Contribution of marine invertebrates to Ciguatera poisoning : the case study of French Polynesia

Reported as early as the 15th century by explorers, ciguatera fish poisoning (CFP) is the most prominent non-bacterial seafood poisoning worldwide. Widespread in tropical and subtropical regions, CFP results from the consumption of coral reef fish contaminated with ciguatoxins (CTXs) produced by *Gambierdiscus* and *Fukuyoa* species. These potent neurotoxins can pose a severe health hazard causing gastrointestinal, neurological and cardiovascular disorders, with cold allodynia being a typical symptom of ciguatera.

Yet, other seafood products such as several species of marine invertebrates highly prized by many island communities in the Pacific region, have also been episodically involved in ciguatera-like poisoning incidents. Indeed, the first mass poisoning outbreak ever documented in the literature following the ingestion of the giant clam *Tridacna maxima* occurred in 1964 in Bora Bora Island (French Polynesia). This poisoning event which affected a total of 33 patients who displayed mainly gastrointestinal and neurological disorders, eventually led to the death of 3 people [1]. Years later, from 2001 onwards, similar incidents were again reported from French Polynesia, but also New Caledonia and the Cook Islands. Of note, besides the digestive and neurological manifestations typically evocative of CFP, patients displayed additional symptoms, such as the rapid onset of the disease, an unusual severity of symptoms including paralysis in some patients, and a rapid burning of their mouth and tongue [2, 3]. Additional poisoning cases following the ingestion of sea urchins, i.e. *Tripneustes esculentus* and *Tripneustes gratilla*, have also been described in the West Indies [4] and French Polynesia [5, 6], respectively, while other marine invertebrates such as the big blue octopus *Octopus scyanea*, the nimble spray crab, *Percnon* spp., and the large worm shell *Dendropoma maxima* have been involved in sporadic poisoning events in the Cook Islands [2]. In addition, the lobsters *Panulirus penicillatus* and octopus from the Republic of Kiribati [7] as well as two starfish from Madeira waters, *Ophidiaster ophidianus* and *Marthasterias glacialis* [8], have been recently found to also bioaccumulate CTXs.

Since many of these edible marine invertebrate species represent a valuable source of protein and revenue in Pacific island countries and territories (PICTs), it is therefore important to document these atypical forms of ciguatera further. The following data describe 2 recent mass-poisoning events which occurred in Anaho Bay (Nuku Hiva Island, Marquesas archipelago, Fig. 1) following the consumption of toxic specimens of *Tectus niloticus,* (gastropods) and *Tripneustes gratilla* (echinoids) in 2014 and 2015, respectively [9-11]. Regardless of the toxic seafood, all patients (9 tourists and 3 tourists, respectively) experienced gastrointestinal and neurological disorders. Cardiovascular symptoms were recorded only in patients that had ingested *T. niloticus,* a likely explanation for this being that patients who consumed toxic sea urchins delayed presenting at hospital, [10, 11]. In the case of the *T. niloticus* poisoning event, the unusual severity of both gastrointestinal and neurological symptoms necessitated the hospitalization of 6 out of the 9 patients.

Unfortunately, in both cases, no food remnants were available for confirmation analysis. Instead, field investigations were undertaken periodically in the toxic area in order to survey the toxicity level in these 2 groups of marine invertebrates and assess the identity

Fig. 1. Study site of Anaho Bay in Nuku Hiva Island, Marquesas Archipelago, French Polynesia

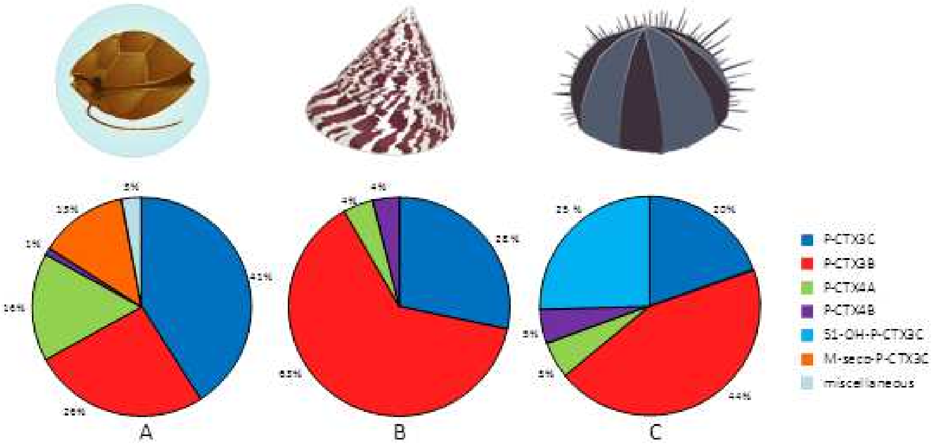

Figure 2. Comparison of Pacific ciguatoxins (P-CTXs) profiles in A) in vitro cultures of Gambierdiscus polynesiensis *(TB-92 [14]), B)* Tectus niloticus *(Nuku Hiva, July 2014 [9]) and C)* Tripneustes gratilla *(Nuku Hiva, July 2015 [11])*

of the toxins involved [9, 11]. Toxicity analysis using the neuroblastoma cell based assay (CBA-N2a) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) revealed the presence of several congeners of P-CTXs in *T. niloticus* and *T. gratilla* toxic samples, at levels consistently above the safety limit recommended for human consumption (0.01 ng P-CTX1B/g of tissue [12]), *i.e.* 11.47 and 20.19 ng P-CTX3C equiv/g of tissue, respectively. These results suggest that *T. niloticus* and *T. gratilla* are able to naturally bioaccumulate P-CTXs in their tissues [9, 11].

Interestingly, these findings were consistent with *Gambierdiscus* abundance data from Anaho Bay obtained using both the natural (i.e. macroalgae) and the artificial (i.e. window-screens) substrate methods [13]. Enumeration of *Gambierdiscus* cells achieved both microscopically and by way of quantitative polymerase chain reaction (qPCR) assays allowed to confirm that *Gambierdiscus* populations were present in this area, while qPCR estimation of the relative distribution of *Gambierdiscus* species in Anaho Bay revealed that *G. polynesiensis* was the dominant species in this toxic area [9, 11]. This species is known to produce P-CTX3C, 3B, 4A, 4B and M-seco-CTX3C as the major CTXs congeners in cultures [14]. The LC-MS/MS analyses performed on *T. niloticus* and *T. gratilla* samples showed that P-CTX3B was the major congener present, followed by P-CTX3C and to a lesser extent, P-CTX4A and P-CTX4B (Fig. 1) [9, 11]. Of note, P-CTX3B is also the major congener found in *Tridacna maxima* experimentally fed *G. polynesiensis* cells [15]. All these observations suggest that *Gambierdiscus* is the likely source of the P-CTXs congeners detected in these marine invertebrates. Sea urchins also contained a significant amount of 51-OH-P-CTX3C (Fig. 2), an analog considered to be favored through metabolization in trophic food webs [11].

Additionally, the field-surveys conducted in Anaho Bay at different time periods emphasized the slow depuration rate for CTXs in both *T. niloticus* and *T. gratilla* toxic samples. The mechanisms controlling the uptake, metabolization and depuration of CTXs in CFP-prone marine invertebrate species are still poorly addressed, thus stressing the need for further investigations. Such knowledge will undoubtedly benefit both ciguatera risk management programs and predictive models of CTX accumulation in these organisms.

In conclusion, these recent results provide evidence that a variety of marine invertebrates actually represent a potential bioaccumulation pathway for ciguatera toxins in marine food webs. Considering that most CFP monitoring programs currently rely on the survey of *Gambierdiscus* cell densities and species composition and/or the monitoring of CTXs in fish, these novel findings highlight the importance of also taking into account the toxicity of marine invertebrates whose consumption may pose a prominent health risk for populations in CFP prone areas.

Acknowledgments

The present work was supported by funds from the countries of France and French Polynesia (Contrat de Projet "Caractérisation et gestion des risques environnementaux et sanitaires liés au développement du phytoplancton ciguatérigène en Polynésie française: CARISTO-Pf" n°7937/MSR/REC of 4th of December 2015 and Arrêté n°HC/491/DIE/BPT of 30th March 2016).

References

1. *Bagnis R 1967. Bulletin de la Société de Pathologie Exotique Tome 60(n°6): 580-592*
2. *Laurent D et al 2012. In: Food Chains: New Research. Chapter 1. Nova Science Publishers, Inc.; pp 1-43*
3. *Rongo T et al 2011. Harmful Algae 10: 345-355*
4. *Earle KV 1980. Transitions of the Royal Society of Tropical Medicine and Hygiene XXXIII(4): 447-452*
5. *Randall JE 1958. Bull Mar Sci 8(3): 236-267*
6. *Pawlowiez R et al 2013. Food Addit Contam Part A 30(3): 567-586*
7. *Mak YL et al 2013. Environ Sci Technol 47: 14070-14079*
8. *Silva M et al 2015. Toxins 7: 3740-3757*
9. *Darius HT et al 2018. Toxins 10(1): 2. doi:10.3390/toxins10010002*
10. *Gatti CM et al 2018. Toxins 10(3): 102. doi:10.3390/toxins10030102*
11. *Darius HT et al 2018. Mar Drugs 16(4): 122. doi:10.3390/md16040122*
12. *FDA: Fish and Fishery Products Hazards and Control Guidance. https://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/seafood/ucm2018426.htm*
13. *Tester PA et al 2014. Harmful Algae 39: 8-25*
14. *Chinain M et al 2010. Toxicon 56: 739-750*
15. *Roué M et al 2016. Harmful Algae 57(Part A): 78-87*

Authors

H Taiana Darius, Jérôme Viallon, Clémence M Gatti & Mireille Chinain, Institut Louis Malardé (ILM), Laboratory of Toxic Microalgae - UMR 241-EIO, PO box 30, 98713 Papeete, Tahiti, French Polynesia

Mélanie Roué, Institut de Recherche pour le Développement (IRD) - UMR 241-EIO, PO box 53267, 98716 Pirae, Tahiti, French Polynesia

Manoella Sibat, Zouher Amzil & Philipp Hess, IFREMER, Phycotoxins Laboratory, F-44311 Nantes, France

Mark W Vandersea & R Wayne Litaker, NOAA, National Ocean Service, Centers for Coastal Ocean Science, Beaufort Laboratory Beaufort, North Carolina, USA

Patricia A Tester, Ocean Tester, LLC, Beaufort, North Carolina, USA

Corresponding author: tdarius@ilm.pf