

## Original article

# CLINICAL DIAGNOSIS AND CHEMICAL CONFIRMATION OF CIGUATERA FISH POISONING IN NEW SOUTH WALES, AUSTRALIA

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## Abstract

Ciguatera fish poisoning is common in tropical and sub-tropical areas and larger fish (> 10 kg) are more susceptible to toxin accumulation with age. Although the coastal climate of northern New South Wales is considered sub-tropical, prior to 2014 there has only been 1 documented outbreak of ciguatera fish poisoning from fish caught in the region. During February and March 2014, 2 outbreaks of ciguatera fish poisoning involved 4 and 9 individuals, respectively, both following consumption of Spanish mackerel from northern New South Wales coastal waters (Evans Head and Scotts Head). Affected individuals suffered a combination of gastrointestinal and neurological symptoms requiring hospital treatment. At least 1 individual was symptomatic up to 7 months later. Liquid chromatography-tandem mass spectrometry detected the compound Pacific ciguatoxin-1B at levels up to  $1.0 \mu\text{g kg}^{-1}$  in fish tissue from both outbreaks. During April 2015, another outbreak of ciguatera fish poisoning was reported in 4 individuals. The fish implicated in the outbreak was caught further south than the 2014 outbreaks (South West Rocks). Fish tissue was unavailable for analysis; however, symptoms were consistent with ciguatera fish poisoning. To our knowledge, these cases are the southernmost confirmed sources of ciguatera fish poisoning in Australia. Educational outreach to the fishing community, in particular recreational fishers was undertaken after the Evans Head outbreak. This highlighted the outbreak, species of fish involved and the range of symptoms associated with ciguatera fish poisoning. Further assessment of the potential for ciguatoxins to occur in previously unaffected locations need to be considered in terms of food safety. *Commun Dis Intell* 2016;40(1):E1–E6.

**Keywords:** ciguatera fish poisoning, New South Wales, Australia, Pacific ciguatoxin 1-B, liquid chromatography-tandem mass spectrometry

## Introduction

Although it is a significantly under-reported food-borne illness,<sup>1–4</sup> ciguatera fish poisoning (CFP) is the most common non-bacterial seafood related illness worldwide.<sup>5</sup> Ciguatoxin and related chemical compounds (CTXs) are naturally occurring. The toxins are produced by marine micro-algal species within the dinoflagellate genus *Gambierdiscus*.<sup>6</sup> Accumulation of CTXs in the marine food chain typically result from herbivorous fish grazing on the toxin-producing micro algae, which are in turn preyed upon by larger carnivorous fish.<sup>7,8</sup> The toxins are metabolised to more toxic forms as they move up the food chain.<sup>9,10</sup>

As with other micro-algal biotoxins, the visual appearance, taste or odours of fish are not affected by the presence of CTXs.<sup>11</sup> There is no process that will remove CTXs from fish prior to consumption, and cooking or freezing the fish will not destroy the toxins. Fatalities from CFP are rare.<sup>2,12</sup> However, documented symptoms are wide-ranging and a combination of gastrointestinal, neurological and cardiovascular effects can occur.<sup>1,13</sup> Regional differences are apparent in reported CFP outbreaks and specific symptoms will depend on the portion size and what part of the fish was consumed, along with the age and wellbeing of the consumer.<sup>3,10</sup> Gastrointestinal indicators include but are not limited to nausea, vomiting, diarrhoea and stomach cramps while neurological features associated with CFP can involve tingling and numbness in fingers, toes, around lips, tongue, mouth and throat, burning sensation or skin pain on contact with cold water, joint and muscle pains with muscular weakness.<sup>3,10</sup> The most characteristic neurological symptom of CFP is that of temperature dysesthesia, although it is not reported in all cases.<sup>3</sup> The onset of the illness is usually within 24 hours of exposure and symptoms generally last 1–4 days but can persist for weeks or months, and in extreme cases years.<sup>14–16</sup> Symptoms can be exacerbated, or individuals may suffer a relapse, by drinking alcohol.<sup>1,17,18</sup> Such a wide array of symptoms, combined with a lack of awareness of CFP, contributes to the infrequent reporting of illness. There is consider-

able overlap of symptoms with other seafood illness (e.g. diarrhetic shellfish poisoning, paralytic shellfish poisoning caused by algal biotoxins or histamine contamination), while the chronic effects of CFP have similar clinical manifestations as chronic fatigue syndrome, brain tumours or multiple sclerosis.<sup>3,19</sup>

In Australia, the majority of CFP outbreaks have resulted from the consumption of fish caught in Queensland and the Northern Territory waters, with the majority of documented cases involving Spanish mackerel.<sup>2,20,21</sup> Cases reported from other Australian states have been linked to imported fish from warm-water tropical regions in Australia or internationally.<sup>22,23</sup> Prior to the 2014 outbreaks, a CFP outbreak was linked to 2 Spanish mackerel caught from northern New South Wales waters (Brunswick Heads) in 2002.<sup>24</sup> Investigations into suspected CFP outbreaks in New South Wales by the NSW Food Authority in 2005 and 2009 were linked to fish originating from Fiji and Queensland, respectively (NSW Food Authority, unpublished data).

## Methods

### Case investigations

The NSW Ministry of Health (NSW Health) and NSW Food Authority officers investigated all cases where individuals had consumed the suspected fish meals and suffered illness. The specifics of neurological and gastrointestinal symptoms were documented and fish samples were collected, where available.

These outbreak investigations were conducted under the *NSW Public Health Act 2010* and thus ethics approval was not required.

### Toxin analysis

The 2 Spanish mackerel involved in the Evans Head case had a combined total weight of 27.8 kg. Although individual weights were not available, following consultation with the restaurant manager by New South Wales Food Authority officers, it was established that the 2 fish weighed approximately 17 kg and 10 kg. Three fish fillets (~1 kg each) were collected but it was not possible to distinguish which fillets were derived from the larger (17 kg) fish.

A single large flesh fillet (~2 kg) was collected following the reported illnesses at Scotts Head. Fish samples from South West Rocks (2015 outbreak) were unavailable for toxin analysis.

Toxin analysis was carried out at the Cawthron Institute, New Zealand. This analytical laboratory has ISO 17025 accreditation across a wide scope of disciplines including food chemistry, microbiology and natural toxins. Samples were frozen (−20°C) prior to shipping on ice. Samples arrived frozen and were stored at −20°C prior to analysis.

All available Spanish mackerel fish fillet samples were screened for the presence of Pacific ciguatoxin-1B (P-CTX-1B) using a liquid chromatography tandem mass spectrometry method developed in-house at the Cawthron Institute and purified P-CTX-1B standard gifted by Dr Mireille Chinain (Institut Louis Malardé, French Polynesia). A manuscript detailing the performance of the methodology is under preparation. Sample extraction followed the procedure described by Lewis et al.<sup>25</sup> The limit of detection of the analysis was 0.1 µg kg<sup>-1</sup> P-CTX-1B. To assess toxin recovery, which may be adversely affected by the sample extraction procedure or by sample co-extractives, an aliquot of one of the fish extracts was fortified with a known quantity of P-CTX-1B at a level of 0.2 µg kg<sup>-1</sup>.

## Results

### Description of outbreaks

#### *Outbreak 1: Evans Head (~29° 06' S)*

On 13 February 2014 the NSW Food Authority received notification from NSW Health of 4 possible CFP cases. The cases were recognised by a local general practitioner and were later reported by a local hospital. Each case had suffered symptoms of myalgia, headaches, tingles and a burning sensation when touching something cold. One individual also experienced vomiting, nausea, diarrhoea bradycardia and hypotension, and was admitted to hospital for 2 days. This individual continued to suffer from neurological symptoms approximately 7 months later.<sup>26</sup>

All cases were employees of a local restaurant and had reported eating Spanish mackerel, 3–4 hours prior to suffering symptoms. The restaurant owner was contacted immediately. The owner advised he had purchased 2 Spanish mackerel with individual weights of approximately 10 kg and 17 kg. The fish had been caught off Chaos Reef, near Evans Head (Figure). A chef prepared the fish into fillets for intended sale to bistro customers. However, none of the fish had been sold to customers. Some of the trimmings were used to make fish cakes for the staff, which were eaten for lunch on 13 February 2014 by 7 staff members including the chef. A third smaller (3.8 kg) Spanish mackerel

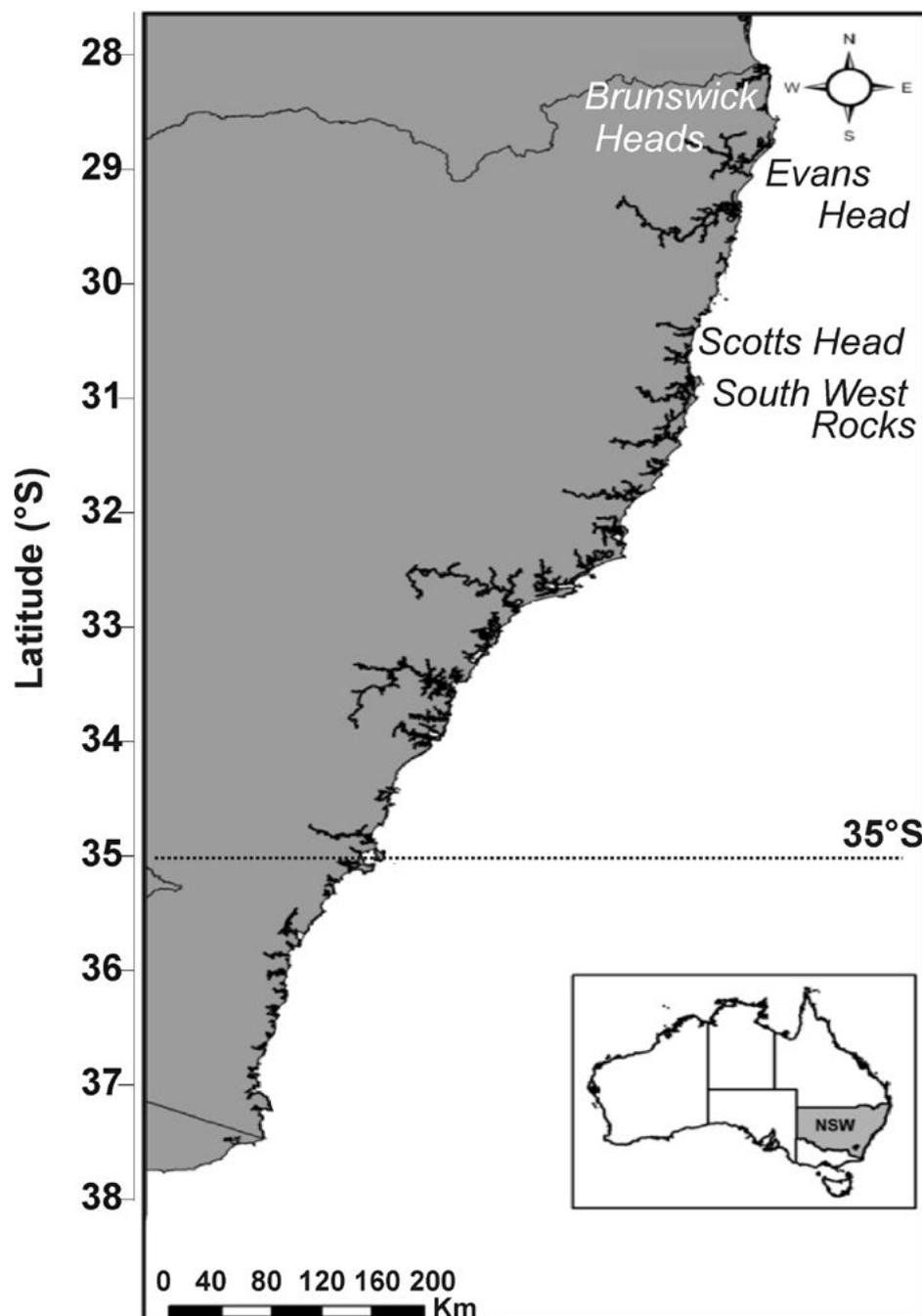
from the same catch, purchased by a different seafood retail outlet, was removed from sale and destroyed following the outbreak.

#### *Outbreak 2: Scotts Head (~30° 45' S)*

On 3 March 2014, NSW Health notified the NSW Food Authority of 9 people who had presented to local hospital emergency departments with suspected CFP. A recreational fisherman had caught a 25.7 kg Spanish mackerel off Scotts Head (Figure) on Sunday 2 March 2014. The fisherman shared the mackerel between himself and 3 family members

and gave half of the mackerel to a friend. His friend did not eat the fish, but gave his share to 2 separate neighbours (resulting in 5 people being affected). All 9 people (ranging in age from 13 to 58) ate the fish for dinner on 2 March 2014. The fish fillets were barbequed prior to consumption and it was estimated that each steak was approximately 2 cm thick and weighed between 250–300 g. Adults ate up to 2 serves, while the younger cases ate smaller portions. The onset of symptoms varied from 1–4 hours after consumption, and cases suffered symptoms of abdominal cramps and paraesthesia of the hands and lips. Cardiac problems occurred

**Figure: Map of New South Wales coastline showing locations of where the implicated Spanish mackerel were caught**



in 1 case. All individuals were advised to avoid alcohol for at least 2–3 months. During follow up interviews, 1 and 2 months post-incident, 1 individual reported a persistent metallic taste for up to 2 months.

### Outbreak 3: South West Rocks (~30° 53' S)

On 9 April 2015, the NSW Food Authority was notified of a suspected CFP in a group of 4 adults affected after eating a Spanish mackerel of approximately 10 kg, caught by a spearfisher off South West Rocks (Figure) on 3 April 2015. At least 1 person was hospitalised on 4 April 2015, and one of the doctors diagnosed the case with classic CFP symptoms.

### Detection of ciguatoxins

P-CTX-1B was unambiguously detected in the fish sample from Scotts Head ( $0.4 \mu\text{g kg}^{-1}$ ) and 2 of the 3 samples from Evans Head ( $0.6\text{--}1.0 \mu\text{g kg}^{-1}$ ) (Table). Overspike recovery, where the fish tissue sample was spiked with a known concentration of toxin standard, was determined to be 41%. This was poor and suggested significant matrix suppression from natural compounds present in the fish tissue and likely means P-CTX-1B levels determined in the samples were underestimated. There was also evidence of other CTX analogues present in the P-CTX-1B positive samples. However, because no reference materials are available for the other CTX analogues they were not able to be accurately quantified as part of this analysis.

**Table: Results of fish flesh analysis by liquid chromatography-tandem mass spectrometry**

Sample	Location	P-CTX-1B	P-CTX-1B ( $\mu\text{g kg}^{-1}$ )
V1207-A	Scotts Head	Detected	0.4
V1207-B	Evans Head	Detected	0.6
V1207-C	Evans Head	Detected	1.0
V1207-D	Evans Head	Not detected	Not detected

### Discussion and outcomes

Following consumption of fish caught in New South Wales coastal waters during 2014, diagnosis of CFP in both cases was based on clinical symptoms and confirmed by chemical analysis and detection of CTXs in the fish tissues. The 2015 case was based on diagnosis of clinical symptoms only, which were consistent with that of CFP. The onset of symptoms was within 4 hours of consumption of the contaminated fish meals. Symptoms were similar in all cases and in 1 indi-

vidual neurological symptoms were still apparent up to 7 months following the consumption of the contaminated fish.<sup>26</sup> In Pacific regions, P-CTX-1B has been documented in many fish species.<sup>27,28</sup> The US Food and Drug Administration has published suggested guidance levels of  $0.01 \mu\text{g kg}^{-1}$  CTX equivalent for Pacific CTX,<sup>29</sup> which represents an extremely low level. Negative effects on human health from exposure to Pacific CTX have been reported at concentrations between 0.08 and  $0.1 \mu\text{g kg}^{-1}$ .<sup>10</sup> The levels of P-CTX-1B detected in 3 of the 4 samples were up to 2 orders of magnitude higher than this value. In addition, matrix suppression from compounds present in the fish tissue likely resulted in an under-estimation of the toxin level in the samples. The variability in concentrations in samples from Evans Head may be partly explained by the difference in sizes of the 2 fish, which were not distinguishable following filleting. However, some variation within individual fish is also apparent. This may explain the lower attack rate in the Evans Head outbreak (4/7) compared with the Scotts Head outbreak (9/9).

To our knowledge, the locations where the contaminated fish implicated in the two 2014 outbreaks were caught were at that time the southernmost confirmed sources of CFP in Australia. The 2015 case further highlighted the potential for CFP incidence to extend further south along the eastern Australian coast. It is generally accepted that potentially ciguatoxic fish occur between the latitudes 35°N and 35°S globally.<sup>29</sup> However, a review by Tester et al.<sup>30</sup> demonstrated the paucity of data south of the Equator regarding CFP incidence for South America, West Africa, the southern Atlantic Ocean and Australia. For south-eastern Australia, an outbreak of CFP in Victoria in 1997 was linked to a consignment of fish from Queensland,<sup>22</sup> while another case in Victoria in 2005 was linked to fish imported from Fiji.<sup>31</sup> Although there have been investigations into CFP associated with imported fish in New South Wales, prior to 2014, only 1 other outbreak (2002, Brunswick Heads) was linked to fish caught in New South Wales waters.<sup>24</sup> At that time, chemical confirmatory testing was unavailable. However, clinical symptoms were consistent with CFP (A. Zammit, personal communication). The cases reported here occurred following the consumption of fish that were caught approximately 70 km (Evans Head), 250 km (Scotts Head) and 265 km (South West Rocks) further south. These cases of CFP occurred within what is considered as the southern CFP boundary (35°S, Figure). However, the 2014 and 2015 outbreaks are consistent with global accounts of CFP, which are increasing in frequency. Additionally, there are increasing reports of CTXs or potentially CTX-producing microalgae in locations outside of tropic reefs regions that were previously con-

sidered not to be at risk.<sup>4,32,33</sup> It is likely that the Spanish mackerel implicated in each of the 2014 outbreaks in New South Wales originated from Queensland waters, particularly as another case of CFP was documented by Queensland authorities in the Gold Coast region during the same period (February 2014), and 67 cases of CFP were reported in Queensland during 2014.<sup>21</sup> While increasing ocean temperatures may be influencing the distribution and migration patterns of fish species, there is also scope for species of microalgae that produce CTXs to increase their geographic range.<sup>34</sup> For example, *Gambierdiscus* species have recently been reported from as far south as Merimbula in New South Wales (36° 53' S),<sup>33</sup> and in coastal waters of Northland New Zealand (35° 15' S).<sup>35</sup> Although it is difficult to ascertain if populations are extant or introduced, these locations are outside of what was originally regarded as the geographic boundary of the species.

There are still many unknowns surrounding the origins and dynamics of CFP from CTX in seafood. The implementation of regulatory criteria for CTXs has been hindered by the limited availability of reference material, which has also restricted the implementation of a commercial test procedure for routine analysis. In the absence of commercial testing, a precautionary approach is applied in Australia whereby certain species of fish are banned or have size restrictions and fish from specific ciguatera 'hotspots' are prohibited for sale.<sup>36–38</sup> Under-reporting of CFP cases can result from misdiagnosis,<sup>19</sup> a limited capability for chemical detection of CTXs and a lack of public awareness particularly in previously unaffected locations. In the wake of the 2014 New South Wales CFP illness outbreaks, material advising consumers to avoid eating large (> 10 kg) Spanish mackerel and fish head, roe, liver and viscera was distributed in the form of media releases and factsheets via the NSW Food Authority and NSW Health websites.<sup>39,40</sup> Educational outreach to 117,000 recreational fishers was facilitated through the NSW Department of Primary Industries (DPI) following the Evan's Head illnesses.<sup>40</sup> This highlighted the location of the outbreak, fish involved and the range of symptoms associated with ciguatera fish poisoning. Further, to protect consumer safety, the regional Fishermen's Co-op at Ballina New South Wales and Sydney Fish Markets updated their risk management strategy to implement a ban on the sale of all fish over 10 kg in the mackerel species. Additionally, a research project at the University of Technology Sydney, supported by NSW DPI and the Australian Government Fisheries Research and Development Corporation, is underway to investigate CTXs in Spanish mackerel from northern New South Wales and to establish testing capabilities.<sup>41</sup> Advancing our understanding of

the risks of CFP, and working towards techniques to reliably and accurately detect if CFP toxins are present in Australian seafood, will reduce the risk to public health and may allow the fishing industry to re-enter the market with fish species that they currently cannot sell.

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