long-term temperatures may become too warm, hindering *Gambierdiscus* growth.

The impact of water temperature and salinity on the growth of two strains of *G. carpenteri,* from two separate locations on the central Great Barrier Reef was examined (Sparrow *et al.*, 2017). In order to mimic actual situations, these strains were co-cultured with the dinoflagellates *Prorocentrum lima* and *Ostreopsis* sp. Population growth was measured over 28 days. In monoalgal growth, the *G. carpenteri* strains showed little variation in growth rate across temperatures of 24 and 28°C and salinities of 36 and 26‰, but showed negative growth at 34°C (all salinities) and salinity of 16‰ (all temperatures). Growth of *G. carpenteri* was generally inhibited in the presence of other dinoflagellates, particularly *P. lima*. While, growth was decreased at salinity of 16‰, increased growth of *G. carpenteri* was observed in the presence of *Ostreopsis* sp. at this salinity.

It has been suggested that increasing SSTs may favour the spread of some *Gambierdiscus* species (e.g. *G. caribaeus*), but hinder the spread of other species (e.g. *G. carolinianus*) (Parsons *et al.*, 2012).

The preference of *Gambierdiscus* spp. for low light, typically 10% of full sunlight (Parsons *et al.*, 2012), was highlighted in a study in the northern Gulf of Mexico, in which *Gambierdiscus* were found to grow at light levels as low as 10 μmol photons/m^2/s, as long as temperature and salinity were favourable (Tester *et al.*, 2013). This level of irradiance corresponds to a depth of 70-80 m in the surveyed region. *Gambierdiscus* has not been found in samples taken below 45 m.

In light-driven growth experiments, a strain of *G. carolinianus* and one of *G. silvae* both achieved maximum growth at irradiance of about 100 μmol photons/m^2/s (Leynse *et al.*, 2017).[4]

Nutrients

A summary of evidence for a relationship between increased eutrophication and prevalence of *Gambierdiscus* spp. was inconclusive (Anderson *et al.*, 2008). Similarly, evidence for a link between eutrophication and toxin production was equivocal. The authors of this study speculated that eutrophication may indirectly contribute to *Gambierdiscus* proliferation by causing mortality of reef-building corals thereby freeing up benthos for the proliferation of macroalgae; increasing availability of suitable substrates for the settlement and reproduction of *Gambierdiscus*.

[4] Full sunlight is typically about 2000 μmol photons/m^2/s

2.1.4 Geographic distribution of ciguatoxic dinoflagellates

PCR analysis of D8-D10 rDNA of isolates from the Atlantic, including the Caribbean, and the Pacific was used to determine the geographical distribution of *Gambierdiscus* species (Litaker *et al.*, 2010). *G. toxicus, G. pacificus, G. australes, G. polynesiensis* and *G. yasumotoi* were found to be endemic to the Pacific, while *G. belizeanus, G.* ribotype 1*, G.* ribotype 2, *G. carolinianus* and *G. ruetzleri* were found to be endemic to the Atlantic/Caribbean. Two species, *G. carpenteri* and *G. caribaeus* are globally distributed. This study further noted that:

- Atlantic CTXs are associated with top predator fish species, while Pacific CTXs are associated with both herbivorous and predatory species
- While little information is available on CTXs from the Indian Ocean, they appear to be more structurally similar to C-CTXs than P-CTXs
- *Gambierdiscus* population densities and frequency of CFP events are associated with stable elevated water temperatures in the range 25-29°C
- Toxin production appears to be under genetic control and major differences in ciguatoxicity are likely to be species-specific, rather than strain-specific. Toxin production appears to be inversely related to growth rate (slower growing species are more toxic) and inversely related to latitude (toxin production is lower further from the equator). It should be noted that this conclusion differs from the conclusions of earlier work, that suggested that toxin production varies between different strains of *G. toxicus* and that not all strains are toxin-producing (Holmes *et al.*, 1991). However, the earlier work should be viewed in the context of the emerging definition of the *Gambierdiscus* genus; it is possible that non-toxigenic strains were not *G. toxicus*.

CFP has not been reported in the Red Sea area. Samples of *Turbinaria* and *Halimeda* macroalgae were sampled from the central Red Sea in 2012-2013 (Catania *et al.*, 2017). *Gambierdiscus* and *Ostreopsis* spp. were isolated from macroalgae, although cell densities were generally low (<200 cell/g wet weight). The Red Sea is one of the warmest (SST up to 32°C) and most saline (36-40+‰) seas in the world. *Gambierdiscus* cell densities were negatively correlated with salinity and SST, although the correlation was only significant for salinity. *Gambierdiscus* isolates were established in culture and were identified as *G. belizeanus* by morphology and molecular analysis. Isolates were determined to be ciguatoxic by N2a, although the maximum toxin content (6.5×10^{-5} pg/cell P-CTX-1 equivalents or 0.065 fg/cell P-CTX-1 equivalents) was very low.

A study was conducted of *Gambierdiscus* species in the Flower Garden Banks National Marine Sanctuary in the northern Gulf of Mexico (Tester *et al.*, 2013). The most common species was *G. carolinianus* (67% of samples), followed by *G. caribaeus* (19% of samples), *G. carpenteri* (12%), *G. belizeanus* (23%), *Gambierdiscus* ribotype 2 (13%) and *G. ruetzleri* (2%). Each sample represented a representative algal collection from a particular site and multiple species were found at some sites.

Some of the highest incidence rates of CFP occurs in the Pacific (Llewellyn, 2010). The closest populations of ciguatoxic dinoflagellates to New Zealand, are located in Australia and the Pacific islands. CTXs have been detected in extracts of *G. polynesiensis* from the Cook Islands (Munday et al., 2017; Rhodes et al., 2014b) and a *Gambierdiscus* isolate from French Polynesia (Pawlowiez et al., 2013). CTX-1 equivalents were detected in *G. australes* from Rarotonga (Rhodes et al., 2010), and *Gambierdiscus* (ribotype 4) from Kiribiti (Xu et al., 2014). Lower CTX-1 like activities were also detected from the same Kiribiti site for ribotype 5 and one isolate of *G. pacificus*. CTX-like activity has also been detected in extracts of unidentified strains of *Gambierdiscus* from Australia, and trace level activity in strains of *G.*

lapillus (Larsson et al., 2018). It is thought these unidentified strains may be causing CFP in that region (See section 4).

2.1.5 Potential impacts of climate and other environmental change

Increased incidence of CFP has been attributed to the expansion of fishing in CFP-endemic regions and the exportation of those fish to non-endemic regions (Dickey and Plakas, 2010). Friedman *et al.* (2017), summarised reports of CFP illness and ciguatoxic fish observations in previously unreported areas, including the Canary Islands (eastern Atlantic Ocean) and the Japanese home islands. *Gambierdiscus* spp. and *Ostreopsis* spp. were also reported to have expanded into new areas that include the western Gulf of Mexico, eastern Mediterranean, the Red Sea, Crete, Brazil, Hong Kong, Thailand, and West Africa (eastern Atlantic Ocean) (Catania *et al.*, 2017; Friedman *et al.*, 2017). In New Zealand, *Gambierdiscus* spp. have been recorded in the northern waters of the Kermadec Islands and the North Island of New Zealand (Rhodes *et al.*, 2014a; Rhodes *et al.*, 2017a). *Ostreopsis siamensis* and other species in this genus were also found in temperate shallow reefs in northern New Zealand and as far south as Wellington (Rhodes *et al.*, 2000; Shears and Ross, 2009). *O. siamensis* blooms have been associated with wave protected environments and calm sea conditions that induce stratification and excessive warming of surface waters that appear to promote their growth (Shears and Ross, 2009).

The link between the expansion of *Gambierdiscus* spp. and ciguatoxic fish with changing climate is difficult to quantify. The difficulty lies in differentiating between the multiple environmental parameters that determine dinoflagellate growth (Friedman *et al.*, 2017). Some studies report positive correlations of CFP and sea surface temperature (SST) (Tester et al., 2010). However, sufficiently high SSTs (>30°C) coupled with other environmental variables, such as salinity and irradiance, have been demonstrated to inhibit growth of some *Gambierdiscus* species (Xu *et al.*, 2016). For New Zealand, average SST is projected to increase by up to 2.5°C by 2100 (Law *et al.*, 2016) under a high emission scenario [RCP8.5] (van Vuuren *et al.*, 2011). This increase may result in a southward expansion of sub-tropical species into temperate fish areas.

There is also the continued risk for the sporadic arrival and (presumed) survival of tropical and sub-tropical species into New Zealand coastal waters via ballast water and other mechanisms (Gordon *et al.*, 2010). The Intergovernmental Panel on Climate Change (IPCC) 5th assessment reported that observed SST has increased on average about 0.07°C per decade over 1909–2009 for New Zealand. The expert opinion of the authors of the assessment is that very high confidence should be associated with this finding. A greater increase in SST was observed for the Tasman Sea region (Reisinger *et al.*, 2014). The projected magnitude of change is expected to follow mean air temperature for coastal waters. Currently, SST trends could not be determined for New Zealand's oceanic, subtropical, and sub-Antarctic waters and the Tasman Sea for 1993 to 2016 (at the 95% confidence level) (Ministry for the Environment and Statistics New Zealand, 2017). The 30 years of climate data collected at Leigh, northern New Zealand, also show no temperature change (Nick Shears, University of Auckland, personal communication). At least for northern New Zealand, climate does not appear to be the key factor in bloom formation or toxin production for *Ostreopsis* despite a number major benthic blooms being recorded in recent years.

Research is still underway to investigate the relationship between *Gambierdiscus* species and other environmental variables including increased atmospheric carbon dioxide concentrations and decreasing ocean pH. Some environmental parameters have been used to predict CFP outbreak locations and develop early warning systems in tropical and sub-tropical countries (Gingold *et al.*, 2014). However, relationships between parameters other than SST for New Zealand waters are yet to be explored.

Establishment of ciguatoxic dinoflagellates is also determined by habitat. In the tropics, dead coral reefs provide a surface for macroalgal growth (Parsons *et al.*, 2011). This in turn, can provide a habitat for dinoflagellates. Coral reefs are particularly vulnerable to both human and natural impacts, such as land-based anthropogenic pollution and sedimentation, overfishing, rising temperatures, and ocean acidification (Burke *et al.*, 2011; Hoegh-Guldberg, 2011; Morrison *et al.*, 2013; Teh *et al.*, 2013). The positive correlation of CFP occurrence with increased tropical storm frequency and severity (Gingold *et al.*, 2014) may therefore, at least in part, be explained by the mass coral mortality events that often occur under these stress conditions, and result in increased macroalgal colonisation.

Ostreopsis cells have been found on drifting macroalgae in New Zealand (Rhodes *et al.*, 2000). The main ocean currents from tropical areas into New Zealand are from branches of the East Australian Current (EAC), that is weakly connected to the Tasman Front (Sutton and Bowen, 2014). The Tasman Front and a tropical source from the subtropical gyre partially sources the East Auckland Current (EAUC) that flows along the eastern coast of New Zealand's North Island.

There is currently no physical evidence that, in the last 20-30 years, the currents around New Zealand are strengthening or significantly changing, with the exception of external factors such as El Niño and Rossby waves. There is some evidence that the East Australian Current extension (south of 34S) and the Tasman Front vary at the expense of each other: when the EAC extension is strong the Tasman Front is weak, conversely when the EAC extension is weak the flows of the Tasman Front are stronger (Fernandez, 2016). Therefore, the important factors to consider are the environmental parameters required to establish a permanent population of ciguatoxic dinoflagellates and whether those populations are capable of producing toxins at a level of human health concern. Rhodes *et al.* (2017b) considered the risk for CFP occurring in mainland New Zealand as currently low. However, increasing SST and expansion of ciguatoxic species throughout the Pacific and Australasia remain possibilities.

2.2 CIGUATOXINS AND THEIR PRECURSORS

CTXs are generally considered to be primary mediators of CFP. While it cannot be ruled out that other dinoflagellate toxins such as MTXs and gambierol (Cuypers *et al.*, 2008) may contribute to CFP, there is no direct evidence to substantiate their involvement.

The genetic basis for CTX production is currently unknown. They appear to be post-translationally biosynthesised by extension of the skeleton ladder structure (see Figure 1) (Kohli *et al.*, 2015). Biosynthesis is believed to involve a range of polyketide synthase enzymes (Kohli *et al.*, 2015; Yang *et al.*, 2016). No information is available on the function of CTXs for the dinoflagellates that produce them.

2.2.1 Structure and nomenclature

CTXs are large polyether molecules, containing multiple 5, 6, 7, 8 and 9-membered rings. The molecules are arranged in a distinctive ‘ladder’ structure (Figure 1). A range of CTXs has been identified, with differences largely related to the degree of oxidation at the termini of the polyether ladder (EFSA, 2010). Several of the toxins occur as stereoisomers (epimers). For example, the algal CTXs, CTX-4A and CTX-4B, have identical chemical formulae, but differ in the orientation of an alkyl side chain.

Ciguatoxic dinoflagellates are believed to contain CTX-4A, CTX-4B and CTX-3C, sometimes referred to as gambiertoxins. Ciguatoxic fish usually contain CTX-1 and the epimeric CTX-2 and CTX-3 (Figure 1). These fish toxins are believed to be produced by oxidative metabolism of the dinoflagellate forms.

There are geographical differences in the structure of CTXs and the names given to congeners are usually prefaced by P-, C- or I- to indicate Pacific, Caribbean or Indian variants.

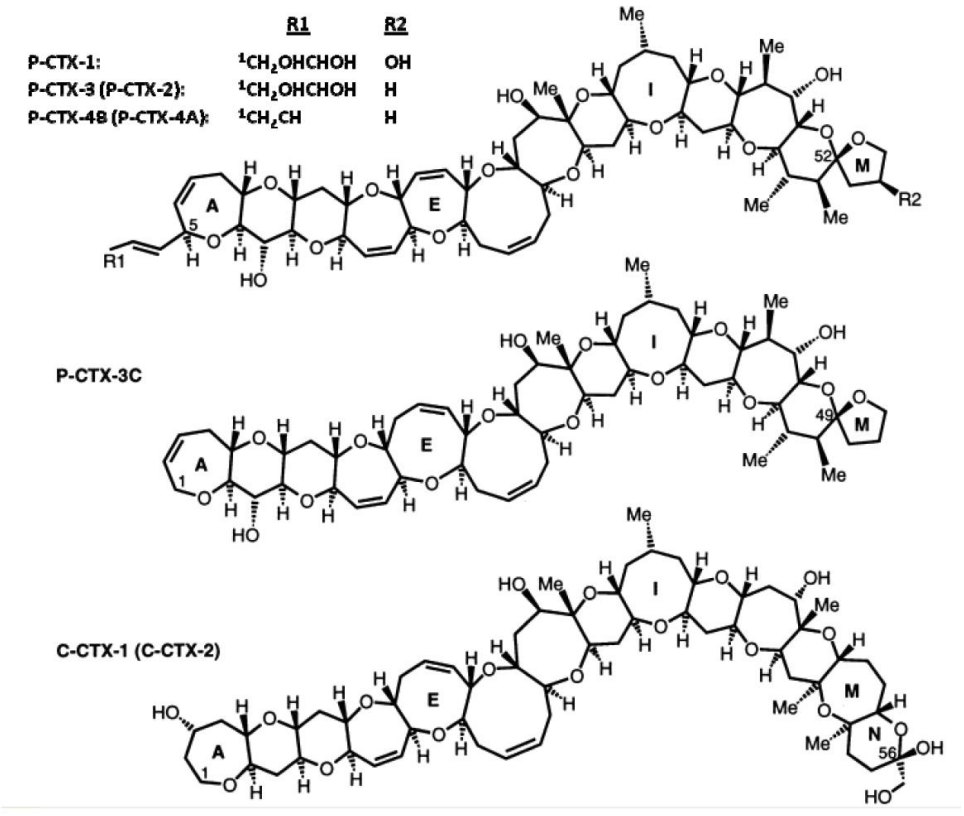
CTXs in 11 *Gambierdiscus* isolates were examined by LC-MS/MS (Roeder *et al.*, 2010). The toxin species 2,3-dihydroxy P-CTX-3C was detected in extracts from all isolates, with P-CTX-3C and P-CTX-4A/B only detected in one isolate. However, based on the genetic reclassification of the genus, only two of the isolates were classified as *G. toxicus*, including the isolate with the expected algal toxin profile.

The gambiertoxins found in dinoflagellates undergo oxidative metabolism in fish to produce the more toxic CTXs associated with CFP, such as P-CTX-1, P-CTX-2, P-CTX-3, C-CTX-1 and C-CTX-2.

A recent study has demonstrated enzymatic oxidation of algal CTXs (CTX4A, CTX4B and CTX3C) by fish liver S9 fractions, human liver enzyme (CYP3A4) and microsomal fractions prepared from ciguatoxic fish species, to produce the CTX analogues found in fish (Ikehara *et al.*, 2017). The authors suggested that this bio-oxidation could result in species-specific patterns of CTX analogues. Fish liver S9 fraction and CYP3A4 oxidised the algal toxins CTX4A and CTX4B to CTX-1B, the most toxic known CTX and the main congener found in carnivorous fish.

High levels of CTXs may cause behavioural and morphological changes in fish and may result in fish mortality (Lehane and Lewis, 2000). It is thought that the toxicity of CTXs to fish may impose an upper limit on the amount of toxin they can carry and the amount that humans may be exposed to. This has been suggested as a possible reason for the low number of CFP fatalities.

Figure 1. Structures of representative Pacific (P) and Caribbean (C) ciguatoxins



Reproduced from EFSA (2010)

Analysis of extracts from a Caribbean barracuda implicated in CFP by LC-MS/MS identified four peaks with fragmentation patterns characteristic of CTXs (Abraham *et al.*, 2012). One of the components was identified as C-CTX-1, which accounted for 60% of the observed toxicity. The remaining three putative CTXs were not identified.

It has been suggested that the profile of CTX congeners may be a function of fish species and may be influenced by the particular enzymes or enzyme isoforms present in each species (Yogi *et al.*, 2014). It was suggested that snappers and groupers produce predominantly CTX-1B, 54-deoxy-CTX-1B and 52-*epi*-54-deoxy-CTX-1B. Spotted knifejaws (*Oplegnathus punctatus*) contained mainly the algal congeners, CTX-4A and CTX-4B, and this species may lack the ability to metabolise CTXs. However, the number of fish analysed in this study was relatively small and further work is necessary to determine if congener profiles are species specific.

2.2.2 Analytical methods

Analytical methods for CTXs are specific to a point in the biological chain (dinoflagellate-fish-human) and may be effect-based or structure-based.

Sample preparation

Sample preparation and the associated purity and cleanliness of the sample extract will depend on sample type (fish or dinoflagellate), the assay type and the intended purpose of the result (screening or quantitation) (Caillaud *et al.*, 2010).

For fish samples, extraction is usually with acetone or methanol, to recover lipophilic components, followed by solvent partitioning with hexane, diethyl ether or chloroform, to separate extracted fatty acids from CTXs. Solid phase extraction (SPE) can be used to further remove fatty acids (Caillaud *et al.*, 2010; Wong *et al.*, 2009).

A CTX rapid extraction method (CREM) has been described, including cooking of fish flesh (70°C for 20 minutes), following by homogenisation in methanol/hexane (3:1), centrifugation, recovery of the aqueous methanol phase and membrane filtration (0.45 μm) (Lewis *et al.*, 2009).

Improved recovery in the clean-up of fish extracts was achieved by increasing the size of primary C18 SPE column from 500 to 900 mg and adding a secondary column using silica SPE columns (Meyer *et al.*, 2015).

For dinoflagellate samples, preparation initially involves extracting cell pellets with methanol (aqueous or absolute) or acetone, followed by solvent partitioning and/or SPE clean up (Caillaud *et al.*, 2010). Dinoflagellate extracts may contain MTXs, in addition to CTXs, which may affect some effect-based assays. Depending on the test to be carried out, a significant part of sample preparation from dinoflagellate material involves separation of the two groups of toxins.

In vivo effect-based assays

The mainstay of marine biotoxin testing for some time was the mouse bioassay (MBA) and this is still reported to be the most widely used method for detection of CTXs in fish (EFSA, 2010; Stewart and Mcleod, 2014). The MBA involves extraction of CTXs from fish flesh with diethyl ether, suspension of the extract in saline containing a surfactant (Tween) and intraperitoneal injection into mice (20 ± 2 g). Mice are observed continuously for two hours and regularly for 24 hours. The relationship between dose, expressed as mouse units (MU) and time to death is approximately log dose (MU) = 2.3 log(1 + T^{-1}).

In vitro effect-based assays

Cell-based assays (CBA), such as the mouse neuroblastoma (N2a) cell assay specifically determines the toxicity of extracts to voltage-gated sodium channels (Manger *et al.*, 1995; Truman and Lake, 1996). Sodium channel specificity is achieved by pre-treating the neuroblasts with veratridine to initiate sodium channel gating and ouabain to block the sodium–potassium pump from compensating for the sodium ion influx. The N2a cell assay can discriminate between toxins that activate or block voltage-gated sodium channel and distinguish between sodium channel specific toxins and those with other mode of actions (Abraham *et al.*, 2012). The assay provides a composite response to all sodium channel toxins in the sample, which may include a range of marine toxins, such as palytoxins, brevetoxins and saxitoxins, in addition to CTXs.

Combining the N2a assay with chromatographic fractionation of seafood extracts has been reported to improve sensitivity, by separating toxic compounds from inhibitors of the assay (Caillaud *et al.*, 2009). The combination of these two techniques also has the potential to allow distinctions to be made between different sodium channel toxins.

Mouse neuroblastoma cells were used to develop a flow cytometric method for detection and quantification of CTXs (Manger *et al.*, 2014). Voltage-sensitive fluorescent dyes are used to detect the change in voltage due to the binding of CTXs to the voltage-gated sodium channels.

A human neuroblastoma cell line (SH-SY5Y) has more recently been used to assess ciguatoxicity and particularly species-specific mechanistic aspects (Coccini *et al.*, 2017; Lewis *et al.*, 2016). P-CTX-3C displayed powerful cytotoxicity against the cell line, requiring the presence of both veratridine and ouabain. The human cell line was much more sensitive to ouabain alone, than the murine cell line (N2a).

An alternative effect-based method for determining ciguatoxicity is the receptor binding assay (RBA) (Van Dolah and Ramsdell, 2001). The assay uses isolated rat brain synaptosomes containing abundant amounts of sodium channels. The assay can be performed as a radioassay, in which toxin activity is determined by competitive binding to site 5 on the sodium channel, against a fixed amount of tritiated-brevetoxin 3. A fluorometric version of the assay has also been developed, based on competitive binding at site 5 against fluorescently-labelled brevetoxin 2 (Hardison *et al.*, 2016; McCall *et al.*, 2014). Results from the RBA_F assay correlated well with the N2a assay.

Structure-based assays

Immunological assays

Immunological methods, utilising monoclonal or polyclonal antibodies to CTXs have been developed. A simple membrane immunobead assay, using monoclonal antibodies to CTXs was developed in the 1990s (Hokama *et al.*, 1998) and was subsequently marketed as a test kit called Cigua Check®.

A direct sandwich enzyme-linked immunosorbent assay (ELISA) was developed with monoclonal antibodies specific to CTX-3C and 51-hydroxy-CTX-3C (Tsumuraya *et al.*, 2010). The assay was shown to not cross-react with other marine biotoxins (brevetoxin, okadaic acid, MTX), although reaction with other CTXs was not tested. The same research group subsequently developed a simpler assay for CTX-1B and demonstrated no cross-reactivity to CTX-3C (Tsumuraya *et al.*, 2012). The monoclonal antibodies used to develop these two assays were raised against synthetic haptens representing a portion of the CTX structure, rather than using purified natural toxins (Tsumuraya *et al.*, 2014).

A further immunoassay for CTX-3C has been described (Zhang *et al.*, 2015b). Antibodies were bound to iron nanoparticles, mixed with a sample extraction and introduced into a capillary electrophoresis column. CTX-3C was detected using a further antibody and horse-radish peroxidase bound to gold nanoparticles and reaction with *o*-aminophenol (OAP). The method was reported to have a superior limit of detection to other CTX immunoassays. A similar system was developed for detection of CTX-1B (Zhang *et al.*, 2015a).

Chromatographic assays

As with many other organic molecules, liquid chromatography-tandem mass spectrometry (LC-MS/MS) has become the method of choice for the separation and quantification of marine toxins. However, LC-MS/MS analysis of CTXs is complicated by a lack of certified standards for many of the toxin congeners.

Nuclear magnetic resonance spectroscopy

Quantification of CTXs was carried out by NMR through careful selection of protons to be used for quantification and use of pyridine-d_5 with a calibrated content of residual protons, as an internal standard (Kato and Yasumoto, 2017). However, the samples analysed were semi-purified toxins rather than fish samples.

Combined strategies

It has become increasing common for analytical strategies to include a step-wise approach involving a combination of analytical techniques. Most commonly, extracts from algae or fish will be analysed for toxicity by an effect-based method, such as the N2a method, with subsequent identification of the CTXs present by a structure-based method, such as LC-MS/MS.

Folk tests

Before the advent of analytical techniques for detecting ciguatoxic fish, a range of 'folk tests' were used in some Pacific Island communities (Darius *et al.*, 2013). Two folk tests used in Raivavae Island, French Polynesia were assessed for consistency across different testing and for agreement with RBA and N2a tests. The folk tests were:

- Rigor mortis test (RMT). The aspect of the fish is examined no more than 2 hours post-catch. If the body of the dead fish appeared flabby, the fish was considered to be toxic.
- Bleeding test (BT). The fish was cut at the tail and the flesh inspected for signs of haemorrhage. Fish with haemorrhagic signs were considered to be toxic.

Based on the RBA test, two different thresholds for toxicity were considered; >1.0 or >0.37 ng eq. P-CTX-3C/g. Agreement between different folk testers was no better than fair. BT gave better agreement with RBA than RMT and also gave better agreement between testers. Positive prediction rates of >80% were achieved when the low toxicity threshold was used, with these rates dropping to 60-70% when the higher threshold was applied. It was concluded that the folk tests were best applied in parallel and that, in combination with knowledge about risk fish species, may help to keep level of intoxication low in Pacific Island communities.

2.2.3 Comparison of analytical methods

As part of an assessment of CTXs, EFSA summarised the strengths and weaknesses of the various analytical methods available for ciguatoxins/ciguatoxicity (EFSA, 2010). A summary of EFSA's conclusions is included in Table 1.

Table 1. Strengths and weaknesses of available analytical methods for ciguatoxins/ciguatoxicity

Analytical method	Strengths	Weaknesses
Mouse bioassay (MBA)	- Provides measure of total toxicity - Does not require complex analytical equipment	- Provides no information on individual toxins - Is not sensitive enough to detect relevant levels of toxin - Cannot be automated - Requires specialised animal facilities and expertise - Inherent variability due to, for example, specific animal characteristics between laboratories - Has not been validated - Ethical concerns
Cytotoxicity assays (e.g. N2a)	- Can be automated - Are simple - Are adequately sensitive	- Unlikely to be cost effective for routine screening - Provide no information on individual toxins - Have not been validated
Receptor-binding assays (RBA)	- Are adequately sensitive - Are more specific than MBA	- Require use of radioactive brevetoxins - Highly dependent on receptor source - Provide no information on individual toxins - Cannot be easily automated - Have not been validated
Immunoassays	- Fast and easy to use - More specific than MBA - Can be used to screen high sample number for further confirmation	- Antibodies may not be useful across regions - Antibodies are not readily available - Provide no information on individual toxins - Due to cross-reactivity results need to be confirmed - Quantification is not reliable - Information on detection capability is scarce - Have not been validated
Liquid chromatography-tandem mass Spectrometry (LC-MS/MS)	- Very specific - Adequately sensitive	- Expensive - Highly trained personnel required

The scarcity or lack of certified standards and the lack of proficiency test programmes were also identified as key issues.

3. HAZARD CHARACTERISATION: ADVERSE HEALTH EFFECTS - CIGUATOXINS

3.1 CONDITIONS

Initial symptoms are intense vomiting, diarrhoea and abdominal pain within hours of fish ingestion, generally lasting 24-36 hours. This is followed, usually within 12-14 hours of onset, by development of neurological disturbance, including paraesthesia (tingling, crawling or burning sensation of the skin) and dysaesthesia (reversal of temperature perception), arthralgia, myalgia, muscle cramping and weakness. Cold allodynia, apparent as a burning sensation when exposed to cold, is also a comment symptom. Pruritis (itching) and sweating are also commonly experienced during this stage of the illness.

Other symptoms that may occur in a proportion of cases include hallucinations, transient paralysis, dysphasia (difficulty in speaking), aching joints, palpitations, dry mouth, disturbed vasomotor regulation including deranged blood pressure control, brachycardia or tachycardia (Chan, 2013b). The neurological disturbance characteristic of CFP usually resolves within weeks of onset, but in some cases may persist for months or even years. The toxin may be stored in adipose tissue for several years and symptoms may recur during periods of stress, such as exercise, weight loss or excessive alcohol consumption (Barton *et al.*, 1995). In one reported case, severe brachycardia was associated with prolonged hypotension, requiring administration of intravenous atropine (Chan, 2013b).

A review of CFP cases in Tahiti (French Polynesia) reported body temperatures below 36.5°C in 48 of 80 cases (Gatti *et al.*, 2008). This phenomenon has been observed in mice exposed to other marine toxins (MTX and brevetoxin) (Gordon and Ramsdell, 2005). In mice that received a single sub-lethal dose of 0.26 μg/kg P-CTX-1 an immediate and transient decrease in body temperature occurred, with subsequent long-term thermoregulatory dysfunction resulting in body temperatures stabilising below 36°C (Dechraoui *et al.*, 2008). A second exposure enhanced the thermoregulatory dysfunction, with 30% of mice dying within 7 hours.

Some cases exhibit an allergy-like syndrome that can persist for several years, in which symptoms typical of CFP are brought on by consumption of non-toxic fish or, occasionally, chicken or pork (Lehane and Lewis, 2000). Sensitivity to alcohol can persist for several years and in some cases alcohol consumption may cause recurrence of CFP symptoms (Gillespie *et al.*, 1986).

Severe cases may result in paralysis, coma and death, although this is rare.

The characteristic features of fatal CFP cases have been reviewed (Chan, 2016). Fatal cases were concluded to be associated with:

- Convulsions and coma, with various focal neurological signs
- Consumption of CTX-rich fish parts (viscera, head) in large quantities
- Consumption of highly ciguatoxic fish species (e.g. yellow-edged moray; *Gymnothorax flavimarginatus*)
- Consumption of reef fish collected after storms
- Individual susceptibility

The whole population is susceptible to intoxication, although in CFP endemic areas susceptibility may increase with age due to accumulation of CTXs in the body, as a result of previous exposures.

3.1.1 Sequelae

It appears that about 5% of CFP cases will develop chronic ciguatera (CC) (Shoemaker *et al.*, 2010), also known as chronic inflammatory response syndrome (CIRS) (Ryan *et al.*, 2015). The chronic condition is a multisymptom, multisystem chronic illness than can last for decades (Shoemaker *et al.*, 2010). A preliminary case definition has been proposed, including measures of immune abnormalities. Transcriptional analysis of blood from CIRS cases demonstrated differential expression of genes associated with wound healing, adaptive immunity and innate immunity compared to transcriptional analysis of blood from normal controls (Ryan *et al.*, 2015).

CFP has also been associated with subsequent development of polymyositis (a chronic inflammatory muscular disease) (Stommel *et al.*, 1991), chronic fatigue syndrome (Barton *et al.*, 1995), and Guillain-Barré syndrome (GBS) (Gatti *et al.*, 2008).

A 53-year-old man in Okinawa, Japan was diagnosed with rheumatoid arthritis (RA), 6 months after experiencing CFP (Ohta *et al.*, 2017). The reporting physician noted the similarity in clinical manifestations between CFP and RA and suggested that increases in tumour necrosis factor-α (TNF-α) and matrix metalloproteinase-9 (MMP-9), associated with CFP, may be related to the onset and progression of RA.

3.1.2 Treatment

CFP treatment is mainly supportive, although intravenous mannitol has been shown to provide benefit in severe cases. However, a double-blind randomised trial in Rarotonga (50 cases) found that mannitol was not superior to saline in relieving symptoms and signs of CFP at 24 hours (Schnorf *et al.*, 2002). Mannitol treatment resulted in an increased rate of side effects.

It is generally considered that mannitol treatment needs to be instituted early on in the disease progression to be effective, but a case report of a 51-year-old male who received IV mannitol after approximately one month of CFP-associated neurological symptoms reported almost immediate resolution of the neurological symptoms (Schwarz *et al.*, 2008).

Injection of the anaesthetic lignocaine into the peritoneum has also been used to reverse the major CTX-induced changes in nerve conduction.

Pregabalin, a gabapentinoid, has been shown to improve the course of CFP (Brett and Murnion, 2015). Pregabalin binds to and blocks the α-2-δ subunit voltage-gated calcium channels and may inhibit neuro-excitory behaviour of CTXs.

In a ‘proof of concept’, monoclonal antibodies to CTX-3C were shown to neutralise the toxin *in vitro* and in an *in vivo* mouse model (Inoue *et al.*, 2009). The antibodies appear to exercise their protective effect by blocking binding of the toxin to the sodium channels.

3.2 TOXICOLOGICAL ASSESSMENT

Only a single toxicological assessment was found for CTXs, carried out by the European Food Safety Authority (EFSA, 2010).

3.2.1 EFSA

The EFSA assessment was limited by the available information. EFSA concluded *inter alia*:

- The MBA has poor specificity and sensitivity and is not considered an appropriate detection method for CTXs
- Biochemical and immunochemical methods do not provide information on toxin profiles and immunochemical methods may be unsuitable in geographical regions

other than the one in which they were developed if antibodies are to region-specific CTXs

- LC-MS/MS would be of value for the quantification of CTXs in fish extracts, subject to further development
- Data do not allow any exposure assessment for the European population
- Relative potency factors were developed, based on intraperitoneal LD_{50}s in mice (see section 3.5), in absence of better information
- Establishment of an acute reference dose (ARfD) was considered, but was not possible due to the very limited quantitative data
- Based on a lowest reported CTX concentration in fish causing mild symptoms of 0.1 μg/kg P-CTX-1 equivalents and a 10-fold uncertainty factor, a concentration of 0.01 μg/kg P-CTX-1 equivalents is not expected to exert effects in sensitive individuals
- The EFSA panel could not comment on the risk associated with exposure to CTXs in fish that reach the European market.

3.3 PROPOSED MECHANISMS OF TOXICITY

CTXs act on voltage-sensitive sodium channels in excitable membranes (Lehane and Lewis, 2000). Sodium channels (and other channels) are membrane proteins that undergo conformational changes to form pores through a cell's plasma membrane in response to a specific trigger. In the case of the sodium channels the trigger is a voltage change. CTXs bind to site 5 of the voltage-gated sodium channels, prolonging their opening, so an influx of sodium ions occurs and the cells involved are depolarised, provoking action potentials. This results in the plasma membrane not being able to control the volume of the cells, due to the alteration of the electrochemical gradient.

The binding affinity of CTXs for the voltage sensitive sodium channels depends on the particular congener, with CTX-1 binding with approximately 30 times higher affinity than the algal analogue CTX-4B (FAO, 2004). CTXs exert a significant lowering of the nerve conduction velocity, as a consequence of the abnormal opening of the sodium channels, resulting in the neurological effects seen in CFP cases (Lehane and Lewis, 2000).

The activity of P-CTX-1 against various isoforms of the voltage-gated sodium channels (Na_v 1.1-1.9) was examined (Inserra *et al.*, 2017). While P-CTX-1 appears to be a non-selective voltage-gated sodium channel toxin, it causes a range of effects in the different Na_v isoforms and the contribution of these isoforms to the excitability of peripheral C- and A-fibre sensory neurons is likely to contribute to the range of neurological effects seen in CFP.

Transient Receptor potential ankyrin 1 (TRPA1) is an ion channel protein, which is a sensor for a wide range of environmental irritants and may mediate sensory responses including pain, cold and itch. Using a mouse model for CTX induced cold allodynia it was concluded that, although P-CTX-1 does not directly act on TRPA1, the sodium channel activation is sufficient to drive TRPA1-dependent calcium influx, that is responsible for cold allodynia (Vetter *et al.*, 2012).

A more recent study examined the mechanism of action of the algal analogue P-CTX-4B on frog myelinated axons (Schlumberger *et al.*, 2010). The toxin acted on both sodium and potassium channels in a dose dependent manner. P-CTX-4B was about four times more effective than P-CTX-1B in its effect on potassium channels, but 50 times less active on

sodium channels. P-CTX-1B is believed to be the metabolic transformation product of P-CTX-4B in fish.

CTXs also exert cardiovascular effects, which are also due to the opening of the voltage-gated sodium channels. As a consequence of the intracellular movement of sodium, the cells are less efficient at exuding calcium. A large part of this increased calcium is buffered by the sarcoplasmic reticulum, which is believed to enhance the force of cardiac muscle contraction; a common symptom of CFP (Paredes *et al.*, 2011). Gastrointestinal effects of CTXs are also due to the intracellular calcium transport that occurs in the epithelial cells.

A primary rat sensory neuron-human keratinocyte co-culture model was used to investigate the mechanism underlying sensory symptoms of CFP; parethesis, cold dysthesis and pruritis (Le Garrec *et al.*, 2016). P-CTX-2 induced a voltage-gated sodium channel-dependent release of neuropeptides (substance P and calcitonin gene-related peptide), which are known mediators of pain and itch sensations.

The mechanisms underlying the cold sensitivity (cold allodynia) and reversed temperature perceptions, often seen with CFP, were investigated (Eisenblatter *et al.*, 2017). The study authors concluded that this phenomenon was due to peripheral sensitisation in Aδ-nerve fibres. It is likely that this sensitisation is mediated through the voltage-gated sodium channels. Another study on this phenomenon suggested the CTXs may confer cold sensitivity to a subpopulation of cold-insensitive afferent nerve fibres (Patel *et al.*, 2015).

3.3.1 Toxicokinetics

Toxicokinetic parameters for P-CTX-1 were determined in rats, based on N2a analysis of blood (Ledreux and Ramsdell, 2013). Following intravenous administration at a dose of 0.13 μg/kg body weight tissue distribution was rapid ($T_{1/2}$ = 6 minutes) and extensive. Elimination from blood was substantially slower ($T_{1/2}$ = 35.5 hours). Bioavailability by oral and intraperitoneal routes of administration was determined to be 39% and 75%, respectively.

3.4 DOSE-RESPONSE

While not strictly dose-response information, it has been proposed that ciguatoxicity within a fish species may be correlated with indicators of fish size (weight or length) (Chan, 2015b). This approach has been incorporated into purchasing specifications for a major Australian fish market (Sydney Fish Market Pty Ltd, 2005). However, relationships of fish size to toxicity also seem to have a strong geographical component.

The relationship between ciguatoxicity and fish size was examined in a study in French Polynesia (Gaboriau *et al.*, 2014). Correlations between ciguatoxicity, as assessed by RBA, and fish length were examined in 45 species-by-location combinations and 32 family-by-location combinations. For the species-by-location correlations, eight were statistically significant ($p < 0.05$), however, the correlation was only positive in one case, while for the remaining seven species-by-location combinations there was a significant negative correlation between ciguatoxicity and fish length. For family-by-location correlations, six were statistically significant, with three positive and three negative associations. The study concluded that fish length was an unreliable indicator of ciguatoxicity, even for a distinct geographical area.

Fish samples associated with outbreaks of CFP in New York city were screened for ciguatoxicity by MBA, with positive samples subsequently analysed by LC-MS/MS (Graber *et al.*, 2013). One meal remnant contained C-CTX-1 and C-CTX-2 at a concentration of 1.1 μg/kg C-CTX-1 equivalents, while a meal remnant from a further outbreak contained 1.9 μg/kg C-CTX-1 equivalents.

Samples of amberjack (*Seriola* spp.) associated with an outbreak of CFP in North Carolina were analysed and found to contain C-CTX-1 at a concentration of 0.6 μg/kg (Langley *et al.*, 2009).

A sample of sawtooth barracuda (*Sphyraena putnamiae*), associated with a fatal case of CFP was analysed by LC-MS/MS and was found to contain 5.6 μg/kg P-CTX-1, 7.9 μg/kg P-CTX-2 and 1.4 μg/kg P-CTX-3 (Hamilton *et al.*, 2010).

Leftover meals associated with CFP outbreaks in Okinawa, Japan were found to contain toxicity of 0.025-0.8 MU/g, equivalent to 0.175-5.6 μg P-CTX-1B/kg (Oshiro *et al.*, 2010).

Fish samples associated with CFP incidents on the east coast of Australia were analysed for P-CTX-1B by LC-MS/MS (Farrell *et al.*, 2017). Four samples were of Spanish mackerel (*Scomberomorus commerson*) caught in Australian waters, with P-CTX-1B concentrations in the range 0.11-1.0 μg/kg. The remaining fish had been caught off the coast of Queensland and imported into New South Wales. Lower toxin concentrations were detected in these samples (0.006-0.069 μg/kg), although in some cases the fish sample was not from the implicated fish, but from the same batch. Only P-CTX-1B was determined, as this was the only CTX for which reference material was available. More severe symptoms were seen in cases where the viscera or head of the fish had been consumed.

A study of fish implicated in CFP in an island region of Japan detected P-CTX-1B in all implicated fish samples, with concentrations in the range 0.13-8.78 μg/kg (Yogi *et al.*, 2013). Two other congeners, 52-*epi*-54-deoxy-P-CTX-1B and 54-deoxy-P-CTX-1B were also detected in all implicated fish samples.

A study in Hong Kong analysed fish samples associated with CFP outbreaks for the presence of CTXs by MBA and LC-MS/MS (Wong *et al.*, 2014). The paper is hard to interpret as LC-MS/MS results have been expressed in terms of mouse units. Using the relationship proposed by Oshiro *et al.* (2010); (1 MU/g = 7 μg/kg P-CTX-1 equivalents), the sum of the concentrations of P-CTX-1, P-CTX-2 and P-CTX-3 in implicated fish samples was in the range 0.1-2.1 μg/kg P-CTX-1 equivalents. This concentration range would be consistent with findings from other studies.

Fish samples associated with CFP outbreaks in Guadeloupe (French West Indies) were analysed by MBA with positive samples then analysed by N2a calibrated against P-CTX-1 (Hossen *et al.*, 2015). CTX concentrations were in the range 0.022-0.47 μg/kg P-CTX-1 equivalents. Combining this information with reported fish consumption amounts and case body weights, a lowest observed adverse effect level (LOAEL) of 48 pg/kg bw of P-CTX-1 equivalents was derived. Two samples were analysed by LC-MS/MS, with C-CTX-1 confirmed as the only toxin present.

With the exception of two studies, all of the studies summarised above express CTX toxicity in terms of P-CTX-1. If a relative potency for C-CTX-1, relative to P-CTX-1, of 0.1 is assumed (see Table 2 below), all estimates of fish toxicity fall in the range 0.02-8.8 μg/kg P-CTX-1 equivalents, with concentrations at the top end of this range having potential to result in fatalities. It should be noted that these values are a range of concentrations, not a range of exposure doses. However, given that the lowest concentration is that from the study of Hossen *et al*. (2015) and that this study used fish consumption and body weight data to calculate a LOAEL, the LOAEL of 48 pg/kg bw of P-CTX-1 equivalents appears to be consistent with other available information.

3.5 RELATIVE POTENCY OF CTX CONGENERS

In the absence of better information, EFSA's CONTAM panel used intraperitoneal median lethal doses (LD_{50}) in mice to assign relative potency factors (RPFs) to the known CTX congeners (EFSA, 2010). The RPFs determined by EFSA are given in Table 2.

Table 2. Relative potency factors of CTX congeners, based on intraperitoneal LD_{50} values

Congener	RPF	Congener	RPF
P-CTX-1	1	51-hydroxy P-CTX-3C	1.0
P-CTX-2	0.3	P-CTX-4A	0.1
P-CTX-3	0.3	P-CTX-4B	0.05
P-CTX-3C	0.2	C-CTX-1	0.1
2,3-dihydroxy P-CTX-3C	0.1	C-CTX-2	0.3

LD_{50}: median lethal dose, RPF: relative potency factor

The toxicity of six CTX congeners was compared using MBA, an ion influx method, RBA and N2a (Yogi *et al.*, 2014). The results of these analyses are summarised in Table 3.

Table 3. Comparative toxicity of six CTXs

Toxin	Toxicity by bioassay (relative toxicity; CTX-1B = 1)				
	MBA (pmol/kg)	Ion influx (pmol/L)	RBA (pmol/L)	N2a (pmol/L)	Mean relative toxicity
CTX-1B	320 (1)	260 (1)	49 (1)	2.6 (1)	1
52-*epi*-54-deoxy-CTX-1B	640 (0.5)	150 (1.7)	21 (2.3)	25 (0.1)	1.2
51-hydroxy-CTX-3C	190 (1.7)	250 (1)	28 (1.8)	8.5 (0.3)	1.2
CTX-3C	1200 (0.3)	390 (0.7)	87 (0.6)	20 (0.1)	0.4
CTX-4A	1300 (0.2)	1010 (0.3)	73 (0.7)	150 (0.02)	0.3
CTX-4B	3400 (0.1)	5400 (0.05)	340 (0.1)	220 (0.01)	0.07

MBA: mouse bioassay, RBA: receptor binding assay, N2a: mouse neuroblastoma assay

While the absolute values of the RFPs/mean relative toxicities are different between the two information sources, the order of congener from most to least toxic is substantially the same.

4. EXPOSURE ASSESSMENT - CIGUATOXINS

4.1 OCCURRENCE AND LEVELS OF CIGUATOXINS IN SEAFOOD

4.1.1 Ciguatoxic dinoflagellates in New Zealand waters

Microalgae isolates (*n* = 17) from the Cook Islands, the Kermadec Islands, mainland New Zealand and New South Wales, Australia were collected and extracts analysed for CTXs and MTXs by LC-MS/MS (Munday *et al.*, 2017). While all but one isolate (*G. carpenteri*) contained MTXs, only an isolate of *G. polynesiensis* contained both CTXs and MTXs. This strain was from the Cook Islands. The only strain from New Zealand (Northland) was of *Fukuyoa paulensis* and contained only putative MTX-3. Extracts from this strain also had the lowest toxicity of any of the strains in mice, by intraperitoneal or gavage administration.

Epiphytic dinoflagellates were isolated from macroalgae and corals from the Kermadec Islands group (1100 northeast of New Zealand) (Rhodes *et al.*, 2014c). A compressed form of *Gambierdiscus* was isolated, but species identification was not possible, as the isolate failed to grow in culture.

A further survey of dinoflagellates in waters of the southern Kermadec Islands (Macauley Island) was carried out in 2016 (Rhodes *et al.*, 2017b). Isolates of *G. polynesiensis* (*n* = 1) and *G. australes* (*n* = 24) were identified by D8-10 large subunit ribosomal RNA sequences. None of the isolates produced CTXs, as detected by LC-MS/MS. Congeners monitored were CTX-3B, CTX-3C, CTX-4A and CTX-4B.

A quantitative polymerase chain reaction (qPCR) method was developed to simultaneously identify all known *Gambierdiscus* and *Fukuyoa* species in environmental samples (Smith *et al.*, 2017a). A dedicated qPCR assay for *F. paulensis* was also developed. The methods were applied to macroalgal and seawater samples (*n* = 31) from Northland, New Zealand (*n* = 7), Kermadec Islands (*n* = 6) and Tonga (*n* = 18). Fourteen samples (2 from Northland, 6 from the Kermadecs and 6 from Tonga) were positive by the multi-species assay. *F. paulensis* was only detected in the samples from Northland. Only samples from the Kermadecs contained DNA of suitable quality to determine the species present. These samples were dominated by *Gambierdiscus* spp., with *G. australes* being the dominant species (>90%), followed by *G. polynesiensis* and *G. honu*. A separate study found that samples from Northland were dominated by *Ostreopsis siamensis* and *F. paulensis* (Smith *et al.*, 2017b).

4.1.2 Ciguatoxins in seafood in New Zealand

While samples of fish implicated in CFP cases in New Zealand have occasionally been tested by N2a, no analyses of CTXs in fish from New Zealand coastal water or exclusive economic zone (EEZ) have been reported.

4.1.3 Ciguatoxic dinoflagellates in Australian waters

A survey of dinoflagellate genera was carried out at 16 Great Barrier Reef (GBR) sites during 2006-2007 (Skinner *et al.*, 2013). Sampling sites covered inshore mid-lagoon and outer lagoon regions. Results were compared to a survey carried out in 1984. At inshore sites *Prorocentrum* or *Ostreopsis* species dominated the macroalgal surface niche, while *Gambierdiscus* dominated this niche at offshore sites. *Gambierdiscus* had dominated at all sites in the 1984 survey. It was speculated that these changes in dinoflagellate population dynamics may be due to ongoing eutrophication.

Microalgae were collected from seagrass in Merimbula Inlet, New South Wales (temperate) and from macroalgae in Heron Island lagoon, Queensland (tropical) (Larsson *et al.*, 2018). Molecular typing identified the temperate strains as *G. carpenteri*, with none of the isolates

producing detectable (LC-MS/MS) CTXs or MTXs. Tropical strains included *G. carpenteri, G. lapillus* and two unidentified species. None of the isolates produced CTXs or MTX-1, but all produced MTX-3. These findings were consistent with an earlier study in which all *Gambierdiscus* from two temperate sites in New South Wales (water temperatures 16.5-17°C) were non-ciguatoxigenic *G. carpenteri* (Kohli *et al*., 2014).

4.1.4 Ciguatoxins in seafood in Australia

It is generally considered that CTXs bioaccumulate up the marine food chain, with sharks expected to be highly ciguatoxic. However, analysis of muscle and liver from sharks (*n* = 22) from CTX hotspots on the east coast of Australia failed to detect P-CTX-1, P-CTX-2 or P-CTX-3 (LOD= 0.05 μg/kg) (Meyer *et al.*, 2016).

As part of the development of a reference laboratory capability in Queensland, 56 fish samples (46 CTX-suspect and 10 control) were analysed for CTXs by LC-MS/MS (Stewart *et al.*, 2010). Of the CTX-suspect fish samples, 27 (59%) were found to contain P-CTX-1 at concentrations in the range 0.04-11.4 μg/kg. None of the control fish samples contained P-CTX-1, while 26 of 27 samples positive for P-CTX-1 were also positive for P-CTX-2 and P-CTX-3.

Spanish mackerel (*Scomberomorus commerson*) were collected from New South Wales (*n* = 71) and Queensland (*n* = 13) and analysed (flesh and liver) for P-CTX-1B by LC-MS/MS (Kohli *et al.*, 2017). For samples from New South Wales, one flesh sample (1.4%, <0.1 μg/kg) and five liver samples (7.0%, <0.4 μg/kg) were positive for P-CTX-1B. For samples from Queensland, six flesh (46%, <0.1-0.13 μg/kg) and five liver (38%, <0.4-1.39 μg/kg) samples were positive. No correlations between toxicity and fish size (length or weight) were found.

4.1.5 Ciguatoxic dinoflagellates in Pacific Island waters

Macroalgae were sampled from 27 sites around Raivavae Island in French Polynesia and tested for the presence of four dinoflagellate genera or species (Chinain *et al.*, 2010b). The most commonly occurring dinoflagellates were *Gambierdiscus* spp., with particularly high cell densities at two sites. There was some evidence for a preference of *Gambierdiscus* to colonise macroalgae of the genera *Jania* and *Halimeda*. It was noted that the density of dinoflagellates at a site did not correlate with the ciguatoxicity of fish from the same site and it was suggested that fish ciguatoxicity may be more dependent on *Gambierdiscus* blooms occurring 6-12 months previously.

The N2a assay was used to test the toxicity of a *Gambierdiscus* isolate sampled from the Australes archipelago, French Polynesia (Rapa Island) (Pawlowiez *et al.*, 2013). The isolate was ciguatoxic with an estimated toxicity of 0.03 pg/cell of P-CTX-3C equivalents. The toxicity was substantially lower than for three reference strains of *G. polynesiensis*.

A survey of toxic dinoflagellates isolated from green *Halimeda* seaweed in Rarotongan lagoons was carried out (Rhodes *et al.*, 2010). A strain of *G. australes* gave a response of 0.04 pg/cell P-CTX-1 equivalents (0.4 pg/cell CTX-3C equivalents) in the N2a assay. However CTXs were not detected by LC-MS/MS.

A further four *Gambierdiscus* strains (*G. pacificus, G. polynesiensis* and two *G. Australes*) were isolated from seawater samples from the Cook Islands and tested for CTXs and MTXs by LC-MS/MS and mouse toxicity (Rhodes *et al.*, 2014b). Only the *G. polynesiensis* isolate contained CTXs (congener not specified) at a concentration of 18.2 pg/cell. The LD_{50} for this extract was 1.0 mg/kg bw by intraperitoneal injection, with a median lethal dose of 7.9 mg/kg bw by gavage.

A survey of dinoflagellate species was carried out in waters around Marakei, Kiribati (Xu *et al.*, 2014). *Gambierdiscus* spp. were the most abundant dinoflagellates at three of the sites

examined, with the distribution of *Gambierdiscus* corresponding to observed patterns of fish toxicity. Representative isolates (*n* = 38) were subjected to DNA analysis, which identified *G. carpenteri, G. belizeanus* and *G. pacificus*, as well as three ribotypes (4, 5 and 6). Ribotype 4 was the most commonly detected type (28 of 38 isolates) and appeared to be similar to the Atlantic ribotype 1. Six isolates were examined for ciguatoxicity by N2a. Four isolates of ribotype 4 had toxicity in the range 2.6-6.0 fg/cell P-CTX-1 equivalents, while an isolate of ribotype 5 and one of *G. pacificus* had very low toxicities (0.010 and 0.011 fg/cell P-CTX-1 equivalents, respectively).

4.1.6 Ciguatoxins in seafood in Pacific Islands

Reef fish specimens (*n* = 171; 68% carnivorous, 24% herbivorous and 8% omnivorous) were collected from two islands (Tarawa and Marakei) in the Republic of Kiribati and tested for P-CTX-1 equivalents by N2a (Chan *et al.*, 2011). CTX activity was detected in 91% of fish samples (LOD = 0.01 μg/kg P-CTX-1). CTX toxicity in fish was found to be species- and location-dependent. The highest CTX concentrations were seen in grouper and moray eel, with mean activities of 2.0 μg/kg (range 0.004-12.4 μg/kg) and 5.7 μg/kg (range 0.02-81.8 μg/kg), respectively. Fish sampled from Tarawa were significantly less ciguatoxic than those from Marakei, with spatial differences in fish toxicity appearing to be related to known density patterns for *G. toxicus*.

Reef fish (*n* = 160, >70% herbivorous) were collected from the Raivavae Lagoon, French Polynesia and tested for ciguatoxicity by RBA (Chinain *et al.*, 2010b). No clear relationship was seen between size and weight of individual fish and their toxicity. Similarly, there was no clear relationship between trophic level and toxicity, as two of the three least toxic species were carnivorous. Herbivorous fish may be classified as browsers or grazers and the authors of this study noted that two highly toxic species in their study (*Scarus altipinnis* and *Naso unicornis*) were both grazers. The highest individual RBA values (4.67 and 5.58 ng P-CTX-3C equivalents/g) were observed in these species. The greater toxicity of grazing species was consistent with other studies. Areas with the highest CFP risk were generally on the windward side of the island, where the greatest reef disturbance due to hurricane or cyclone damage occurred.

Fish were sampled from Raivavae Island (a low risk CFP area, *n* = *7*) and Rapa Island (*n* = 13) in the Australes archipelago of French Polynesia and tested for ciguatoxicity by N2a (Pawlowiez *et al.*, 2013). None of the samples from Raivavae Island exhibited N2a toxicity. Five fish samples from Rapa showed ciguatoxicity with concentrations in the range 13-124 ng/kg P-CTX-3C equivalents (0.013-0.124 μg/kg). Giant clams (*Tridacna maxima*) and sea urchins (*Tripneustes gratilla*) from the archipelago also tested positive for CTXs by N2a, with estimated toxin concentrations in the range 0.023-2.2 μg/kg P-CTX-3C equivalents.

There was some conjecture over whether the toxicity observed in giant clams was due to *Gambierdiscus* or to cyanobacterial mats formed in the same area (Roue *et al.*, 2013). However, accumulation of CTXs by giant clams was demonstrated in the presence of a highly ciguatoxic strain of *G. polynesiensis* (Roue *et al.*, 2016). Giant clams were demonstrated to accumulate ciguatoxicity to 2.9-3.3 μg/kg P-CTX-3C equivalents, based on N2a analysis. LC-MS/MS confirmed the presence of P-CTX-3B, as the major CTX congener in giant clams. Congeners P-CTX-3B, -3C, -4A, -4B and M-seco-4A were all present in the *G. polynesiensis* strain. This suggests that giant clams may accumulate CTXs, but there is no evidence of metabolism.

As part of a study of CFP on Marakei Island, Kiribati, coral reef fish (*n* = 205) and invertebrates (*n* = 15) were sampled from two ciguatoxic sites and one reference site and analysed for CTXs by LC-MS/MS (Mak *et al.*, 2013). Standards were available for P-CTX-1, -2 and -3. P-CTX-1 was detected in two invertebrates; one octopus (0.003 μg/kg) and one lobster (0.003 μg/kg). No CTXs were detected in invertebrates from the reference site. CTXs

were detected in 54% of herbivorous reef fish, with total CTX concentrations in the range <0.0005-1.67 μg/kg wet weight, with P-CTX-2 being the dominant CTX present. CTXs were detected in 72% of omnivorous fish (<0.0005-1.81 μg/kg wet weight) and 76% of carnivorous fish (<0.0005-69.5 μg/kg wet weight). P-CTX-1 was the dominant species in carnivorous fish. The highest CTX concentrations were found in moray eels, followed by snapper and grouper.

4.1.7 International context

Invasive Indo-Pacific lionfish (*Pterois volitans*) have proliferated throughout the Caribbean and development of a commercial fishery has been proposed as a control strategy (Litaker *et al.*, 2014). Lionfish (*n* = 193) were collected from 11 Caribbean locations and analysed by RBA_F, using P-CTX-3C and C-CTX-1 as standards. Approximately 11% of samples contained CTXs at concentrations greater than 0.1 μg/kg C-CTX-1 equivalents. The geographical distribution of lionfish CTX concentrations matched the known distribution of CFP cases in the Caribbean.

Another study collected lionfish (*n* = 153) from waters surrounding the US Virgin Islands and tested them for ciguatoxicity by N2a, with LC-MS/MS confirmation (Robertson *et al.*, 2014). Of these specimens, 19 fish were considered toxic, as assessed by N2a, exceeding the FDA guideline level of 0.1 μg/kg C-CTX-1 equivalents. Presence of C-CTX-1 and C-CTX-2 was confirmed by LC-MS/MS. A further 43 fish tested positive by N2a, but at toxicity levels below the FDA guideline, for a total prevalence of ciguatoxicity of 41%. The maximum toxicity found was 0.3 μg/kg C-CTX-1 equivalents.

Lionfish (*n* = 120) were collected from waters surrounding the French Antilles (Caribbean) and tested for ciguatoxicity (Solino *et al.*, 2015). Toxicity was detected in 27 samples (23%) by N2a. All positive samples were from one of the three sampling locations. Toxicity of samples was in the range 0.005-0.333 μg/kg P-CTX-1 equivalents. Samples with toxicity ≥0.157 μg/kg P-CTX-1 equivalents were submitted for LC-MS/MS confirmation and were confirmed to contain C-CTX-1.

Barracuda (*Sphyraena barracuda*; *n* = 38) were collected from sites in the Bahamas (O'Toole *et al.*, 2012). Liver, muscle and blood samples were taken and analysed for CTXs by N2a with C-CTX-1 as a standard. Concentrations across the three tissue types were in the range 2.5-212 ng/kg C-CTX-1 equivalents (0.0025-0.212 μg/kg C-CTX-1 equivalents). Blood and liver toxin concentrations were significantly positively correlated. Barracuda were subsequently caught, blood drawn, tagged and released. Tagged fish were followed by telemetry. Preliminary results suggested that ciguatoxic fish may have smaller home ranges than non-toxic fish.

Barracuda (*n* = 20) were collected from the north-western Gulf of Mexico and analysed for ciguatoxicity by N2a (Villareal *et al.*, 2007). CTX activity was detected in 11 samples, but was only present in trace amounts in most fish (9 of 11). The maximum concentration found was 0.14 μg/kg C-CTX-1 equivalents.

A random survey of fish (*n* = 227, representing 21 species) from fish markets in Bangkok, Thailand was carried out, using the antibody-based Cigua Check® test kit (Sintunawa *et al.*, 2014). Positive results were reported for two samples. However, these positive results were not confirmed by MBA.

Samples of suspected ciguatoxic fish (*n* = 13) from the Canary Islands were analysed by N2a (Caillaud *et al.*, 2012). Four samples tested positive with concentrations in the range 0.058-6.2 μg/kg P-CTX-1B equivalents. Six of the samples that were negative by this assay had previous tested positive by the Cigua Check® method.

An outbreak of CFP, affecting 124 people, including 11 deaths, occurred in Madagascar in 2013 after consumption of flesh, liver, head and parts of the viscera of a bull shark (*Carcharhinus leucas*) (Diogene *et al.*, 2017). Samples from the shark were positive by MBA and N2a, with stomach samples showing very high toxicity (83 μg/kg P-CTX-1 equivalents by MBA and 93 μg/kg P-CTX-1 equivalent by N2a). Liquid chromatography-high resolution mass spectrometry (LC-HRMS) detected I-CTX-1/2 (6.5 μg/kg P-CTX-1 equivalent) and I-CTX-3/4 (9.7 μg/kg P-CTX-1 equivalent). Toxins were not detected in flesh or fin samples. A further two unidentified CTXs (I-CTX-5 and I-CTX-6) were also identified in stomach fractions. Gambieric acid was identified in the flesh, but not stomach and fin samples.

4.1.8 Ciguatera in aquaculture

The captive nature of fish raised in aquaculture has the potential to place fish in a situation where they are not able to avoid ciguatoxic food. A survey of two aquaculture facilities in Hawai'i detected *Gambierdiscus* spp. on three open cages at one facility and one open cage at the other facility (Campora *et al.*, 2010). Cell densities were quite low (mean 142 cells/g algae). Ciguatoxicity was analysed in almaco jack (*Seriola rivoliana, n* = 60) and Pacific threadfin (*Polydactylus sexfilis, n* = 10) from the facilities by immunoassay and N2a. None of the cultured fish contained detectable ciguatoxicity. Wild caught almaco jack (*n* = 81) were also examined, with 7.4% positive for ciguatoxicity.

4.2 CONSUMPTION OF SEAFOOD

4.2.1 New Zealand

Information on food consumption in New Zealand can be derived from 24-hour dietary recall records, that have been collected as a component of national nutrition surveys (Ministry of Health, 2003; Russell *et al.*, 1999; University of Otago and Ministry of Health, 2011). Information from these surveys has been reviewed and is summarised in Table 4 (Cressey, 2013). No data are presented on cephalopod consumption, due to the low prevalence of consumption of this food type.

Table 4. Summary of marine food consumption by New Zealand children (5-14 years) and adults (15+ years)

Metric	Children (5-14 years)	Adults (15+ years)	
	2002	1997	2009
Finfish			
Consumers (% of total respondents)	14.9	18.0	20.3
Consumer mean (g/person/day)	89	99	134
Population mean (g/person/day)	13.3	17.8	27.2
Crustaceans			
Consumers (% of total respondents)	0.8	0.9	1.2
Consumer mean (g/person/day)	78	130	82
Population mean (g/person/day)	0.6	1.2	1.0
Shellfish (molluscs)			
Consumers (% of total respondents)	0.5	2.4	1.5
Consumer mean (g/person/day)	49	106	85
Population mean (g/person/day)	0.2	2.5	1.2

Marine foods are generally less commonly consumed by children than adults. While finfish are consumed by about 20% of the adult population on any given day, crustaceans and shellfish are not commonly consumed, with just over 1% of the adult population and less than 1% of children consuming these foods on any given day.

4.2.2 Australia

Information on foods consumed by Australians are available from the 1995 National Nutrition Survey (McLennan and Podger, 1999) and the 2007 Australian National Children's Nutrition and Physical Activity Survey (ANCNPAS).[5] A more recent survey of Australians aged 2 years and above has also been carried out; 2011-13 Australian Health Survey (AHS): 2011-12 National Nutrition and Physical Activity Survey (NNPAS)[6], however limited data from this survey are available in the public domain.

The 1995 national nutrition survey included approximately 13,000 respondents, with 18.3% reporting consumption of fish and seafood products and dishes on the survey day (McLennan and Podger, 1999).[7] While it is not possible to extract separate figures for finfish, crustaceans and molluscs, this total appears very similar to that for New Zealand in 1997 (Table 4). Crustaceans and molluscs (excluding canned) were consumed by 2.7% of the survey population on the survey day. The population average consumption of fish and seafood products and dishes was 25.4 g/person/day, with consumption of crustaceans and molluscs accounting for 2.5 g/person/day.

Data from the ANCNPAS (2-16 years) were accessed through the FAO/WHO Chronic individual food consumption database – summary statistics (CIFOCOss).[8] Food consumption data are presented for individual fish species, so it is not possible to determine the overall frequency of finfish consumption. The mean consumption of all finfish species is 14.3 g/person/day. Mean consumption of crustaceans is 1.6 g/person/day, molluscs 0.2 g/person/day and cephalopods 0.5 g/person/day.

4.2.3 International context

The CIFOCOss database contains food consumption information from approximately 30 countries. Data in this database must be derived from a minimum of two days of dietary information per respondent. It should also be noted that this is the only current international database that provides estimates of food consumption. This contrasts with food balance sheets, which provide information on food available for consumption.

Table 5 summarises information from CIFOCOss on the mean population consumption of finfish, crustaceans, molluscs and cephalopods. For concision, data are only presented for adults or the general population.

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[5] http://www.health.gov.au/internet/main/publishing.nsf/Content/phd-nutrition-childrens-survey Accessed 19 July 2017

[6] http://www.abs.gov.au/websitedbs/d3310114.nsf/home/australian+health+survey Accessed 19 July 2017

[7] Survey days were randomised across the study cohort, so that dietary practices that may be associated with a particular day of the week did not bias the study results

[8] https://extranet.who.int/sree/Reports?op=vs&path=/WHO_HQ_Reports/G7/PROD/EXT/CIFOCOSS_Country&userid=G7_ro&password=inetsoft123 Accessed 19 July 2017

Table 5. Daily food consumption information from various countries (CIFOCOss)

Country	Mean consumption (g/person/day)			
	Finfish	Crustaceans	Molluscs	Cephalopods
Bangladesh	3.0	0.3		
Belgium	12.5	4.0	1.6	0.2
Brazil	7.4	0.6	0.1	
Burkina Faso	0.1			
China	8.0	4.1	1.6[c]	
Czech Republic	4.9	0.1		
Denmark	14.8	2.1		0.1
Finland	16.3	0.9	0.2	
France	13.6	1.6	2.0	0.5
Germany	9.4	0.6	0.3[c]	
Hungary	6.2			
Ireland	17.7	0.8	0.2	
Italy	23.3	4.4		9.9[d]
Japan	25.8	6.1	11.2[c]	
Latvia	6.5			
Netherlands	3.6	1.2	0.4	
Philippines	26.5	1.4	3.5[c]	
Republic of Korea	23.7	3.2	1.4	7.4
Spain[a]	40.0/49.0	4.2/5.2		9.8/12.0[d]
Sweden	2.3	4.2		0.1
Thailand	15.9	5.2	7.7[c]	
Uganda	2.8			
United Kingdom	19.2	2.6	0.5[c]	
USA[b]	5.7	3.2	1.2[c]	

[a] Data are available from two food consumption surveys

[b] Food consumption figures were on a 'g/kg bw/day' basis and have been recalculated to a 'g/day' basis using a nominal body weight of 70 kg

[c] For these countries it is not possible to completely separate mollusc and cephalopod consumption, as some information is entered for particular mollusc or cephalopod types, while other information is entered for 'molluscs, including cephalopods nes', where nes is an acronym for not elsewhere specified.

[d] See footnote c above. For these countries, there is sufficient information to conclude that this figure mainly represents consumption of cephalopods

While few countries consume substantially greater amounts of finfish than New Zealand, many countries consume greater amounts of crustaceans, molluscs and cephalopods. Countries in southern Europe and Asia are particularly notable for their levels of consumption of these food types.

An attempt has been made to estimate the total number of reef fishers worldwide (Teh *et al.*, 2013). Two approaches were used:

- Reef fishers as a proportion of total fishers in a country, based on the ratio of reef-related to total marine fish landed values, or
- Reef fishers as a function of coral reef area, rural coastal population and fishing pressure.

Total reef fishers (6.1 million) were calculated as the average of estimates from these two approaches (5.2 and 6.8 million). The greatest number of reef fishers were estimated to be in southeast Asia (3.35 million), while Indonesia was the single country with the greatest number of reef fishers (1.7 million).

4.3 ESTIMATES OF DIETARY EXPOSURE

4.3.1 New Zealand

No estimates of dietary exposure to CTXs have been carried out for New Zealand.

4.3.2 Australia

No estimates of dietary exposure to CTXs have been carried out for Australia.

4.3.3 Overseas estimates of dietary exposure

No overseas estimates of dietary exposure to CTXs were found. In a 2010 assessment, EFSA noted that "the few data on occurrence of CTX-group toxins in fish do not allow any exposure assessment for the European population" (EFSA, 2010).

4.3.4 Biomarkers of exposure

No biomarkers of exposure have been validated for humans (Azziz-Baumgartner *et al.*, 2012).

Toxicokinetic studies in rats with P-CTX-1 showed peak blood concentration, as measured by the N2a assay, within two hours of administration, followed by slow elimination (half-life of 82 hours following oral administration and 112 hours following intra-peritoneal administration (Bottein *et al.*, 2011). CTX activity was detectable in liver, muscle and brain up to 96 hours post-administration. The faeces were the primary route of excretion, with highest CTX activity in faeces seen 48-72 hours post-administration. CTX activity was still detectable in faeces 96 hours post-administration.

Studies in mice dosed with a single dose of 0.26 μg/kg bw P-CTX-1 resulted in measurable concentrations of CTXs in blood up to 3 days post-dosing (Dechraoui *et al.*, 2008). CTX concentration in blood one hour after dosing was 9 ng/L P-CTX-1 equivalents, decreasing to 2.3 ng/L after 4 hours and remained fairly constant up to 3 days.

5. RISK CHARACTERISATION - CIGUATOXINS

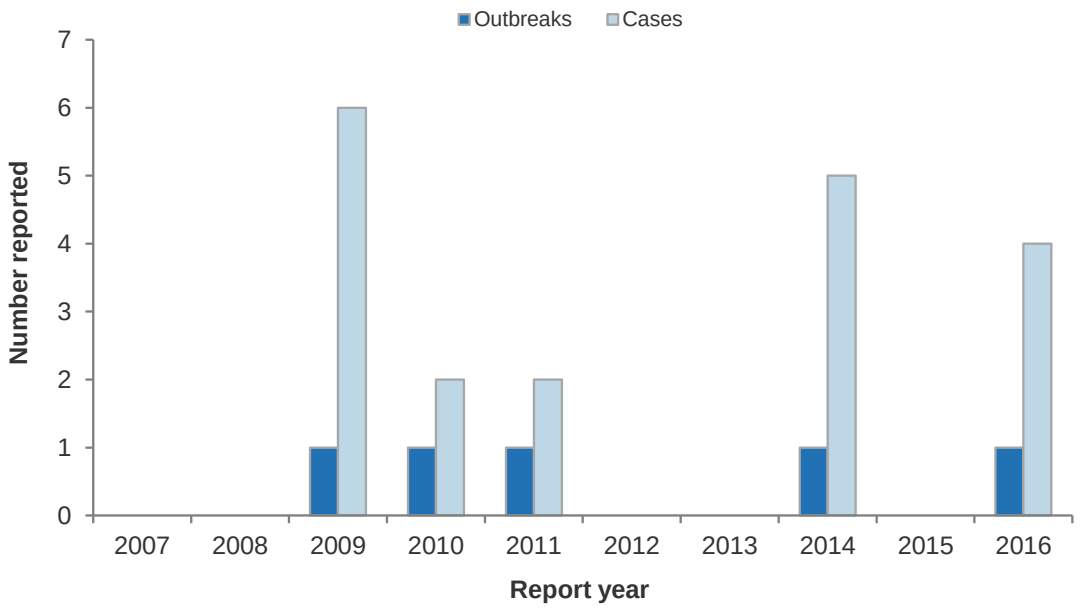
5.1 ADVERSE HEALTH EFFECTS IN NEW ZEALAND

5.1.1 Incidence of ciguatera food poisoning

No systematic estimates of the incidence of CFP in New Zealand have been derived. Individual CFP cases and outbreaks may be reported to the EpiSurv database, while hospitalisations due to CFP are reported through the National Minimum Data Set (NMDS).

In the most recent reported year (2016), five cases (0.1 per 100,000 population) and eight hospital admissions (0.2 per 100,000 population) were reported (Pattis *et al.*, 2017). A single outbreak of CFP, involving four people, was reported in 2016. A summary of CFP outbreaks and outbreak-associated cases in New Zealand is presented in Figure 2.

Figure 2. Ciguatera fish poisoning outbreaks and associated cases reported by year, 2007–2016



Reproduced from Pattis et al. (2017)

5.1.2 Outbreaks

A detailed report was published of four cases of CFP presenting at an emergency department (ED) following consumption of moray eel self-imported from Samoa (Armstrong *et al.*, 2016). At presentation, three of the cases were brachycardic (heart rates 30-32 bpm) and hypotensive (blood pressures 72/35, 72/35 and 61/42 Hg). All cases reported gastrointestinal symptoms (diarrhoea and vomiting). One case developed paraesthesia of the lips while in ED. For the three brachycardic cases, treatment with atropine was given at 4-6 hourly intervals. Cardiotoxicity persisted for 3-4 days. The implicated eel tested positive for CTX-1B.

5.1.3 Epidemiological studies

No epidemiological studies of CFP in New Zealand have been carried out.

5.1.4 Risk assessment

No risk assessments of CFP in New Zealand have been carried out.

5.2 ADVERSE HEALTH EFFECTS OVERSEAS

5.2.1 Case and outbreak reports

A French visitor to the Dominican Republic developed abdominal cramps and diarrhoea four hours after eating fish (Develoux *et al.*, 2008). The gastrointestinal symptoms persisted for three days. General pruritis, headache, arthralgia, myalgia and paraesthesia developed 24 hours after initial symptoms. Upon return to France routine laboratory tests were normal. Pruritis and asthenia persisted for approximately seven weeks after exposure.

While the adverse effects of CTXs have historically been referred to as CFP, shellfish are increasingly being associated with a CFP-like syndrome, termed ciguatera shellfish poisoning (CSP) (Lonati *et al.*, 2015). In June 2014, an outbreak of nine cases was associated with consumption of troca (*Tectis niloticus*) in French Polynesia.

The impact of overseas travel on the incidence of CFP in non-endemic countries was highlighted by two case reports from the Netherlands (Slobbe *et al.*, 2008). The cases had travelled to Mexico and Queensland, Australia, respectively and had eaten CTX-risk species prior to returning to the Netherlands.

In 2009, an outbreak of CFP occurred on a vessel in the port of Hamburg (Schlaich *et al.*, 2012). Of the 15 sailors on board, 14 were affected. All affected sailors had consumed fish from the ships Caribbean catch. Symptoms (gastrointestinal and neurological) persisted for at least 14 days.

CFP is usually associated with consumption of flesh from large fish. Consequently, CFP will often occur as an outbreak, rather than as a sporadic case. After identification of a CFP case by the Florida Department of Health, multiple data sources (surveillance systems, emergency departments) were used to identify a further five cases (Klekamp *et al.*, 2015). Five cases had consumed black grouper head, while the sixth consumed black grouper fillet.

Two clusters of CFP were identified in Paris, France in 2011 (Epelboin *et al.*, 2014). The two outbreaks were related to two fish, caught in Guadeloupe (French West Indies) and air-transported to Paris. CTXs were confirmed in both fish by MBA. The first cluster involved eight cases; five with gastrointestinal symptoms and four with neurological symptoms, following consumption of barracuda (*Sphyraena barracuda*). Three of the cluster were symptom-free and had not consumed fish, but were present at the meal. Cases recovered fully within a period ranging from two days to more than two months. The second outbreak involved a couple with both gastrointestinal and neurological symptoms after eating grey snapper (*Lutjanus griseus*). One case only ate the fish once, while the other ate it every day for a week. The second case still had subjective symptoms one year after exposure. The severity of symptoms was generally correlated with the self-reported amount of fish consumed.

An outbreak of CFP (19 cases) was reported in Germany in 2012 (Mattei *et al.*, 2014). Samples of implicated fish were analysed at the European Union Reference Laboratory for Marine Biotoxins (EURLMB) in Vigo, Spain, with 7 of 11 samples testing positive for P-CTX-1B. Other putative CTXs were also detected, but could not be confirmed as CTXs due to lack of standards. Fish samples originated from Sri Lanka, from waters affected by heavy cyclones and associated reef damage. Cases had consumed 80-300 g of red snapper (*Lutjanus malabaricus*).

CFP outbreaks are increasingly being reported caused by fish caught off the New South Wales (NSW) coast of Australia, rather than more traditionally associated with fish caught off Queensland (Farrell *et al.*, 2016). Two outbreaks of four and nine cases occurred in early 2014, following consumption of Spanish mackerel from northern NSW waters. A combination of gastrointestinal and neurological symptoms were reported, with one case still symptomatic after seven months. P-CTX-1B was detected in fish flesh from both outbreaks at concentrations up to 1 μg/kg (LC-MS/MS). A further outbreak (4 cases) occurred in April 2015, with the implicated fish caught even further south. The fish was unavailable for testing, but the symptoms were consistent with CFP.

The emergence of CFP on the West African coast (Canary Islands) has been described (Boada *et al.*, 2010). CFP was not reported in this region prior to 2004, when an outbreak occurred linked to consumption of lesser amberjack (*Seriola rivoliana*). In 2008-2009, a further two outbreaks were identified, involving 10-40 cases. In all three outbreaks a sample of the suspected fish was obtained. All samples were positive by immunoassay (Cigua-Check®) and when tested by the N2a assay were found to contain 0.08-1 μg C-CTX-1 equivalents/kg. LC-MS/MS analysis identified C-CTX-1 in all three samples.

Five large outbreaks of CFP (29-132 cases) occurring in China, due to consumption of brown marbled grouper (*Ephinephelus fuscoguttatus*) were reviewed (Chan, 2014b). A combination of gastrointestinal, neurological and occasionally cardiovascular symptoms occurred within 4-8 hours of fish consumption and generally subsided after 1-2 days. The same author also reviewed five case series of CFP following consumption of humphead wrasse (*Cheilinus undulates*), with generally similar symptoms observed (Chan, 2013a). However, it was further noted that residual symptoms associated with consumption of this apex predator could persist for up to six months. The most common residual symptoms were neurological or neuropsychiatric complaints and skin pruritis.

5.2.2 Incidence of ciguatera fish poisoning

Pacific

A questionnaire was sent to health and fisheries authorities of Pacific Island Countries and Territories (PICTs) eliciting information on cases of CFP in the period 1998-2008 (Skinner *et al.*, 2011). Responses were received from 85% (*n* = 17) of the PICTs contacted. Annual incidence rates within the PICTS ranged from zero (Nauru, Wallis and Fortuna) to 1576 cases per 100,000 population (Tokelau). Even higher rates were reported for particular geographical locations within the PICTs. For example, the annual CFP rate for the Gambier island group of French Polynesia was 4504 cases per 100,000 population. The overall incidence was 194 cases per 100,000 population. This compared to a rate of 104 cases per 100,000 population for the period 1973-1983. Information on incidence of environmental disturbance (coral bleaching, cyclone incidence, perceived coral reef condition) was also collected. While all three types of environmental disturbance were positively correlated with CFP incidence, the correlations didn't reach statistical significance. There was evidence from some PICTs that CFP had resulted in dietary changes within the community (avoidance of reef fish).

The Cook Islands have been reported to have the highest reported incidence of CFP (Bailey and Withers, 2014). Annual case numbers have been in the range 55-469 cases per annum during the period 1992-2013. Based on a reported population of 13,700 these case numbers would equate to crude rates in the range 400-3400 cases per 100,000 population. During the period 1992-2013, hospitalisation rates for CFP in the Cook Islands were in the range 9-33%.

The island of Raivavae (Australes) in French Polynesia has been described as a CFP 'hot spot' with an estimated incidence in 2007-2008 of 140 cases per 10,000 population (1400 case per 100,000) (Chinain *et al.*, 2010b). The Gambier archipelago had an even higher incidence of CFP during 2007-2008, with a mean of 470 cases per 10,000 population. For the period 2000-2008, the overall incidence of CFP in French Polynesia was estimated to be 23 cases per 10,000 population.

An intensive study of CFP was carried out on Moorea, the second most populous island in French Polynesia (Morin *et al.*, 2016). An annual incidence of 8 cases per 10,000 was estimated for the period 2007-2013, based on standardised declaration forms. These forms also included reference to family members who also became ill. This information was used to estimate an under-reporting rate of 54%. Health-related costs were estimated at USD $1613 for reported cases and USD $749 for unreported cases.

Asia

During the period 1989-2008, the annual incidence of CFP in Hong Kong was reported to be in the range 3.3 to 64.9 cases per million people (Chan, 2014a). However, these estimates appear to have been based on reported outbreaks only and are likely to represent an underestimate. Groupers were the predominant fish species implicated in CFP outbreaks. Reanalysis of 27 fish samples, positive by MBA, found mainly P-CTX-1, P-CTX-2 and P-CTX-3.

The same author also reported regional incidence estimates for the People's Republic of China, in the range 1.1 to >130 cases per million people (Chan, 2015a). However, the limitations of these estimates noted in the previous paragraph also apply.

Data from the years 2008-2012 were used to estimate the incidence of CFP in South Korea (Park and Bahk, 2015). The estimated incidence was 0.012 cases per 100,000 population (0.12 cases per million), with 29% of cases hospitalised. While the study accounted for diagnostic specificity and sensitivity, no attempt was made to estimate cases that did not interact with the health system.

Americas

Using outbreak and poison control centre call data and applying under-reporting and under-diagnosis multipliers, it was estimated that the annual burden of CFP in the USA was 15,910 cases (90% credible interval 4140-37,408), including 343 hospitalisations (90%CI 69-851) and three death (90%CI 1-7) (Pennotti *et al.*, 2013). The multipliers used were the same as previously used for bacterial disease outbreaks (Scallan *et al.*, 2011). The authors noted that it is uncertain how applicable these multipliers are to non-infectious fish poisoning incidents. Based on a US population of about 327 million[9] the annual incident CFP cases equate to a crude rate of 49 cases per million population (4.9 cases per 100,000 population).

A study focused on Florida state used case reports and an e-mail survey of recreational fishers to estimate an incidence rate for CFP in Florida of 5.6 cases per 100,000 population, adjusted for under-reporting (Radke *et al.*, 2015). The crude estimated incidence amongst fishers was 400 per 100,000 population, with Hispanic much more likely than non-Hispanics (relative risk = 3.4) to suffer CFP. Barracuda and grouper were the fish types most commonly associated with CFP.

[9] https://www.census.gov/popclock/ Accessed 15 February 2018

5.2.3 Epidemiological studies

A cohort study including occupied households (*n* = 340) on the island of Culebra, Puerto Rico was carried out during 2005 and again in 2006 (Azziz-Baumgartner *et al.*, 2012). Across the two survey years 13 household members exhibited symptoms following fish consumption that were sufficiently typical of CFP to be classified as probable cases (7.5 per 1000 person-years), while a further 7 people were considered to be possible CFP cases (4 per 1000 person-years). Of three fish samples retained and submitted for analysis, one was positive for C-CTX-1, one was negative and for the third there was insufficient sample for analysis. Households reporting CFP symptoms were more likely to have consumed barracuda (p = 0.02), a fish species that is often found to be ciguatoxic.

A US study analysed fish-associated illness outbreaks for the period 1998-2015 (Barrett *et al.*, 2017). Of 857 outbreaks, 637 had a confirmed aetiology, with CFP accounting for 227 (36%) of outbreaks with confirmed aetiology. These outbreaks included 894 cases (mean 3.7 cases per outbreak, median 3 case per outbreak) and 96 hospitalisations (11%). Of the CFP outbreaks, 54 involved consumption of grouper and 49 involved consumption of barracuda.

A cohort study was conducted with participants recruited from attendees at a nursing clinic in Noumea, New Caledonia (Baumann *et al.*, 2010). Of 559 patients who completed the study questionnaire, 210 (37.8%) had experienced symptoms consistent with CFP at least once in their lives. Reported prevalence was higher in those aged 40+ years. However, given that the study was measuring lifetime prevalence, this is not surprising. Neurological symptoms were the most frequently reported symptoms, with paraesthesia of the extremities (95%) the most commonly reported symptom. Gastrointestinal and cardiovascular symptoms were much less commonly reported. Of the participants reporting CFP symptoms, 56 (27%) reported multiple CFP episodes, with the maximum being 15 episodes. About one-third of cases reported recovery times longer than a year. Grouper were the fish most often (48% of episodes) implicated.

Concerns regarding CFP in the EU have increased since the occurrence of outbreaks of CFP in the Canary Islands (Nunez *et al.*, 2012). While European outbreaks have occurred, associated with imported fish, the outbreaks in the Canary Islands were associated with amberjack (*Seriola* spp.) caught in Canarian waters. At the time of publication of this paper, 9 outbreaks had been identified, affecting 68 people.

A small case control study (12 cases, 12 matched friend controls) was conducted to examine potential neuropsychological effects of CFP (Friedman *et al.*, 2007). At baseline (within one month of intoxication) and at follow-up (six months after baseline), there was no significant difference between cases and controls across a wide range of cognitive measures, but the cases identified significantly greater prevalence of subjective toxicity at baseline (e.g. fatigue, tingling sensations). Follow-up suggested resolution of subjective toxicity symptoms.

5.2.4 Risk assessments and other studies

The socioeconomic impact of CFP in Rarotonga was assessed (Rongo and van Woesik, 2012). Household surveys indicate that the per capita consumption of fish in Rarotonga had decreased from 149 g/person/day in 1989 to 75 g/person/day in 2006. The resultant decline in CFP incidence has resulted in an increase in fish consumption to 103 g/person/day in 2011. It was estimated that the cost of CFP (health costs, loss of labour, monitoring costs) amount to approximately NZD $750,000 per annum, while the costs associated with the dietary shift away from reef fish were estimated to be NZD $1 million per annum. It was

further speculated that the move from consumption of fresh fish to consumption of processed foods may have long-term health-related consequences.

A study in Rarotonga considered the relationship between CFP incidence (as measured by hospital admissions), coverage of coral by algae, as a proxy for reef state, densities of herbivorous fish and reef disturbance (Rongo and van Woesik, 2013). High densities of the herbivorous striated surgeonfish (*Ctenochaetus striatus*) and reef disturbance were found to be strong predictors of CFP. The two predictors were correlated, as densities of striated surgeonfish increase after disturbance of the reef by major cyclones. Based on these findings, the decrease in CFP cases in Rarotonga from 2006 to the present was ascribed to the climatic shift towards the negative phase of the Pacific Decadal Oscillation (La Niña), with its associated decrease in cyclone frequency.

6. RISK MANAGEMENT INFORMATION - CIGUATOXINS

6.1 RELEVANT FOOD CONTROLS: NEW ZEALAND

6.1.1 Establishment of regulatory limits

No regulatory limits have been established for CTXs in New Zealand (MPI, 2016a).

6.1.2 Imported Food Requirements (IFR)

Fish are classified as foods of high regulatory interest with respect to the chemical contaminants histamine and tetrodotoxin (puffer fish), but not CTXs (MPI, 2016b).

The previous version of this risk profile identified private importation of reef fish from the Pacific islands as the predominant source of CFP in New Zealand. It has been confirmed that the private importation of finfish, included reef fish, is still permitted (Dr Andrew Pearson, NZFS, personal communication).

6.1.3 Codes of Practice

While codes of practice have been developed for seafood processing, no reference to CTXs is made in these codes. This is consistent with existing information that CTXs are not an issue within New Zealand's EEZ.

6.1.4 Travel advisories

The Safetravel website, operated by the Ministry for Foreign Affairs and Trade (MFAT) includes advice on CFP for travellers to the Pacific.[10] The website states that:

"Ciguatera, or fish poisoning, is an illness caused by eating fish containing certain toxins. It can cause symptoms like nausea, vomiting, and tingling fingers or toes and can be found in many areas of the tropical Pacific region. There is no way to tell whether fish has been contaminated, so if you are visiting a Pacific island and want to avoid ciguatera, avoid eating reef fish. Deep water fish like tuna are a better option."

It should be noted that no equivalent advice is issued for travellers to the Caribbean or Indian Ocean destinations.

6.2 RELEVANT FOOD CONTROLS: OVERSEAS

6.2.1 Establishment of regulatory limits

The US Food and Drug Administration (USFDA) has established action levels for CTXs in fish; 0.01 μg/kg for P-CTX-1 equivalents and 0.1 μg/kg for C-CTX-1 equivalents (USFDA, 2011).

A 'safe level' of P-CTX-1 of 0.01 μg/kg for P-CTX-1 equivalents had previously been proposed in a 2000 publication (Lehane and Lewis, 2000).

[10] https://safetravel.govt.nz/pacific Accessed 8 March 2018

Within the EU, Commission Regulation (EC) No. 854/2004[11] specifies that "Checks are to take place to ensure that the following fishery products are not placed on the market: fishery products containing biotoxins such as Ciguatera or other toxins dangerous to human health". However, no limits or details of analytical methods are specified.

6.2.2 Codes of practice

In addition to codes of practice identified in the previous version of this risk profile, the Hong Kong Food and Environmental Hygiene Department have developed a code of practice for the import and sale of live marine fish for human consumption (Food and Environmental Hygiene Department, 2004). The Code is similar to other control measures in specifying particular fish species and sources to be avoided. The Code further comments that laboratory testing is unlikely to be an effective control measure, due to: the shortage of qualified laboratories, the long time required to perform tests and the difficulty of interpretation of tests.

It has been noted that, with respect to the activities of the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO), The General Standard for Contaminants and Toxins in Food and Feed (CODEX STAN 193-1995), the Code of Practice for Fish and Fishery Products (CAC/RCP 52-2003) and other Codex standards for fishery products, currently do not provide any guidance on ciguatera management and control.[12]

The East African Community has produced a Standard in the form of a code of practice for fish handling, processing and distribution (East African Community, 2000). The Standard specifies that the placing on the market of "fishery products containing biotoxins such as *ciguatera*, scombroid toxins or muscle-paralysing toxins" shall be forbidden. Species covered and approved methods of analysis are to be specified in National standards.

6.2.3 Trade prohibitions

The Sydney Fish Market, the largest seafood market in the southern hemisphere, places species, geographical and size restrictions on purchasing of fish from high CFP risk locations (Sydney Fish Market Pty Ltd, 2005). These measures include prohibitions on six fish species, prohibition of certain fish species or types (e.g. all warm water ocean fish) from several locations (Kiribati, the Marshall Islands, Fiji, the Northern Territory and Queensland) and size restriction on certain species from Australian states or the Pacific Islands. For example, Spanish mackerel (*Scomberomrous commersoni*) from northern New South Wales, Queensland and Pacific countries are only purchased if they weigh no more than 10 kg.

An assessment was carried out of the potential to implement a CFP management programme in the Noumea fish markets (Clua *et al.*, 2011). It was proposed that a management system be implemented based on trade prohibitions based on fish species, size and origin. The recommendations included:

- Banning sale of high risk species, such as giant moray (*Gymnothorax javanicus*) and the two-spot red snapper (*Lutjanus bohar*)

[11] http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32004R0854&from=en Accessed 19 February 2018

[12] http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-735-11%252FWD%252Fcf11_03_Add2e.pdf Accessed 8 March 2018

- Banning sale of moderate risk species for fish over a certain size threshold, such as barracuda spp. (*Sphyraena* spp.)
- Identification of high risk areas and tracking of the origin of fish entering the fish market.

It is uncertain whether these recommendations were implemented.

6.3 INFLUENCE OF FOOD PROCESSING ON CIGUATOXINS IN MARINE FOOD SPECIES

In 2010, EFSA concluded that there were “insufficient data to draw conclusions on the influence of processing on the levels of CTX-group toxins in fish” (EFSA, 2010).

Sample extracts from a Caribbean barracuda (cooked and uncooked) were fractionated and the fractions analysed by N2a (Abraham *et al.*, 2012). The cytotoxicity profile of the fractions was very similar, suggesting minimal impact of the cooking process on toxicity.

7. CONCLUSIONS - CIGUATOXINS

7.1 DESCRIPTION OF RISKS TO NEW ZEALAND CONSUMERS

The risk of New Zealanders suffering CFP following consumption of ciguatoxic fish includes two potential routes of exposure; incursion of ciguatoxic dinoflagellates into New Zealand's EEZ and coastal water, resulting in ciguatoxic fish being caught for consumption in New Zealand, or importation of ciguatoxic fish into New Zealand.

While *Gambierdiscus* species have been found in waters around the far north of New Zealand and a known ciguatoxic species (*G. polynesiensis*) has been found in the Kermadec Islands, these isolates did not produce CTXs. To date, there is no evidence of CTX-producing dinoflagellates in waters near (within 1000 km) to New Zealand. However, the occurrence of CFP cases in Australia associated with fish caught in temperate waters suggests that the possibility cannot be discounted.

Private importation of fish from Pacific islands into New Zealand is still a permitted activity and it appears likely that this activity will be the primary risk factor for CFP in the near future. Intoxications occurring shortly before return to New Zealand may also contribute to notifications in New Zealand.

7.2 COMMENTARY ON RISK MANAGEMENT OPTIONS

Risk management of CFP internationally appears to be problematic. While the USFDA has established guideline levels for Caribbean and Pacific CTXs in fish, there is no evidence that testing of fish for CTXs is being applied as a risk management measure anywhere.

Specification of risk species and upper size limits for fish of those species has been used as a risk management measure. However, the relationship between fish size and ciguatoxicity does not appear to be generally applicable and, at best, may be useful within a very localised context, that is for people harvesting fish from a single location.

Currently, there is no evidence of ciguatoxic fish being caught within New Zealand's EEZ or of the occurrence of CTX-producing dinoflagellates within this zone and application of CFP control measures within the fishing industry does not currently appear to be necessary.

Private importation and consumption of ciguatoxic fish remains the most likely cause of CFP in New Zealand. Given the comments above concerning CTX testing and fish categorisation, the only possible control measure for this source of CFP would be a ban on private imports.

7.3 DATA GAPS

Assessment of CFP risks for New Zealanders is beset by data gaps. However, some of these data gaps will be more critical than others. In particular:

- Information on the frequency of personal importation of reef fish into New Zealand and the species involved would provide a useful denominator for the notified cases of CFP. At present it is unknown whether the CFP cases that come to the attention of the public health system in New Zealand are a small or large proportion of people privately importing reef fish.
- Testing for ciguatoxicity in New Zealand and worldwide suffers from a lack of certified standards and a lack of validated methods. This means that the results of analytical testing are often difficult to interpret.

- The lack of standardised, validated methods also impacts on other areas where data are sparse. While information on the dose-response relationship for CFP is improving, definition of the dose and in particular the concentration of CTXs in the implicated fish is still method dependent. It is uncertain how comparable doses derived through different methods are.

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