Ciguatera and man's influence in New Caledonia.

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Abstract *Ciguatera is a marine intoxication caused by the consumption of coral reef fish which accumulate ciguatoxins (CTXs) through their diet. This ecotoxicological phenomenon is induced by natural or anthropogenics disturbances of coral reefs ecosystems that result on new available substrates which are followed by the proliferation of naturally present toxinogenic micro-organisms. It is established that certain strains of the dinoflagellate Gambierdiscus spp. are able to produce CTXs. More recently, specific benthic cyanobacteria were also incriminated as potential progenitors of CTX-like compounds. In order to improve the management of ciguatera outbreaks risk, surveys were undertaken in two sites, considering in account these micro-organisms. Here, we present the survey methodologies that were used and some preliminary results.*

Introduction

Ciguatera is a common foodborne intoxication resulting from the consumption of coral reef fish that have accumulate potent naturally occurring lipid-soluble neurotoxins (ciguatoxins, CTXs) through their diet (Friedman, 2008). Environmental conditions are of prime importance in promoting ciguatera outbreaks. This ecotoxicological phenomenon appears induced by natural or man-made disturbances of coral reef ecosystems, followed by the proliferation of macro-algae which are ideal substrates for various micro-organisms. Among these, certain strains of the dinoflagellate *Gambierdiscus* spp. are known to produce CTXs, responsible for human intoxications (Bagnis, 1980). CTXs are transferred trough the food web as the algae is consumed by herbivorous fishes (Lehane and Lewis, 2001; Nicholson et al., 2006).

More recently, pelagic and benthic cyanobacteria were also suspected as potential progenitors of CTX-like compounds (Laurent et al, 2008; Kerbrat et al, 2009 (submitted)). These new findings suggest that ciguatera risk assessment programs should now include both the monitoring of such cyanobacteria and also well-established ciguatera-causing dinoflagellates.

Environmental and anthropogenic factors enhancing the proliferation of these micro-organisms, resulting in ciguatera outbreaks, are still unclear. In order to improve the management of the ciguatera outbreaks risk, two on-going surveys were undertaken at a large scale in New Caledonia, in two sites chosen for their particular situation: (i) the Bay of Prony, currently in development of port facilities for mining construction and (ii) the lagoon of Ouvea reputed to be free of ciguatera.

Here, we present the methodologies that were used for this study carried out as part of a PhD project, as well as some of the preliminary results obtained.

Materiel and Methods

1. Study sites

The bay of Prony

Located in the south of New Caledonia, the bay of Prony is currently in development of port facilities for nickel plant construction. Site collections are defined according anthropics or non anthropogenics factors (port, erosion, mouth of river...). To our knowledge, only some few cases of ciguatera intoxications were recorded in the bay of Prony near the "Ilôt Gabriel" (North Rade) located 3,5 km from the port facilities.

Ouvea atoll

The second survey site was conducted in Ouvea (Loyalty Island), 100 km northeast of New Caledonia. This island is a crescent-shaped coral atoll and the lagoon of Ouvea (166°30′E, 20°30 S) is 50 km long and 7 km wide with a narrow strip of land in the centre, extended by a rosary of islets that surround a shallow lagoon.

Fishery activities are important part of local economy and consisting on the main source of food. Ouvea has been regarded as non-ciguatoxic by the local population. At the time of the study, only one area could be supported for anthropigenics impacts: the port located at Wadrilla.

2. Sample collection and treatmant

Micro-organisms

i. Sampling method

Gambierdiscus spp. and cyanobacterial populations were monitored on a monthly basis, following the method proposed by Chinain et al. (1999a). Briefly, around 150g of epiphytic macro-algal were collected in a plastic bag sealed underwater. After vigorous shaking to dislodge *Gambierdiscus* cells from their host-alga, the sea-water was filtered successively through 500 µm, 250 µm and 45 µm-mesh sieves. The resulting retentate was preserved in 5% formaldehyde solution

for further microscopic observations. Cyanobacteria forming colonies were visible to the naked eye; a sample from batch was directly collected and preserved in the same condition.

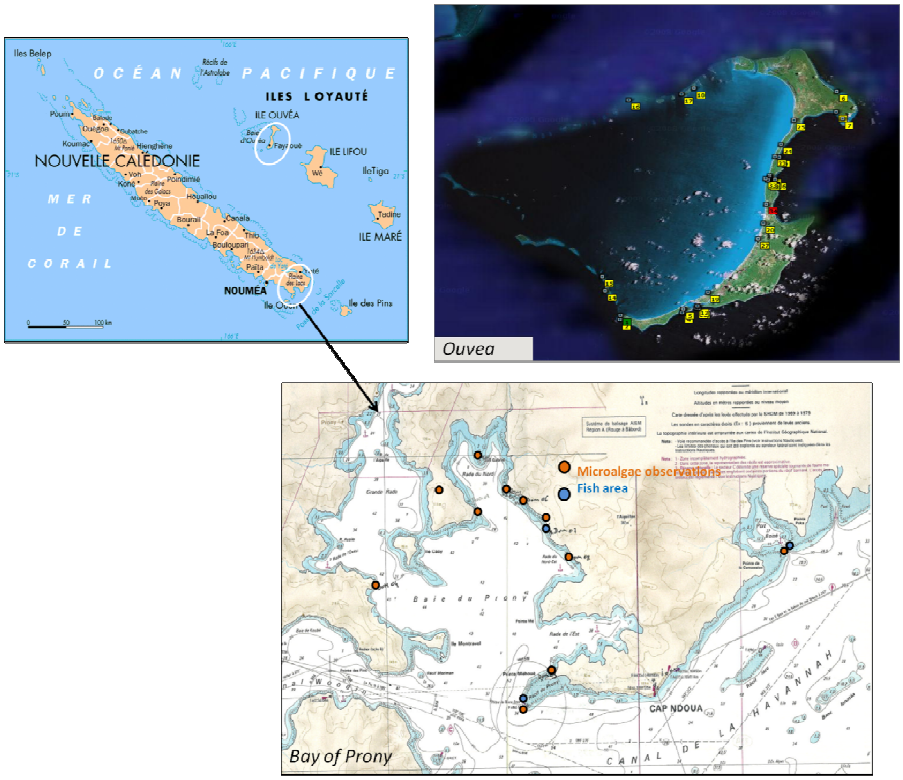

Fig.1: The collection sites of the presented survey in New Caledonia: Bay of Prony located in the south of New Caledonia and Ouvea the northern island of the Loyalty Island.

ii. Extraction method

When blooms of micro-algae or cyanobacteria were observed, materials were collected in quantity and were extracted as described by Chinain et al. (1999a). Pellets obtained from samples were extracted using solvent partition, in order to obtain lipid-soluble compounds. Briefly Freeze-dried bundles were extracted twice with methanol under ultra-sonication (1 h) and agitation overnight. The methanol extract were filtered and dried. The evaporated residue was then partitioned between aqueous methanol (60%) and diethyl ether, and the two phases were dried. The lipid-soluble extracts were tested in the mouse neuroblastoma cells assay (N2A assay).

Fish

i. Sampling method

The sampling areas were selected according to the absence or presence of potential anthropogenic disturbances (fig.1). In each sampling site, two species of herbivores (*Scarus* spp. and *Naso* spp.) versus one of carnivores (*Plectropomus* spp.) were sampled. Five individuals per genus were spear-fished, measured (total length to the nearest cm) and weighted (to the nearest

g).Their flesh (minimum 200g) was conditioned in fillets and stored at-30°C until CTX extraction and analysis.

ii. Extraction method

Samples were extracted following a method developed in Louis Malardé Institute (Papeete, French Polynesia) (Darius et al., 2008). Filleted flesh samples were mixed and homogenized. And 3 aliquots of 5 g were extracted in 7 mL of methanol under sonication durind 2 h, and let set overnight at - 4°C. After centrifugation (3000 rpm, 5'), the supernatant likely to contain CTXs, passed through a 0.45 µm Millex membrane filter (Millipore) was mixed with 3 mL of distilled water (70/30) and the resulting crude extract loaded on Sep-Pak®C18 cartridges (Watters) pre-conditioned with 7 mL aqueous methanol (70/30). The cartridge was then washed twice with 7 mL aqueous methanol (70/30). The fraction eluted with 7 mL aqueous methanol (90/10) was further dried and stored at 4°C until tested for their toxicity using the N2A assay.

3. Toxicity analysis

The Neuroblastoma (Neuro-2A) cytotoxicity assay was initially developed by Manger to detect sodium channel-enhancing marine biotoxins (Manger et al, 1993). For experiments, cells were seeded at 2.5×10^5 cells/well in 96-well plates. After a 24 h recovering-time, cells were treated with ouabain (0.5 mM) and veratridine (0.05 mM) and incubated 14 h with extracts. Each extract was tested in triplicate at 3 distinct concentrations (6.3 to 25.0 fresh flesh geqv/mL). After treatment, cell viability was assessed using the tetrazolium salt MTT method (Mosmann, 1983). The absorbance of its reduced form produced by active cells was measured at 490nm. Results were expressed as inhibitory concentration at 50% (IC_{50}).

Preliminary results and conclusions

1. Micro-organisms populations

The Prony Bay Over the past 4 years, no bloom was detected following the monthly monitoring of either *Gambierdiscus* spp or cyanobacterial populations.

Ouvea During the two missions conducted in this atoll, both during the cold season, no bloom of *Gambierdiscus* spp. was observed; although the presence of small cell densities of this dinoflagellate were noticed near the wharf of Wadrilla. Some large mat-forming filamentous benthic cyanobacteria morphologically identified as *Hydrocoleum cf. lyngbyaceum* and *Phormidium cf. laysanense* on the basis of their morphological characteristics were collected and are currently under analysis. These two benthic species of cyanobacteria were collected in Lifou Islands (Loyalty Island) in a survey carried out in this PhD project (Laurent et al, 2008). Chemical structures of CTX-like compounds are underway.

2. Ciguatoxicity in the fish food web

Analyses of potential ciguatoxicity of fishes are currently underway and will be treated in a subsequent publication.

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