Ability of giant clams to bio-accumulate ciguatoxins from *Gambierdiscus* cells

Ciguatera Fish Poisoning (CFP) is a seafood poisoning classically related to the consumption of tropical coral reef fish contaminated with ciguatoxins (CTXs). These polyether neurotoxins are produced by dinoflagellates in the genus *Gambierdiscus* [1]. Pacific Island Countries and Territories (PICTs) communities, who are dependent on seafood for their subsistence but also for fishery and tourism industries, are among the populations most affected by CFP worldwide [2]. In these countries, marine invertebrates such as giant clams also constitute highly prized food resources. Unfortunately, in several of these PICTs such as French Polynesia, New-Caledonia, Cook Islands and Republic of Vanuatu, atypical ciguatera-like poisonings incidents following the consumption of giant clams *Tridacna maxima* (Fig. 1) have been reported in recent years [3,4]. As an example, in French Polynesia, official reports of these atypical forms of poisonings following the consumption of various marine invertebrates including *Tridacna max*ima (giant clam) [4], *Tripneustes gratilla* (sea urchin) [5], and *Tectus niloticus* (gastropod) [6] exist, although they represent less than 10 cases/year *versus* an average of 300 fish poisoning cases officially reported annually (*www.ciguatera-online.com*). These statistics about poisonings triggered by consumption of marine invertebrates may however be largely underestimated, as marine invertebrate meals are often omitted in clinical reports whereas fish meals are incriminated [5]. During these ciguatera-like intoxications, classical symptoms of CFP are observed (gastrointestinal disorders, reversal of hot and cold sensations, itching, paresthesia, asthenia, muscular pain, dizziness), in addition to atypical symptoms (alteration of the taste, burning sensation on the tongue and the throat, paralysis) [4], suggesting the involvement of several toxin families, including CTX-like toxins.

Giant clams are bivalve molluscs capable of filtering seawater and retaining particles that are in suspension in the water column such as microalgal cells. *Gambierdiscus* cells have a tychopelagic life style, meaning that this benthic dinoflagellate can temporarily become free-swimming in the water column, especially in high-energy environments. In the process, senescent *Gambierdiscus* cells can release dissolved CTXs in the surrounding water. It is thus likely that giant clams living in areas contaminated with toxic *Gambierdiscus* blooms can potentially accumulate CTXs in their tissues, either by direct ingestion of toxic *Gambierdiscus* cells dispersed in the water column, or by filtration of seawater containing dissolved CTXs. To test this hypothesis and to assess the ability of giant clams to bioaccumulate CTXs upon an episodic exposure to high densities of *Gambierdiscus* cells, *ex situ* contamination experiments of giant clams with *in vitro* cultures of *Gambierdiscus* strains were conducted (Fig. 2) [7].

*Fig. 1. Giant clam (*Tridacna maxima*). © M. Roué.*

Giant clams were exposed for 48 h to live cells or lyzed cells homogenates of *Gambierdiscus*, to mimic natural exposure to free-swimming *Gambierdiscus* cells and dissolved CTXs, respectively. Two distinct strains were used: (i) *G. polynesiensis* TB92, a highly toxic strain containing approximately 5.83 ± 0.85 pg P-CTX-3C equiv. $cell^{-1}$; and (ii) *G. toxicus* HIT0, a strain 2,850-fold less toxic than TB92, containing approximately 2.05 ± 1.16 x 10^{-3} pg P-CTX-3C equiv. $cell^{-1}$. Exposure experiments were conducted at an overall concentration of 150 cells mL^{-1}, equivalent to 0.86 µg P-CTX-3C equiv. L^{-1} and 0.31 x 10^{-3} µg P-CTX-3C equiv. L^{-1} in the case of TB92 and HIT0 strains respectively. The presence of CTXs congeners in giant clams tissues was further assessed using the mouse neuroblastoma cell-based assay (CBA-N2a). Results showed that giant clams exposed to either live cells or lyzed cells homogenates of TB92 were able to bio-accumulate similar levels of toxins, *i.e.* 2.92 ± 1.03 and 3.28 ± 1.37 ng P-CTX-3C equiv. g^{-1} flesh (wet weight) respectively (Table 1). These concentrations are well above the safety limit of 0.01 ppb P-CTX-1B (or 0.02 ppb P-CTX-3C) recommended for human consumption in the Pacific region [8]. In contrast, control animals (no exposition to *Gambierdiscus* cells) and giant clams exposed to cells of the weakly toxic strain HIT0 were found to be free of toxins (Table 1), suggesting that the nature, the risk of contamination of these bivalves is established only in the presence of highly toxic blooms of *Gambierdiscus*. Liquid chromatography mass spectrometry (LC-MS/MS) analyses confirmed CBA-N2a results and revealed that P-CTX-3B was the major CTX congener retained in the tissues of giant clams fed with TB92 cells.

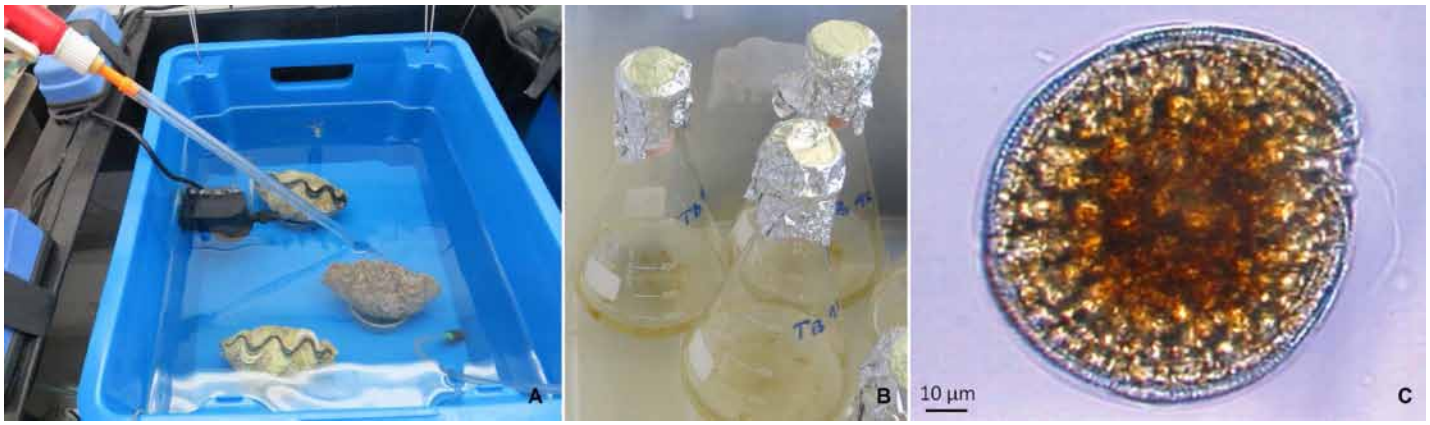

Fig. 2. Ex situ *contamination experiment: giant clams were first acclimated in experimental tanks containing 20 L of seawater (A) and further exposed to in vitrocultures of* Gambierdiscus *spp. (B-C). © M. Roué (A, B); © ILM (C).*

The results of this study [7] provide evidence of the bioaccumulation of CTXs from *Gambierdiscus* cells in giant clams and thus confirm that these molluscs, which are part of the diet of many populations in PICTs, can actually constitute another pathway in ciguatera poisonings in areas where toxic *Gambierdiscus* populations are endemic.

These findings highlight the need for an improved global strategy on ciguatera risk assessment and management programs currently on-going in PICTs. These programs, so far limited to the survey of lagoon fish, should also take into account all major seafood resources consumed within island communities, including those, such as giant clams, commonly regarded as being at low risk of ciguatera.

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Table 1. Estimation of toxin contents in giant clams exposed to live cells or lyzed cells homogenates of Gambierdiscus*, using CBA-N2a. ND: not detectable.*

Condition of exposure	Strain	Giant clams per tank	Giant clams total wet weight (g)	Toxin content ± SD (ng P-CTX-3C equiv g^{-1} wet weight of flesh)
Lyzed cells	control	3	213	ND
	HIT0	3	180	ND
	TB92, tank 1	3	176	3.74. ± 0.26
	TB92, tank 2	3	159	4.96 ± 0.58
	TB92, tank 3	3	137	2.02 ± 0.10
	TB92, average of tanks 1-3	**9**	**157**	**3.58 ± 1.32**
Live cells	control	2	95	ND
	HIT0	3	172	ND
	TB92, tank 1	3	170	3.94 ± 1.21
	TB92, tank 2	3	224	2.48 ± 0.29
	TB92, tank 3	3	197	2.34 ± 0.54
	TB92, average of tanks 1-3	**9**	**197**	**2.92 ± 1.03**