



National ciguatera fish poisoning research strategy

Reducing the incidence of ciguatera in Australia through improved risk management



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Executive summary

Ciguatera fish poisoning (CFP) accounts for the majority of food safety outbreaks related to seafood consumption in Australia, with more than 1,650 cases reported since 1965, including 2 fatalities [1-9]. Taking into account an estimated reporting rate of <20% [3], this equates to an approximated incidence of 148 CFP cases per year. A nationally consistent approach to management and reporting is therefore urgently required. The critical need to better understand CFP is reflected internationally, with the World Health Organisation (WHO) and Food and Agricultural Organisation (FAO) highlighting the apparent climate-change driven global increase of cases as an emerging threat to seafood safety.

We here present a national CFP research strategy aimed at mitigating risk for the seafood industry and reducing CFP illness in Australia. This strategy was developed at a multidisciplinary workshop by national and international experts to identify and prioritise immediate research needs (March 27-28th 2019, Brisbane). It complements the international interagency ciguatera strategy (FAO, WHO, IAEA, IOC-UNESCO) to provide a summary of the current state of knowledge of CFP in Australia and identify key research needs as discussed at the workshop.

Although Australian research objectives closely align with the broad aims of the international inter-agency ciguatera framework, the detailed research needs of the Australian region are distinct. Recent reports of *Gambierdiscus* species and CFP cases in NSW associated with significant loss of income from the local Spanish Mackerel fishery highlight the need to understand regional fish movements, environmental conditions associated with CTX occurrence, the identification of local causative organisms, development/validation of rapid analytical tools for screening of fish before market, and improved CFP reporting/epidemiology. Current best management practice is informed by limited data and restricted to avoidance strategies of fish of certain size, species or origin. No consistent national response to CFP incidents and reporting currently exists.

As a first step to address these limitations, several issues slowing work in this field were identified. Consistent with other international research efforts, fundamental research in Australia has been hampered by the lack of commercially available ciguatoxin standards and poor epidemiological data (the latter subject to significant underreporting). Nationally consistent epidemiological reporting across all states, in concert with improved risk factor data collection, was identified as a core priority to better approach the true incidence of ciguatera in Australia and inform future funding decisions. Indeed, the development of (inter)-nationally validated and standardised sampling and reporting guidelines is a priority across all fields of ciguatera research, presenting opportunities to link Australian research to international validation efforts. Important work on the ecology, toxicology and epidemiology of ciguatera has historically been conducted in Australia by a small Queensland research team (80's and 90's), which has since been dismantled, and few Australian researchers are currently active in the field, largely due to the limited availability of reliable, continued research funding. This has left Australia at risk of losing available expertise.

Several of the priorities identified by the working group could be immediately progressed over relatively short time periods (<1 year) to initiate long-term improvements if funding were to become available (e.g. nationally consistent epidemiological reporting, health care worker and recreational fisher awareness, ciguatera risk map), while other key objectives, such as analytical method development, will require substantial investment.

In the absence of a sizeable funding source to support a substantiative research and education/awareness program, future progress will likely be restricted to individual projects. This often necessitates researchers to focus on localised areas, leading to partial collection of the vital information required to assess state overlapping risks (e.g. Spanish Mackerel migration). Potential for the development of a larger project to align NSW, QLD and NT interests exists, as CFP is highly topical to commercial and recreational fishers in all of these states. The authors are hopeful that the action plan here presented as the consensus of a multidisciplinary working group ([Table 1](#)) will serve to inform funding bodies on how best to invest into existing & emerging Australian expertise to build capability and improve ciguatera management in Australia.

Introduction

Ciguatera fish poisoning

Ciguatera fish poisoning (CFP) has been identified as an emerging threat to seafood safety by the Food and Agricultural Organisation (FAO) and World Health Organisation (WHO). CFP, a well-known illness in tropical regions, is caused by the consumption of fish that have accumulated naturally occurring toxins produced by benthic dinoflagellate species of the genus *Gambierdiscus* or possibly *Fukuyoa* spp. Ciguatoxins (CTX) are cyclic polyether toxins that are known to accumulate both in herbivorous fish and the larger predatory species feeding on them. CFP can be caused by consumption of fish containing analytically challenging low levels of CTX and the impact of prolonged exposure to subacute levels of CTX remains unknown. Three different groups of CTXs are distinguished based on their region of prevalence: the Indian (I-CTX), Caribbean (C-CTX) and Pacific ciguatoxins (P-CTX), with the latter the most potent and present in Australia [10]. These toxins are odourless, tasteless, not removed by cooking or freezing and require sophisticated and expensive analytical techniques for reliable detection.

Internationally, an estimated 10,000-500,000 people are impacted by CFP every year, with less than 10-20% of cases reported. In Australia, CFP accounts for the majority of food safety outbreaks related to seafood consumption (>1600 cases reported since 1965, including 2 fatalities [1-9]). Taking into account an estimated reporting rate of <20% [3], this equates to an approximated incidence of 148 CFP cases per year). While localised industries may be the first affected (e.g. New South Wales Spanish Mackerel fishery), CFP outbreaks carry a substantial risk of reduced consumer confidence in all seafood products, extending both directly and indirectly to the tourism industry (e.g. restauranteurs, charter boat operators, resorts). The globalisation of trade has increased market exposure to potentially affected fish (e.g. the Rapid Alert System for Food and Feed of the EU notified the presence of CTXs on four occasions in 2016 and several NSW cases were linked to imported fish [6]). An apparent global expansion of CFP outbreaks has highlighted the paucity of scientific information available for informing effective management practices currently limited to avoidance strategies rejecting certain fish based on species, size, and origin [11]. Climate change has the potential to both expand the range of the causative dinoflagellates as well as CTX contaminated fish and thereby increase CFP risk and confound risk management strategies.

International research initiatives

An inter-agency strategy has been formulated by the UNESCO Intergovernmental Oceanographic Commission (IOC), International Atomic & Energy Association (IAEA), Food and Agricultural Organisation (FAO) and the World Health Organisation (WHO) to develop a coordinated strategy for improved research and management of ciguatera on a global scale [12]. This strategy incorporates existing CFP research programs and aims to improve causative organism detection and sampling methods, toxin detection, epidemiological data collection, reporting and assessments. Several large-scale international collaborations as well as individual research projects currently feed into the global inter-agency (IOC-IAEA-FAO-WHO) ciguatera strategy framework.

Following the request from the 11th Session of the Codex Committee on contaminants in Food (CCCF11), the Joint FAO/WHO Expert Committee for Food Additives (JECFA) released an international call for data on ciguatera fish poisoning (CFP) in 2018 to conduct an international risk assessment (to be released late 2019). To ensure the relevance of the outcome of the FAO/WHO risk assessment to Australia, SafeFish¹ established an Australian working group on CFP.

SafeFish ciguatera working group

The SafeFish ciguatera working group identified, collated and submitted all available Australian data to the FAO/WHO for risk assessment purposes. It was decided to continue working towards forming a strong Australian ciguatera network with ties to international research. Acknowledging the contributions of working group members and the incidence of CFP in Australia, the SafeFish partnership funded a two-day workshop with the overarching goal of focusing research efforts, identifying knowledge gaps, and thereby reducing the incidence of CFP in Australia through improved management.

Objectives & output

The multidisciplinary workshop brought together representatives from the fishing industry, state health departments, researchers, regulators and food authorities to develop a national ciguatera strategy by:

- Identifying and grouping knowledge gaps
- Identifying and discussing actions to resolve these
- Prioritising actions based on need, duration, cost and available expertise

This document presents the output from this workshop to detail a national ciguatera research strategy aimed at reducing the incidence of CFP in Australia through improved management. It coherently outlines major knowledge gaps in ecology, epidemiology and toxicology. Essential responses to current and emerging ciguatera risks/critical knowledge gaps are identified and prioritised to present a coordinated, national research plan. Key objectives of the strategy are to increase our understanding of ecological drivers and improve collection, storage and accessibility of epidemiological and toxicological data to inform management approaches. The following section outlines the current state and future directions of Australian ciguatera research within the fields of ecology, toxicology and epidemiology, as discussed at the SafeFish ciguatera workshop in Brisbane (March 27– 28th 2019).

¹ SafeFish

The SafeFish program provides technical advice to support Australia's seafood trade and market access negotiations, helping to resolve barriers to trade. SafeFish is funded by the Australian seafood industry and the Fisheries Research & Development Corporation (FRDC). It is the leading platform in the FRDC program for dealing with food safety, trade and market access issues affecting Australian seafood. The SafeFish partnership identified CFP to be of high technical research importance, leading to the initiation of the Australian ciguatera working group.

Current Australian research consensus

Epidemiology

In Australia, CFP accounts for the majority of food safety outbreaks related to seafood consumption (>1600 reported cases since 1965 [1-9], impacting an estimated 148 people per year). CFP is currently only notifiable in the NT and QLD, with outbreaks being reported through OzFoodNet in the other states and territories (an outbreak is defined as two or more cases linked to the same contaminated food source). Ciguatera case questionnaires are currently only employed by the states and territories where reporting is mandatory (QLD and NT) and outbreaks in other jurisdictions are reported through the OzFoodNet reporting template. This form has previously not captured CFP specific information and no nationally consistent questionnaire or approach to reporting/collating of data existed (efforts by OzFoodNet and QLD Health are currently underway to update the outbreak questionnaire and implement nationally). Since 2006, isolated ciguatera cases (single person only affected) in QLD that would normally not be included in the OzFoodNet database, are also recorded in the Queensland Enhanced Ciguatera Surveillance Database (QLD Health).

The vast majority of cases are reported in QLD, where QLD Health Forensic and Scientific Services (QLD HFSS) upon request by the Public Health Units, conduct ciguatoxin analysis (P-CTX-1,-2, and -3) of remaining meal or fish samples. At present, difficulties exist in linking epidemiological data to risk, with reports of laboratory results to the requesting Public Health Unit (PHU) often filed away in paper format and no copy provided to OzFoodNet. No clear guidance currently exists on trace back of fish samples or data flow and case reports at some of the 13 PHUs in QLD may not be followed up with CTX analysis or molecular fish identification, partly due to a lack of awareness of the availability of such services at QLD HFSS. Queensland Health is currently reviewing their CFP case questionnaire to roll out through OzFoodNet for the nationally consistent collection of improved epidemiological risk factor data. NSW is the only other state in Australia where CFP cases are followed up with CTX analysis. Reports of CFP by GPs are directly forwarded to the NSW Food Authority (NSW DPI) to investigate and collect samples for CTX testing (if available). Since 2014, there have been more than 10 ciguatera fish poisoning (CFP) outbreaks in New South Wales (NSW), affecting 41 individuals. These incidents were linked to both local catch (Spanish Mackerel) and fish sourced outside the state (Green Jobfish, Purple Rockcod, Redthroat Emperor, Grouper). Historically, outbreaks of CFP in the state were few and mainly associated with imported fish [6].

As in other countries, CFP in Australia suffers from significant underreporting by both health care workers and those affected. It is estimated that less than 20% of cases are reported [3]. Reasons for underreporting by health care workers include difficulty in reporting (time required to file reports, often remote locations), misdiagnosis (individual experience of health care workers, often complicated with ambiguous symptoms) and lack of awareness of reporting system/requirements. CFP sufferers often do not consult their health care workers due to the lack of effective treatment options and/or distance to the nearest health care provider. Mannitol is given in some cases to relieve symptoms, but effectiveness is highly variable.

Anecdotal reports of CFP cases often appear on social media (e.g. online fishing forums), offering the opportunity for targeted awareness, information and broader data collection.

There currently is no national data platform to collate epidemiological data raised from health care professionals, laboratory results and/or the public, but efforts through OzFoodNet are currently underway to link patient data to toxicological reports in a single database. A limited amount of easily accessible information is currently available to acute and chronic CFP sufferers and no centralised source of advice/guidance exists. CFP patients currently rely on the experience/skill level of their respective health care providers and are often limited to consulting more detailed overseas online resources.

The working group acknowledges that no medical professionals were present at the workshop to lead a discussion on the development of potential treatment options. The working group recognises that improved quality and collection of epidemiological and toxicological data would represent a vital first step and will seek to involve a clinician on any future discussions on the topic. CFP is listed in the International Catalogue of Diseases (ICD) curated by the WHO (code “XM1DD9 Ciguatera fish oral poison”). This manual guides all hospital/health practitioner coding and statistics and CFP is therefore included in the Australian Institute of Health and Welfare statistics (CFP accounted for approximately 50% of all seafood poisoning incidents in the 2016-17 period).

Ecology

Five different *Gambierdiscus* , (*G.carpenteri*, *G. honu*, *G lapillus*, *G. holmesii*, *G. lewisi*) and one *Fukuyoa* species (*F. yasumotoi*), have been identified in Australia to date. However, the species responsible for producing the ciguatoxin precursors in Australian waters remains unknown and the identity of ciguotoxic cultures used in older Australian studies (identified as *Gambierdiscus toxicus* by Holmes et al. 1990) can no longer be verified. In the Pacific region, *Gambierdiscus polynesiensis* has been identified as producing P-CTX-4A, -4B and -3C [14], which in the presence of fish liver enzymes convert to more oxidised forms often implicated in CFP [15]. This species has not been recorded in Australia to date, and an Australian Research Council funded project led by Shauna Murray at the University of Technology Sydney is currently underway (2019-2022) to locate the source of Australian P-CTX-1. This work presents a vital first step to isolate, culture and characterise benthic microalgae and associated toxin production from two field sites in Queensland. Identification of the causative organism(s) will open further avenues of research, including the development of targeted molecular monitoring techniques and determination of environmental conditions required for growth of the source organism(s). Once developed, advanced monitoring techniques would allow for estimations of the time lag between blooms of the toxin producing microalgae and the presence of ciguatoxins in fish flesh.

Additional US National Science Foundation funded efforts (NSF 1743802, 1841811) coordinated by Alison Robertson (USA, director of CiguaPIRE) are aiming to better understand the ciguatera risk in North East and North Western Australia as part of a large-scale (international) research program. The work represents a collaborative effort by the University of Western Australia, Edith Cowan University, James Cook University, Great Barrier Reef Marine Park Authority, and Commonwealth Scientific and Industrial Research Organisation. Although the scale of the Australian aspect of this project remains to be confirmed, Alison has indicated that the NSF is open to the idea of supporting Australian research projects through in-kind contributions and leveraging of data collected through the CiguaPIRE work.

The key collaborations for Australia to further develop are with the French Polynesian Institut Louis Malardé (ILM) and New Zealand's Cawthron Institute. Both parties were present at the SafeFish workshop and expressed interest in working together with Australian researchers and regulators. Areas of possible future collaboration include toxin analysis and CTX standard production, as well as ecological and epidemiological aspects of CFP.

Traditionally, Queensland and the Northern Territory have been considered as the regions of highest ciguatera risk, yet *Gambierdiscus* species have now been reported as far south as Merimbula in New South Wales. Whether this represents an extension of the existing *Gambierdiscus* spp. distribution or increased awareness in response to recent CFP cases in New South Wales cannot be answered with the currently available data. Indicators are that local conditions in NSW appear to be changing to create favourable environmental settings for *Gambierdiscus* species. To date, *Gambierdiscus* spp. isolated from NSW have not been recorded to produce ciguatoxins and CFP cases are dominated by migratory fish like Spanish Mackerel that likely accumulate the toxins elsewhere (e.g. QLD waters). Regional investigations linking fish ecology (movement and feeding) with microalgal ecology (proliferation and toxin production) in light of environmental conditions (including anthropogenic and natural disturbance events) are required.

Initial 2014 FRDC funded work gathered the largest Australian data set on CTX in fish to date (84 fish) to explore the potential relationship between the length/weight ratio and ciguatoxin concentration in Spanish Mackerel [16]. CTX was present in both flesh and liver samples (1-7% incidence), but was not clearly correlated with the weight or length of individual fish. This work warrants substantiation through increased sample sizes to more accurately estimate the true prevalence of CTX in Spanish Mackerel, particularly in light of likely inter-annual variation. Few investigations have linked fish movements, causative microalgal blooms, environmental conditions and disturbance events internationally, let alone within Australia. Little information exists nationally on the transfer of ciguatoxins through the food web, including studies on toxin uptake, transformation and depuration rates at different trophic levels.

Toxicology

The quantification of ciguatoxins in Australia has long been restricted by the limited availability of toxin standards, yet considerable expertise in a range of analytical techniques exists within Australia and New Zealand. These include non-specific cell line screening tests like the neuroblastoma N2a and fluorescent imaging plate reader assays (FLIPR) that detect overall toxic activity in sample extracts. Any detection events can be followed up with targeted confirmatory analysis via liquid chromatography-tandem mass spectroscopy (LC-MS/MS) or high resolution mass spectrometry (LC-HRMS) to quantify select analytes. The restriction of the latter approach is the high cost of analysis (high initial set-up cost, ongoing maintenance and requirement for highly skilled staff), as well as the critical requirement for purified reference materials/toxin standards. In Australia, facilities capable of LC-MS analysis of CTXs currently exist at the Sydney Institute for Marine Sciences, Queensland Health Forensic and Scientific Services and the University of Queensland. Cawthron Institute in New Zealand also maintains CTX analytical facilities.

It should be noted that the conservative US Food and Drug Administration (FDA) guidance value of 0.01 ppb of Pacific ciguatoxin (P-CTX-1) is below the current limit of detection for most analytical techniques, although an enzyme-linked immunosorbent assay (ELISA),

recently developed in Japan, claims targeted detection of P-CTX-1 down to 0.0005 ppb (quantification limit [17]). This new CSI sandwich hybridisation assay offers promise for the detection of low levels of CTX, but remains to be validated for specific fish samples and the economic viability of routine testing confirmed. It is noted that a previous Cigua-Check test kit, developed by the University of Hawaii and marketed by Oceanit, was recently removed from sale after cases of illness in recreational fishermen relying on this product [18]. The commercial fishing industry is highly interested in the development of a rapid test kit for routine screening of fresh fish to allow fishers, retailers and consumers to access resources currently restricted under the Sydney Fish Market management guidelines currently in use (e.g. >10 kg Spanish Mackerel). Improved data on the prevalence of CTXs in different fish species will form a vital component of the validation and refinement of these guidelines.

Of the Pacific ciguatoxins, P-CTX-1, -2 and -3 have all been detected in fish implicated in human poisonings in Australia, including one fatality [2, 19]. P-CTX-2 and -3 have not been reported in fish flesh in the absence of P-CTX-1, the principal and most potent of the ciguatoxins tested to date [10]. While not yet quantified/detected in Australian fish, the congeners P-CTX-4A and B have been reported in fish in the Pacific region [14], most likely contributing to total toxicity in combination with the other P-CTXs listed above. P-CTX-1 has been identified by the working group as the prime analytical target for Australia due to its high potency and prevalence in all CFP implicated Australian fish samples tested by QLD Health (59 samples 2013-2018). A standard for this congener is available as part of the new CSI ELISA test kit, but not on its own. Where quantification techniques are employed that can detect multiple specific analytical targets (LC-MS/MS, LC-HRMS) and standards are available, the working group recommends additional quantification of P-CTX-2 and P-CTX-3. P-CTX-2 was the major CTX implicated in a fatal case of ciguatera in Australia (and potentially its epimer P-CTX-3 [2]). Since no toxin standards for P-CTX-4A or B are currently commercially available and their reported potencies are significantly lower than P-CTX-1, these congeners are regarded as emerging analytical targets for Australia. As additional ciguatoxin standards are released in reliable supply (likely >5 years away), the new data becoming available will require review to adjust the list of analytical targets for Australia. The cost for the limited ciguatoxin standards currently available remains high (e.g. P-CTX-3C: AUD 500 for just 100 ng, Wako Chemical Inc.) and supply in the face of international demand is uncertain. The team led by Mireille Chinain (Institut Louis Malardé, French Polynesia) are planning to make additional ciguatoxin standards available in 2019 and are interested in collaborating with Australian researchers. Other overseas initiatives to produce CTX standards are currently underway in France and New Zealand, but release date and availability remain uncertain.

There is currently no health based guidance value for ciguatoxins in Australia. The US FDA guidance (<0.01 ppb P-CTX-1) is based on limited data and therefore conservative. Furthermore, this level could not be implemented with currently available confirmatory analysis (e.g. LC-MS/MS limit of quantitation = 0.05 ppb). The working group sees value in establishing an interim Australian guidance level before adopting a level that cannot be implemented. Its development would require the collection of improved epidemiological data linking toxicology to epidemiology (i.e. QLD Health CTX in fish flesh data linked to patient questionnaires and Australian seafood consumption data held by Food Standards Australia and New Zealand). Data improvements include recording of the severity of individual symptoms on patient questionnaires, as well as nationally consistent approaches to epidemiological and toxicological data collection and reporting.

Australian research priorities

Informed by the above described Australian research status collated during the workshop, the working group identified actions to meet the most critical requirements for advancing the national understanding of ciguatera distribution, incidence, reporting and management (listed in *Table 1*). The criteria on which the prioritisation process was based are described in the Appendix (*Table 2*). The requirement for analytical CTX standards was not included in the initial prioritisation voting, as it was unknown what/if standards were available at the time. Since only P-CTX-3C and P-CTX-1 (as part of the ELISA test kit) are commercially available (in unknown quantities), CTX standards have been included here to be of critical importance as the backbone of future research endeavours.

The first-tier priorities centre on the collection of epidemiological and toxicological data (details in *Table 1*):

- Nationally consistent approach for ciguatera related sampling and analysis.
- Nationally consistent collection of epidemiological data.
- Analytical CTX standards
- ELISA rapid CTX test kit validation & implementation².

The second-tier priorities include:

- Identification & culturing of CTX producing microalgae.
- Communication strategy - increased and targeted awareness.

Investments required and time frames of completion

The identified research priorities differ in the amount of funding and time required to achieve their respective outcomes (*Table 1*). A major hurdle for progressing toxicological and ecological aspects of the prioritised ciguatera actions remains the limited supply of commercially available ciguatoxin standards, as well as lack of continued funding for what is considered to be highly challenging analytical research.

Several priorities identified as leading to long-term improvements were deemed to be immediately progressable (<1 year) at relatively low cost (\$<50K). Examples include the generation of awareness materials/surveys or developing a nationally consistent ciguatera questionnaire (currently being progressed by OzFoodNet/QLD Health and supported by this working group). Potential exists to further scientific outcomes through citizen science projects that in turn generate increased awareness (e.g. provision of fish samples in size classes that cannot be obtained from commercial fishers or entering ciguatera cases into a

² The ELISA rapid test kit validation & implementation priority was based on the limited information available on the assay at the time of the workshop. Subsequent enquiries revealed that current availability and cost (\$500 per sample) would prohibit routine use of this specific test kit. Nonetheless, this priority has been included to highlight continued industry desire for a rapid CTX test kit.

real time, online ciguatera risk map). To ensure a continued return from the initial investment into certain projects (<\$50k), ongoing/incremental, small scale funding will be required to support maintenance and updating of content (websites, awareness material, databases). The requirement for (online) databases to effectively collate, store, access and analyse data, along with validated, standardised sampling and reporting guidelines to feed into these databases, is a recurrent theme across epidemiology, ecology and toxicology. Of most critical importance in this regard is the linking of improved epidemiological data to the toxicological analysis of implicated fish samples. Nationally consistent epidemiological reporting across all states is regarded as a core priority to approach the true incidence of ciguatera in Australia as this information will serve as a vital background statistic to inform future funding decisions. Indeed, most of the proposed actions in *Table 1* will not be progressed without additional funding into Australia's research expertise and capability.

Australian research expertise and capability

Current research on CFP in Australia is restricted by the availability of funding and Australia at risk of loss of expertise, as few individuals with significant CFP research experience have remained actively working in the field. Historically (80's and 90's), a considerable amount of research was conducted on the toxicological aspects of CFP at the Queensland DPI (Richard Lewis, Noel Gillespie, Lyndon Llewellyn and Mike Holmes). Today, expertise on CFP within Australia essentially resides with two individuals leading long-term CFP research: Prof Richard Lewis at the University of Queensland (toxicologist working on CFP since the 80's) and A. Prof Shauna Murray at the University of Technology Sydney (molecular ecologist working on CFP since 2010). Additionally, new CTX analytical capability (LC-MS) exists at the Sydney Institute of Marine Sciences, QLD HFSS and continues to be available at the University of Queensland (Richard Lewis), with CFP outbreak investigations conducted by government institutions (i.e. QLD HFSS and NSW DPI). It should be noted that complementary expertise topical to ciguatera research exists in other fields of marine ecology, such as fish biology (e.g. movements of migratory fish) and benthic ecology (e.g. data sets on seaweed distribution).

While expertise to progress the priorities identified in this strategy currently exists within Australia, it is evident that substantial investment is required to not only address these priorities, but to strengthen existing, and developing new CFP research capability in the process. In terms of developing capability to support long-term ciguatera research in Australia, the production of CTX toxin standards is a critical priority. While this would require a substantial amount of investment (i.e. purification and extraction from 50-200 kg of CTX fish, or bulk culture of CTX *Gambierdiscus* cultures), it would alleviate the reliance of Australia on the continued uncertainty of limited supply/availability of CTX standards from overseas. There is no doubt that ciguatoxin standards will be key not only to the quantification of CTXs, but also to world-leading research on ciguatoxin accumulation and trophic transfer rates required to inform risk management strategies.

Future funding sources

Although CFP accounts for the majority of food safety outbreaks related to seafood consumption, at this point in time the National Health and Medical Research Council (NHMRC) has had limited involvement in supporting CFP research. This funding body requires consideration of Australian public health in relation to other food safety concerns, such as *Salmonella* outbreaks (1000s of cases, 100s of hospitalisations and several deaths

over the last decade), as well as other prominent maladies (e.g. diabetes, obesity, cancer). The impact of CFP, however, extends beyond the underreported incidence of ciguatera to the entirety of the seafood industry. While localised industries may be the first affected (e.g. NSW Spanish Mackerel fishery), media coverage of CFP outbreaks carries substantial risk of reduced consumer confidence, with the risk extending both directly and indirectly to the tourism industry (e.g. restauranteurs, charter boat operators, tourist resorts).

In the absence of a single, sizeable funding source to support a program that consists of both substantiative research and education/awareness, progress will be slow and restricted to few individual research providers either with a stake in seafood safety or interest in funding academic research (e.g. Fisheries Research and Development Council, Australian Research Council). This tends to drive isolated research projects to focus on regional areas of concern and runs the risk of deviating from a nationally consistent approach. For example, studies of migratory fish, such as Spanish Mackerel, would benefit greatly from coordinated research across NSW, QLD and the NT. The authors are hopeful that the action plan presented here as the consensus of a multidisciplinary working group ([Table 1](#)) will provide a focal point to align Australian research activities for a national approach to ciguatera research. Furthermore, we hope that this work will serve as a guide to inform funding bodies on where best to invest into existing & emerging Australian expertise to build capability and improve ciguatera management in Australia.

Future communications

The SafeFish partnership continues to view ciguatera fish poisoning as an issue of high technical research importance and has agreed to provide small scale funding to facilitate the continued coordination of the working group, as long as the action plan is being implemented. As such, the role of SafeFish will be to:

1. Continue to maintain and where possible grow the Australian ciguatera network,
2. Where possible ensure Australian research is represented internationally,
3. Facilitate the development of new projects and sharing of CFP related information.

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Table 1 Australian Research priorities and actionable items identified at the SafeFish ciguatera workshop. Estimated cost and timeframes of research objectives are listed and criteria informing the priority ranking/voting at the workshop are detailed in the appendix ([Table 2](#)). Parties potentially interested in progressing aspects of the action plan and/or collaborating on future projects are indicated.

Priority	Requirement	Description	Cost (\$ '000)	Time (years)	Action	Parties interested in progressing
Critical	Nationally consistent approach for ciguatera related sampling and analysis	Internationally validated standard methods for ecological and toxicological sampling, including sample preparation, screening & confirmatory analysis, reporting and database formatting, data accessibility.	High >250K	Long >3 years and ongoing refinement	Maintain watching brief on IOC-UNESCO reference manual development and outcomes of FAO/WHO risk assessment to identify opportunities for Australia to contribute to international method validation programs. Secure funding for a project to build the framework for an Australian best practice manual. Future additions/reviews of the manual would pass through the SafeFish working group.	SARDI, SafeFish, UTS, QLD HFSS
Critical	ELISA rapid test kit validation & implementation (see notes in text)	Validation of the CSI ELISA test kit for use with species of interest. Development of suitable protocol for rapid screening of fish samples, including a policy on use and required training.	Medium 50-250K	Medium 1-3 years	1. Secure funding for pilot study to identify suitability (FRDC will release EOI April 2019). 2. If pilot successful, secure funding for full validation for fish samples & training manual preparation.	SARDI, NSW DPI, UTS, QLD HFSS

Priority	Requirement	Description	Cost (\$ '000)	Time (years)	Action	Parties interested in progressing
Critical	Analytical standards	Although some JFRL standards are now becoming commercially available, supply and release are uncertain. Incentive to isolate more standards for national use.	Medium 50-250K	Long >3 years and ongoing production	Working group to maintain a watching brief on release of additional ciguatoxin standards.	All
Critical	Nationally consistent collection of epidemiological data	Nationally consistent case definition, patient questionnaire, reporting and fish sample provision guidelines	Low <50K	Short 1 year	<ol style="list-style-type: none"> 1. QLD Health is reviewing patient questionnaire to circulate to working group for comment before national distribution through OzFoodNet 2. Identify ciguatera clinician interested in opening discussion on national ciguatera case definition and what is required to reach national definition. 3. National guidelines for GP's, PHUs detailing how to consistently report ciguatera cases, including questionnaire, fish sample provision for molecular ID, CTX testing, linking of epidemiological data to toxicological data as well as collation, storage and analysis thereof. 	SafeFish, Qld Health, OzFoodNet, NSW DPI

Priority	Requirement	Description	Cost (\$ '000)	Time (years)	Action	Parties interested in progressing
High	Identification & culturing of CTX producing microalgae	Locating the microalgal source of ciguatoxin in Australia. Subsequent studies on isolated strains.	High >250K	Long >3 years & ongoing maintenance of isolated strains	1. ARC Linkage funded research (2019-2022) aimed at identifying and isolating CTX producer(s) from two sites in QLD is currently underway (led by Shauna Murray, UTS), including development of molecular methods to detect and monitor CTX producing microalgae. 2. Develop funding proposals to identify and isolate CTX producing microalgae from other CFP impacted regions e.g. NSW, NT, other sites in QLD.	UTS, NSW DPI, Institut Louis Malardé,
High	Communication strategy increased and targeted awareness	Media selected for respective audience, including GPs, recreational & commercial fishers, EHOs, charter boat and resort operators, public.	Medium 50-250K	Medium 1-3 years	Additional working group required to identify all issues involved in planning a communication strategy (e.g. stakeholders, audience specific & technically correct information, type of media, opportunities for citizen science projects)	SafeFish, NSW DPI, UTS

Priority	Requirement	Description	Cost (\$ '000)	Time (years)	Action	Parties interested in progressing
Medium	Epidemiological data platform	Focused on the input and output of data: real time risk map, data collection and data visualisation.	Low <50K	Short 1 year to set up, but ongoing maintenance	Epidemiological & risk factor data will be collated through OzFoodNet, with QLD Health and OzFoodNet working towards a national database combining toxicological and epidemiological data.	Institut Louis Malardé, OzFoodNet, QLD Health
Medium	Analytical target identification for potential & emerging analogues	Review of toxicological data as new ciguatoxin standards/analytical methodologies become available. Prime target for Australia is P-CTX-1 and where possible also -2 and -3. Other toxin congeners considered to be emerging.	Medium 50-250K	Long >3 years & ongoing watching brief	1. Whole working group to maintain watching brief of potential and emerging analogues in the Pacific region. 2. Short review of available data Australian data and international information on Pacific CTX.	UTS, SARDI, SafeFish
Medium	Identification of implicated fish species & their movements	Desk based collation of information to link to cyclones/environmental conditions/climate change	Low <50K	Short 1 year	Requires scoping and development of funding application with links to fisheries scientists.	UTS
Medium	Length to weight fish ecology & citizen science survey (QLD and NSW)	CTX in fish survey to investigate whether Spanish Mackerel length:weight ratio correlates with CTX concentration	High >250K	Medium 1-3 years	Project to be developed in response to NSW FRDC EOI (release April 2019).	UTS, NSW DPI

Priority	Requirement	Description	Cost (\$ '000)	Time (years)	Action	Parties interested in progressing
Low	Health based guidance value for Australia	Definition of an implementable CTX level based on Australian exposure data (food consumption, epidemiological and toxicological)	Medium 50-250K	Long >3 years & revision as required/new information available	Long-term need requiring additional data originating from validated, nationally consistent sampling, analysis, data reporting. Working group to maintain watching brief on outcomes of FAO/WHO risk assessment.	UTS
Low	Fish feeding study	Estimate uptake & depuration rates of CTX in fish and their food sources to inform management	High >250K	Medium 1-3 years	More information and international collaboration required before progressing.	UTS

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Appendix

Research objective details used to inform prioritisation process

Table 2. Prioritisation of Australian CFP research requirements as informed by their individual importance, cost, timeframe and required capability. Workshop attendees cast three individual votes (1-3), with 1 being the highest priority. The total score represents the sum of the individual votes for each research requirement and the priority score the total score divided by the number of votes cast. Based on the priority score, each issue component was assigned a priority (low, medium, high, critical) as detailed at the bottom of the table.

Issue components	Importance			Cost			Timeframe			Capability in Australia			Prioritisation Votes			
	Low	Medium	Critical	Low (<50K)	Medium (50-250K)	High (>250K)	Short (1 year)	Medium (1-3 years)	Long (>3 years)	None	Requires overseas advice	Existing	Total No. of votes	Total score	Priority score	Assigned priority
Nationally consistent approach for seafood toxin analysis			X	X Plan		X Fully implemented	X Single lab val.		X Inter lab val.			X	18	35	1.9	Critical
ELISA rapid test kit validation & implementation			X	X Pilot	X Full		X Pilot	X Full				X	9	15	1.7	Critical
Health based guidance value for Australia		X			X		X Initial assess.		X Ongoing			X	0	0	0.0	Low
Analytical target identification for potential & emerging analogues		X			X		X		X			X	1	3	3.0	Medium
Identification & culturing of CTX producing <i>Gambierdiscus</i>			X			X		X				X	10	22	2.2	High
Identification of implicated fish species & their movements (Desk based collation of information to link to cyclones/environmental conditions/climate change)			X	X			X					X	5	13	2.6	Medium

Issue components	Importance			Cost			Timeframe			Capability in Australia			Prioritisation Votes			
	Low	Medium	Critical	Low (<50K)	Medium (50-250K)	High (>250K)	Short (1 year)	Medium (1-3 years)	Long (>3 years)	None	Requires overseas advice	Existing	Total No. of votes	Total score	Priority score	Assigned priority
Length to weight fish ecology & citizen science survey QLD and NSW for CTX			X			X		X				X	3	5	1.7	Medium
Fish feeding study to measure uptake & depuration of CTX		X				X		X			X	X	0	0	0.0	Low
Communication strategy increased and targeted awareness			X		X		X	X				X	15	31	2.1	High
Nationally consistent data collection Case definition & questionnaire review & guides for fish sample provision			X	X			X					X	10	14	1.4	Critical
Data platform Input and output of data: risk map & data collection		X			X		X					X	2	9	4.5	Medium

Assigned priority	Criteria
Critical	More than 5 votes and a priority score <2
High	More than 5 votes and a priority score > 2
Medium	Between 1- 5 votes
Low	No votes

SafeFish ciguatera workshop (March 27– 28th 2019, Brisbane)

Table 3. List of workshop attendees and their contact details.

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Ciguatera Science Day presentation abstracts

Opening keynote presentation

How coordinated international efforts can benefit ciguatera research? The French Polynesian experience

Dr. Mireille Chinain

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Ciguatera Fish Poisoning (CFP) is a well-known illness caused by the ingestion of fish and marine products naturally contaminated with ciguatoxins (CTXs), with strong impacts on human health/well-being and fish trade. CTXs are produced by benthic dinoflagellates in the genera *Gambierdiscus* and *Fukuyoa* whose expansion from tropical to temperate regions has been linked to emerging CP incidents in previously non-endemic areas. This globalization of ciguatera has resulted in a resurgence of interest among the scientific community and regulatory agencies, and voices have been raised for more coordinated international research efforts in this field, as attested by recent inter-agency initiatives (e.g. IOC/IPHAB Global Ciguatera Strategy, FAO/WHO data call).

French Polynesia, a long-standing ciguatera hotspot in the Pacific, is the only nation among PICTs with a small unit devoted to ciguatera research since the early 70s. Following a brief presentation of the response to outbreaks currently in place in FP, practical examples of how past and present collaboration opportunities have allowed significant achievements in ciguatera control programs will be provided.

Great strides have been made in the understanding of ciguatera over the past several years. However, several methodological/technical impediments as well as critical knowledge gaps remain, that limit both our forecasting capabilities and effective management of CP incidents. Suggested areas for future research efforts will be discussed. Improved networking at an international level is also needed to help foster trans-regional, multidisciplinary comparative studies that will assist in the implementation of a global response to a global issue.

Session 1 Ecology

Chair Gustaaf Hallegraeff

Ciguatera Fish Poisoning in Australia: What do we know about the identities, distribution and abundance of causative *Gambierdiscus* species and vector fish?

Dr. Shauna Murray

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Ciguatera Fish Poisoning (CFP) is an illness occurring worldwide, endemic to tropical regions, particularly associated with coral reefs. In Australia, more than 1400 cases were reported between 1965 and 2010, including 2 fatalities, with a reporting rate of ~20%. Prior to 2014, only one CFP event was linked to a fish caught south of the QLD border. Over the past four years, 24 cases of CFP have occurred due to fish from sub-tropical NSW waters,

including a fish caught at Crowdy Head (32°S), the most southerly reported fish containing CTXs. The fish species most commonly reported to contain CTXs in more southerly sites are Spanish Mackerel (*Scomberomorus commersoni*), and the relationship between fish size and CTX amount has been investigated. The warming East Australian Current and its southward intrusion appears linked to the increased and more southerly distribution of CFP. This expanding public health threat has highlighted the need to assess our knowledge of CFP, CTXs *Gambierdiscus* in Australia. Since 1991, when the first *Gambierdiscus* spp from Australia were isolated and cultured, knowledge of the diversity and CTX production of *Gambierdiscus* has greatly expanded internationally. Most *Gambierdiscus* species cultured worldwide do not produce P-CTX 4A, 4B, 3C, which are the analogs that have been demonstrated to bioconvert to P-CTX-1B in the presence of fish liver enzymes. However, in Pacific countries close to Australia, the species *G. polynesiensis* consistently produces high levels of P-CTX 4A, 4B, 3C, and its abundance was significantly positively correlated with CFP incidences in reef fish in French Polynesia, with a lag time of ~3 months. In Australia, none of the cultured *Gambierdiscus* species found to date (*G. carpenteri*, *G. lapillus*, *G. honu*, *G. holmesi* sp nov, *G. lewisi* sp nov, *Fukuyoa cf yasumoto*) produce detectable quantities of P-CTX 4A, 4B, 3C. For this reason, the identities, toxicologies, distribution, and abundance of CTX-related *Gambierdiscus* species remains to be investigated. We are currently conducting field trips to investigate and culture *Gambierdiscus* at sites in QLD, and will engage fishing communities in order to investigate CTX uptake in co-occurring fish populations. This work will build on our knowledge of CTX causative and vector species and provide basic information to manage the increasing risks of CFP in Australia.

Field ecology of *Gambierdiscus* and *Fukuyoa*: insights from Tongan studies and relevance to Australia

Phoebe Argyle¹²³, Kirsty Smith³, Lesley Rhodes³, Tim Harwood³, Tuikolongahau Halafihi⁴, Islay Marsden²

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Ciguatera poisoning has been reported in Tonga for a number of several (or in recent years) years, resulting in a demand for research into the cause of this phenomenon. In the first study of *Gambierdiscus* and *Fukuyoa* in Tonga we demonstrated the high spatial variability and species diversity present in different coastal ecosystems such as seagrass, macroalgae, coral, and mangrove dominated systems. We also showed how molecular tools can be applied to field monitoring, the limitations of current sampling methodologies, and potential areas of improvement to achieve successful microalgae-based monitoring program for toxic benthic dinoflagellates.

Ecological approach to understanding ciguatera occurrence in Queensland, Australia

Leanne Sparrow

James Cook University

To improve current understanding of ciguatera occurrence in Queensland, an ecological approach was undertaken that investigated temporal and spatial drivers of ciguatera incidence, key environmental factors that trigger population growth of the causative

organism, *Gambierdiscus* spp, and dietary importance of predatory fish in trophic transfer of ciguatoxins. A temporal shift in scombrid- and serranid- associated reported cases in Queensland (Qld) showed a strong geographic delineation at 23°S, which delineates the southern boundary of the Great Barrier Reef (GBR) and may suggest that marine habitat is relevant in the likely fish caught and consumed. The Austral wet season was a temporal driver, which was thought to indicate the potential importance of climatic systems that result in the frequent occurrence of environmental disturbances, such as cyclones, heavy rainfall and thermal-induced coral bleaching. Climatic systems and environmental disturbances are known to impact environmental factors, including sea surface temperatures, salinity and nutrient availability. Interactions between such environmental factors were studied to identify potential ecological impacts to benthic toxic dinoflagellates within marine habitats of the central GBR, with focus on *Gambierdiscus* spp. Temperature, salinity and nutrient uptake impacted final population, relative population growth and doubling times of *Gambierdiscus*, *Prorocentrum* and *Ostreopsis* species.

Session 2 Epidemiology

Chair Shauna Murray

Epidemiology of ciguatera fish poisoning: the example of French Polynesia

Dr. Clémence Gatti

Institut Louis Malarde, French Polynesia

Where does Ciguatera Fish Poisoning (CFP) occur? Why is reporting CFP incidents important? How to report CFP? Despite the progress achieved in ciguatera research over the past five decades, having a precise idea of the extent of CFP occurrence globally is still very challenging. Why? Mainly because of a poor knowledge of the disease in non-endemic regions and the absence of a biological diagnosis tool, which contribute to the high underreporting of CFP.

After a quick overview of the worldwide distribution of CFP and the complexity to obtain data at a global scale, Dr. Gatti proposes to put the spotlight on French Polynesia, a historically Ciguatera endemic territory, which has developed an epidemiological surveillance program in collaboration with Health Care Workers and the general population. She will share her views on both its benefits and weaknesses, and will also present efforts currently undertaken to improve this reporting system (development of an online declaring system, real-time ciguatera risk map, extend this model to neighboring Pacific Islands Countries and Territories, etc).

Lastly, she will present the results of a 2018 survey conducted among Health Care Workers, to better document : i) their relationship with both the disease (level of knowledge, access to information, involvement in the surveillance program) and CFP patients (how do they diagnose, treat and inform them), and ii) the patients' behavior regarding CFP. Data collected through this study can greatly help identify blocking points and orient future targeted actions/communications campaigns in order to improve awareness and reporting of CFP.

The epidemiology of ciguatera fish poisoning in Australia

Russell Stafford

Epidemiologist, OzFoodNet, Communicable Diseases Branch, Department of Health, Queensland.

Ciguatera Fish Poisoning (CFP) is a notifiable condition in Queensland and Northern Territory but not in other jurisdictions. However, outbreaks of two or more cases of CFP are investigated by public health agencies in each state and territory. OzFoodNet records all reported CFP outbreaks in a national outbreak register. In addition, Queensland Health maintains an enhanced ciguatera surveillance database that records epidemiological risk factor data for all ciguatera cases notified in that state. This presentation will include a review of Queensland notification data between 2000 and 2018, a summary of epidemiological risk factor data between 2006 and 2015 from the enhanced surveillance database, and a review of national outbreak data from the OzFoodNet outbreak register.

Ciguatera fish poisoning in New South Wales – State Summary

Hazel Farrell¹, Alan Edwards¹, Anthony Zammit¹, Shauna Murray², Chowdhury Sarowar³ and Tim Harwood⁴

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Since 2014, there have been 10 ciguatera fish poisoning (CFP) outbreaks in New South Wales (NSW), affecting 41 individuals. These incidents were linked to both local catch (Spanish Mackerel) and fish sourced outside the state (Green Jobfish, Purple Rockcod, Redthroat Emperor, Grouper). Historically, outbreaks of CFP in the state were few and mainly associated with imported fish. The growing awareness of CFP in Australia and other locations around the world is in part due to incidence of CFP in regions that were not previously impacted. With limited options for diagnosis and without the capacity for efficient, cost effective, routine testing of ciguatoxins, seafood safety risk management strategies are challenging. The current risk management approach to CFP relies heavily on fishing bans and consumer awareness. Testing of Spanish Mackerel implicated in CFP reported in NSW in 2014 was carried out at the Cawthron Institute, New Zealand. The capability to test ciguatoxins was established in NSW at the Sydney Institute of Marine Science in 2016, and supported the subsequent NSW illness investigations. We used data from the recent NSW outbreaks to develop practical risk management guidelines. These focus on ways to reduce potential ciguatoxin exposure for consumers and to improve surveillance methodologies to increase our understanding of the disease

A personal account of ciguatera fish poisoning

Dianne Johnson

Dianne Johnson will share a short account of her diagnosis with ciguatera fish poisoning (March 2017) after consuming reef fish in New Zealand in October 2016. Herself a registered nurse, Dianne will be elaborating on the symptoms she experienced as well as her road to diagnosis.

Session 3 Toxicology

Chair Hazel Farrell

Ciguatera: Detection methods and research activities in New Zealand

D. Tim Harwood, J. Sam Murray, Michael J. Boundy, Andrew I Selwood

Cawthron Institute, New Zealand.

Although ciguatera is not currently problematic in New Zealand it does represent a credible and increasing risk to inshore fisheries. This is heightened by the apparent range expansion of *Gambierdiscus* spp and occurrence of ciguatera poisonings in similar latitudes to those found in the northern part of New Zealand. To help address this emerging risk, ciguatera research is being performed with support from the Seafood Safety programme and other funding sources, such as the Pacific Fund. Some examples of ciguatera research being performed in New Zealand will be presented and future initiatives discussed.

The analysis of marine samples for toxins associated with ciguatera is challenging. This is due to a variety of reasons, including complex sample types, multiple toxins implicated, existence of many toxin analogues, extremely low levels observed in samples and lack of well described detection methods. This presentation will touch on these challenges and focus specifically on the methodology typically used for confirmatory analysis, liquid chromatography tandem mass spectrometry (LC-MS/MS).

Ciguatera toxicology

Richard J. Lewis

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Ciguatera is a global food poisoning caused by the consumption of warm-water fish that have accumulated toxic levels of dinoflagellate-produced ciguatoxins through the marine food chain. These potent, heat stable, polyether sodium channel activator toxins cause a range of gastrointestinal, neurological and cardiovascular signs and symptoms that remain difficult to treat and resolve slowly. This overview will provide an update of our understanding of the pathophysiology and detection of ciguatoxins.

Investigating secondary metabolites produced by *Gambierdiscus* and cohabitating benthic dinoflagellates

J. Sam Murray, D. Tim Harwood, Michèle R. Prinsep, Lesley L. Rhodes, Alistair L. Wilkins, Michaela E. Larsson, Martina A. Doblin and Priscilla T.Y. Leung

Benthic dinoflagellates within the *Gambierdiscus* genus produce a complex array of secondary metabolites that show varying levels of toxicity. The most potent of these are ciguatoxins (CTXs) and maitotoxins, with many others having been described; gambieric acid, gambierol, gambieroxide, gambierone and most recently, 44-methylgambierone, previously reported as MTX-3.

To investigate the distribution, and potential use of 44-methylgambierone as a biomarker for toxic bloom events, 77 isolates from 7 *Gambierdiscus* species were analysed. Production was ubiquitous to all species, but in the case of *G. carpenteri* not all strains. In addition, it was identified that isolates of co-habitating benthic dinoflagellates of the genera *Coolia* and *Fukuyoa* also produce this metabolite, raising the question 'Do other genera of marine microalgae produce 44-methylgambierone?'

Characterising novel compounds from new *Gambierdiscus* species is important in assessing their contribution to CFP intoxication events. Two recently described species, *G. cheloniae* and *G. honu*, display high acute toxicity via intraperitoneal injection to mice, however do not produce any of the algal ciguatoxins routinely monitored. Using toxicity guided fractionation and tandem mass spectrometry, attempts will be made to isolate and characterise the compound(s) responsible for the observed toxicity.

Ciguatera fish poisoning: an Australian perspective

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Ciguatera Fish Poisoning has been an issue in Australia for many years. While our understanding of this human illness on a global and domestic level continues to grow, many aspects remain to be examined. In this talk, I present a summary of our research into the toxicology and ecology of *Gambierdiscus* and *Fukuyoa* populations in Australia, and offer suggestions for focusing future research efforts, to assist the development of an effective and applicable management strategy.