



Ciguatera risk management in French Polynesia: The case study of Raivavae Island (Australes Archipelago)

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ABSTRACT

Based on epidemiological data available through long-term monitoring surveys conducted by both the Public Health Directorate and the Louis Malardé Institute, ciguatera is highly endemic in French Polynesia, most notably in Raivavae (Australes) which appears as a hot spot of ciguatera with an average incidence rate of 140 cases/10,000 population for the period 2007–2008. In order to document the ciguatera risk associated with Raivavae lagoon, algal and toxin-based field monitoring programs were conducted in this island from April 2007 to May 2008. Practically, the distribution, abundance and toxicity of *Gambierdiscus* populations, along with the toxicity levels in 160 fish distributed within 25 distinct species, were assessed in various sampling locations. Herbivores such as Scarids (parrotfish) and Acanthurids (unicornfish) were rated as high-risk species based on receptor-binding assay toxicity data. A map of the risk stratification within the Raivavae lagoon was also produced, which indicates that locations where both natural and man-made disturbances have occurred remained the most susceptible to CFP incidents. Our findings also suggest that, locally, the traditional knowledge about ciguatera may not be scientifically complete but is functionally correct. Community education resulted in self-regulating behaviour towards avoidance of high-risk fish species and fishing locations.

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1. Introduction

Ciguatera Fish Poisoning (CFP) is a complex form of human illness caused by the consumption of fish that have accumulated toxins originating from a benthic dinoflagellate, *Gambierdiscus* spp. These toxins, namely ciguatoxins (CTXs), are transformed into more potent forms as they move through the marine food web.

According to Bruslé (1997), as many as 10,000–50,000 individuals are affected annually, while Fleming et al. (1998) estimate the global number of poisonings to 50,000–500,000 per year.

Originally limited to the tropics and subtropics in a belt between latitudes 35°N and 35°S (Lewis, 1993; Quod and Turquet, 1996; Pottier et al., 2001), CFP now affects a diverse population in previously non-endemic areas owing to the expansion of travel, tourism, and modern transportation which favours importation of fish from the tropics (De Haro et al., 1997; Van Dolah, 2000). It has become the most frequently reported seafood-related

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disease in the United States (Fleming et al., 1997; Lipp and Rose, 1997), and is an increasingly serious problem in many inland locations where toxic fish may be sold, including countries in Canada and even Europe (Todd, 1992; Bentur and Spanier, 2000; Chateau-Degat, 2003; Perez-Arellano et al., 2005; Aligazaki and Nikolaidis, 2008). Overall, 5–600 cases per 10,000 people are reported annually depending on the geographic region (Lange, 1994).

Ciguatera is highly endemic throughout much of the tropics, most notably in the island nations of the Pacific and the Caribbean (for reviews, see Bruslé, 1997; Lehane and Lewis, 2000 and references therein), where it constitutes a significant health problem and a major impediment to the economy of these island highly dependent on fish for subsistence, export and tourist trade. Although there is scattered information on the true extent of the global impact of ciguatera (Bagnis et al., 1992; De Sylva, 1994; Todd, 1997; Shuval, 2000; Anderson et al., 2000), five major adverse impacts have been identified, i.e. loss of a food source, loss to fishing industries, loss to tourism, cost of public health, and cost of monitoring and management (Epstein and Rapportt, 1996). In addition to the direct effects of ciguatera on public health, fear of ciguatera often leads to reduced fishing in coral lagoons in many of these island countries (Dewailly et al., 2008) and to a corresponding increased reliance on lower quality imported food, which, in turn, may contribute to a rising prevalence of chronic diseases such as diabetes, hypertension and cardiovascular diseases in indigenous Pacific populations (Lewis and Ruff, 1993).

Amongst the factors contributing to a dramatic increase in CFP incidence within Pacific island countries (Lewis and Ruff, 1993) is the rate at which marine tropical ecosystems are modified in many of these developing nations. As a matter of fact, many CFP events have been more or less directly associated with natural as well as human-made disturbances of coral reefs, including, hurricanes, coral bleaching, dredging, blasting, construction, pollution, sewage, etc. (for a complete list, see Bruslé, 1997; Lehane and Lewis, 2000 and references therein). Global climatic changes such as ocean temperature increases were also proposed as potential influences on ciguatera incidence (Tosteson et al., 1988; Epstein et al., 1993; Hales et al., 1999; Chateau-Degat et al., 2005).

CFP literature is rich in epidemiological data from worldwide survey studies. However, in many instances, morbidity statistics are highly unreliable due to the tendency of many individuals not to report such illness and the wide geographic distribution of islands in ciguatera endemic areas. According to Tosteson (1995), generally 0.1% or less of intoxicated persons actually consult a physician in the Caribbean islands, while in the South Pacific, the incidence figures are likely to represent only 20% of actual cases (Lewis, 1986).

In French Polynesia, ciguatera is an officially notifiable disease as part of a governmental surveillance program involving 82 public health centres, supervised by the Public Health Directorate (Chateau-Degat et al., 2007). Notification of CFP cases by private physicians,

however, remains voluntarily. Aside from this governmental survey, the Louis Malardé Institute (ILM) is also conducting an ongoing epidemiological research program on ciguatera since 1965 (Bagnis et al., 1985a; Chateau-Degat et al., 2007), as well as algal and toxin-based field monitoring programs in several islands of French Polynesia since 2004 (Darius et al., 2007). Statistical analysis of CFP epidemiological data available from both sources (Public Health Directorate and ILM) for the periods 1960–1989 and 1992–2001 by Bagnis et al. (1985a, 1992) and Chateau-Degat et al. (2007), respectively, found no Polynesian archipelago completely safe from ciguatera, although the morbidity was unequally distributed between islands with the most remote archipelagos showing the highest incidence rates (Chateau-Degat et al., 2007). It is noteworthy that until 1984, the Australes were still regarded as the least affected archipelago, with an average incidence rate <30 cases per 10,000 people (Bagnis et al., 1985a). In Chateau-Degat et al. (2007) study, however, the Australes ranked second behind the Marquesas archipelago, with an annual incidence rate of almost 200 cases per 10,000 population. Interestingly, the period covered by this study also coincided with the construction of airport landing strips in three of the five islands forming this archipelago, as part of a governmental policy aiming at opening up these outlying islands for a “lasting and well-balanced development” (Malogne-Fer, 2004).

The present research was conducted in Raivavae Island (Australes) from April 2007 to May 2008 following hearsay information by the locals that they have not been consuming fish from the lagoon since 2002 due to its high risk of ciguatera, while in the same time, official data from the Public Health Directorate of French Polynesia indicated very low CFP incidence rates for this island. The main objectives of this study were to (i) reactivate the CFP reporting program in this island, for a more accurate quantification of ciguatera locally, (ii) document the distribution, abundance and toxicity of *Gambierdiscus* populations within the Raivavae lagoon, (iii) assess the ciguatera risk associated with the main fishing areas of the population, and (iv) initiate community education and outreach. Following community interviews, a variety of biological samples were collected from various locations of the Raivavae lagoon and tested for their toxicity in the laboratory. The results of this research were presented to the population and local authorities of Raivavae through restitution meetings and educational workshops in schools.

2. Materials and methods

2.1. Study area

Located in the South Pacific Ocean, French Polynesia is composed of 118 islands (among which 67 inhabited islands) scattered over a surface as large as Europe, and distributed among five archipelagos: Society, Marquesas, Tuamotu, Gambier and Australes (Fig. 1A). Raivavae, along

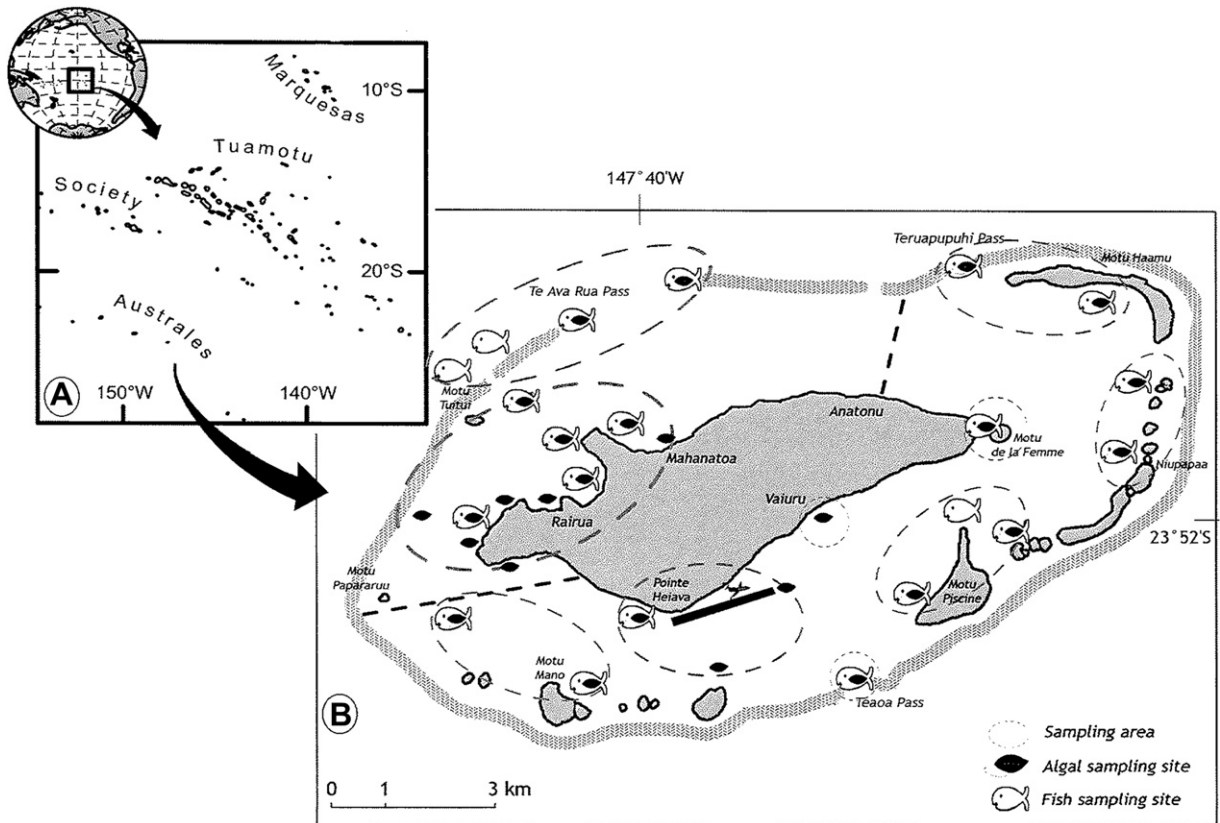


Fig. 1. Maps of French Polynesia (A) and of the island of Raivavae (Australes) (B) showing the locations of the sites sampled for this study.

with Tubuai, Rurutu, Rimatara and Rapa, are part of the Australes archipelago. Located 710 km from Tahiti, it consists of an ancient volcano with 16 km² of emerged land and a fairly large lagoon. According to the last census of 2007, Raivavae counts 905 inhabitants who rely primarily on agriculture and subsistence fishing for their resources. Raivavae also exports around 30 tons of giant clams annually to neighbouring islands (Tubuai) and Tahiti, and has had an airport since 2002 located in the south part of the island, the construction of which was started in 1999 (Fig. 1B).

2.2. CFP case recording and incidence rates

CFP data were available through the Public Health Directorate (PHD) and the Louis Malardé Institute (ILM). PHD receives monthly reports of notifiable diseases including ciguatera fish poisonings from its 82 public health centres. These health centres include 3 public hospitals, several dispensaries, medical centres and first-aid posts distributed among the five archipelagos of French Polynesia. These monthly reports consist of case counts regrouped according to age categories (Chateau-Degat et al., 2007). Concurrently, ILM has been conducting an epidemiological research program since 1965, based on the voluntary participation of public health physicians. For each reported case of ciguatera, these

physicians complete a standardized individual clinical record elaborated by ILM, very similar to the one proposed by the South Pacific Commission for the SPEHIS database (SPC, 1991). These records, which provide a detailed description of the cases according to gender, age, symptoms experienced by the patients, number of past episodes of food poisoning, type of seafood responsible for the intoxication, etc., are sent to ILM on a regular basis.

In 2006, however, the need for reactivating these long-term surveys became apparent when PHD failed to provide incidence data for most of the islands of French Polynesia. Hence, in 2007, the decision was made by both PHD and ILM to share a common database and to transfer the supervision of this survey to ILM. Since June 2007, the updated case report form presented in Appendix 1 is used by all public health structures of French Polynesia.

Crude incidence rates presented throughout this study correspond to new cases of CFP incidents per 10,000 population per year, and were calculated based on population estimates provided by the Institut National de la Statistique et des Etudes Economiques (INSEE) and Institut de la Statistique de Polynésie française (ISPF). Except for 2002 and 2007 for which official data from general census were used, for all other years, the population distribution per archipelago

was estimated to be 87.60%, 3.41%, 6.0%, 0.48% and 2.51% for the Society, Marquesas, Tuamotu, Gambier and Australes, respectively, based on data provided by INSEE and ISPF.

2.3. Community interviews

Three field missions were conducted in Raivavae, in April 2007, September 2007 and May 2008, respectively. Prior to samplings, community interviews were conducted to collect historical information on the natural and man-made disturbances undergone by the Raivavae lagoon, the different types of seafood poisoning experienced by the population, and their local knowledge and understanding of ciguatera.

Since the occurrence of toxins is known to vary according to fish species and locality, a questionnaire made by ILM, and translated in French and Tahitian languages, was also used to assist us in identifying the (i) infected reef areas versus “low risk” zones of the lagoon, and (ii) fish species most popular with the population versus those deemed not edible.

2.4. Sampling site selection and samples preparation

Ten sampling areas were selected within the lagoon of Raivavae, following community interviews (Fig. 1B). Four of these areas were on the windward side of the island (Te Ava Rua Pass (A), Rairua and Mahanatoa locality (B), Teruapupuhi Pass (C) and Motu de la Femme (D)) and the remaining six were leeward sites (Niupapaa (E), Motu Piscine (F), Teaoa Pass (G), Motu Mano (H), the Airport zone (I) and Vaiuru locality (J)). Since ciguatera is notoriously patchy even on spatial scales of a few meters, a subset of several sampling sites was screened within each of these areas (Fig. 1B).

2.4.1. Dinoflagellate samples

Turf-like agglomerated macroalgal hosts (e.g. *Halimeda* sp., *Jania* sp., *Amphiroa* sp.) were collected via snorkeling at water depths of approximately 1–5 m (depending on the sampling site), and examined for the presence of various groups of dinoflagellate (*Gambierdiscus*, *Ostreopsis*, *Prorocentrum*). Samplings took place during the hot season when populations of *Gambierdiscus* tend to reach maximum abundance (Chinain et al., 1999). Between 180 and 400 g of algal samples were picked from each sampling site, sealed within plastic bags underwater, and shaken and kneaded vigorously to dislodge dinoflagellate cells. The detrital suspension was successively filtered through 125, 40 and 20 μm -mesh sieves, and the 40 and 20 μm fractions preserved in 50 mL of 5% formalin-seawater. Cell densities were assessed microscopically from 100 μL aliquots of these two fractions, in Quick Read 10 counting chambers (Globe Scientific Inc[®]) using a field Specwell[®] microscope 35 \times . At each sampling site, values expressed in cells g^{-1} algal wet weight represented the mean number of dinoflagellate cells enumerated on n ($=1$ –3) subsamples of the same host algae, with values rounded to the nearest whole number. When densities exceeded

1000 cells g^{-1} wet wt, an additional 1 kg of macroalgae was sampled and treated as described above, for confirmatory counting back at the laboratory, using a Leica DMLB microscope. Following filtration, these wild cells were concentrated by centrifugation at 2000g for 20 min, and the resulting cell pellets stored at -20°C until further extracted for toxicity analysis. Whenever possible, the establishment of clonal cultures from single-cell isolates was also attempted, and the cultured strains screened for CTXs production.

2.4.2. Fish samples

Species most often cited by the population as either ciguatoxic or edible were preferentially targeted. As fish toxicity is likely to vary depending on age, several individuals per species were spear-fished to screen the largest range of size as possible. Each fish was measured and weighted, and its flesh conditioned in the form of fillets stored at -20°C until further CTX extraction. Fish specimens were tested individually for their toxicity.

2.4.3. Other biological samples

Following information that giant clams were possibly toxic in Raivavae, several specimens of giant clams and substantial mat-forming cyanobacterial samples found nearby these molluscs were collected in various areas of Raivavae lagoon, and subjected to toxicity analysis.

2.5. CTXs extraction

2.5.1. Gambierdiscus samples

Toxic extracts were prepared from *Gambierdiscus* cell pellets following the mass extraction procedure described in Chinain et al. (2010). Briefly, each cell pellet was extracted twice in methanol and twice in 50% aqueous methanol under sonication, for 30 min each. After the extract was evaporated, a solvent partition was applied to the resulting residue using dichloromethane and 60% aqueous methanol, according to the procedure recommended by Satake et al. (1993). The dichloromethane soluble fractions (DSFs), in which CTXs are recovered, were dried under vacuum and stored at 4°C until tested for toxicity via the receptor-binding assay.

2.5.2. Fish samples

Fish extracts intended for the receptor-binding assay were prepared following the procedure described by Darius et al. (2007). For each individual, 2×5 g of flesh was homogenized in a Stomacher 441[®] waste disposal unit for 2 min, and re-suspended in 5 mL methanol under sonication for 2 h, prior to incubation overnight. After centrifugation at 850g for 5 min, the supernatant in which CTXs are yielded was subjected to subsequent purification using Sep-Pak C₁₈ silica cartridges (Waters[®]). The resulting extract was further dried under vacuum for 3 h at 60°C in a SpeedVac concentrator, and stored at 4°C until tested for its toxicity.

2.6. Toxicity analysis and ciguatera risk stratification according to sampling areas

All extracts were analyzed for their toxicity via the receptor-binding assay, a test specifically devised to detect the presence of toxic compounds such as brevetoxins and CTXs, which display a differential affinity for the site 5 of the voltage-sensitive sodium channels (Poli et al., 1986).

2.6.1. Receptor-binding assay (RBA)

Aliquots equivalent to 5000 cells or 5 g of fish tissue were tested for their toxicity using the RBA performed in a test tube format, following the protocol previously described by Darius et al. (2007). For ease of comparison, data were expressed in Pacific CTX-3C (P-CTX-3C) equivalents, as this CTX congener is the only one consistently present along the ciguatera food chain. P-CTX-3C was used as an internal standard for sample calibration using its $IC_{50} = 0.62 \pm 0.16$ ng/ml ($r^2 = 0.9796$, Hill slope -0.84) obtained from sigmoidal dose–response (variable slope) curve, where IC_{50} is the concentration of ciguatoxic extract which causes 50% inhibition on the binding of tritiated brevetoxin. Quality control of the assay was performed by testing each receptor-binding assay with a toxic extract of *Gambierdiscus polynesiensis* with a known concentration of 3.1 pg P-CTX-3C equivalent. Briefly, eight different dilutions ranging from 6 to 2000 cells or 0.006–2 g of flesh were assayed in duplicate (limit of detection (LOD) <0.31 pg P-CTX-3C equivalents cell $^{-1}$ and <0.31 ng P-CTX-3C equivalents g $^{-1}$ for *Gambierdiscus* and fish extracts, respectively). Each toxic extract yielded a binding curve with an IC_{50} value determined using Multicalc software 2.6 (Wallac, Perkin Elmer France) and GraphPad Prism software v4. Integrated toxicity values were expressed in pg P-CTX-3C equivalents cell $^{-1}$ or ng P-CTX-3C equivalents g $^{-1}$ for *Gambierdiscus* and fish extracts, respectively. For each sample, values are the mean toxicity estimated from duplicate assays.

2.6.2. Risk rank definitions

At the individual level, each fish specimen was arbitrarily assigned a risk rank based on its RBA values: “(–)”:

RBA < 0.31 ng P-CTX-3C equivalents g $^{-1}$; “(+)”: RBA = [0.31–0.36] ng P-CTX-3C equivalents g $^{-1}$; “(++)”: RBA = [0.37–0.8] ng P-CTX-3C equivalents g $^{-1}$; “(+++)”: RBA > 0.8 ng P-CTX-3C equivalents g $^{-1}$. Practically, based on data obtained from both mouse bioassays and RBA analysis conducted on either field samples or fish remnants responsible for CFP incidents in patients, only samples with RBA values in the range (++) and (++++) are likely to cause symptoms of intoxications. For these samples, the clinically effective harmful dose to humans may also vary according to individuals, e.g. heavy versus occasional fish consumers, or individuals who have experienced previous CFP episodes, etc. Of note, all fish samples responsible for CFP incidents in consumers displayed RBA values > 1.4 ng P-CTX-3C equivalents g $^{-1}$, regardless of the number of previous intoxications experienced by the patient. At the species level, a given species group was regarded as generally safe/infrequent poisoner when at least 70% of tested specimens were rated (–) or (+) and, conversely, was regarded as a frequent poisoner/high-risk species if at least 70% of individuals were rated (++) or (+++). At the spatial level, the ciguatera risk associated with each sampling area was defined by a range from 0 to 2, where 0 means absence or low risk of ciguatera, i.e. no *Gambierdiscus* blooms were detected and $\geq 70\%$ of the fish tested in that area were rated (–) or (+), with no more than 20% of fish in the (++++) group, and 2 maximum risk, i.e. presence of toxic blooms and $\geq 70\%$ of the fish tested in that area rated (++) or (+++).

3. Results

3.1. CFP reporting program

Table 1 summarizes the crude incidence rates of ciguatera per 10,000 population per year, from 2000 to 2008, for each of the five archipelagos of French Polynesia and, more specifically in Raivavae Island (Australes). During this period, the mean incidence rate of CFP in French Polynesia as a whole was around 23 ± 6.5 cases per 10,000 population per year, although quite a large variability was observed through time according to archipelagos and even islands. Overall, the highest incidence rates reported within the timeframe of 2002–2007 were consistently observed in the Gambier

Table 1

Crude incidence rates of ciguatera per 10,000 population per year, reported from 2000 to 2008 in the five archipelagos of French Polynesia and Raivavae Island (Australes). Crude incidence rates were inferred from the total number of CFP cases reported annually by the Public Health Directorate (PHD) of French Polynesia. Data in brackets for 2004, 2005 and 2006, respectively, represent the actual number of individual clinical records sent to ILM by public health physicians on a voluntary basis. Data in bold characters are from the common CFP database shared by PHD and ILM since 2007.

Year	2000	2001	2002	2003	2004	2005	2006	2007	2008
Society	5	7	5	3	3	4	NA ^a	3	2
Marquesas	346	242	212	197	125	NA	NA	66	77
Tuamotu	108	136	250	209	165	177	NA	121	112
Gambier	497	157	304	186	790	369	NA	539	397
Australes	209	128	131	85	113	34	NA	65	70
Raivavae	497	355	464	376	233	42	NA	199	71
Total CFP cases	702	640	779	620	583 [188]	438 [180]	NA [150]	420	383
Population estimates ^b	235,100	239,300	244,830	247,300	251,000	254,600	NA	259,800	262,800

^a NA: data not available.

^b Source of data: INSEE and ISPF.

Table 2

Distribution of CFP cases reported in Raivavae by fishing area and fish species involved. Data are from the medical records filled out by patients at the Rairua medical centre in 2007–2008. Figures in parenthesis indicate the number of poisoning incidents in which each species was involved.

Sampling site	2007			2008		
	Case reports	Number of guests ^a	Species involved	Case reports	Number of guests	Species involved
N/A ^b	3	4	<i>Naso unicornis</i> (1) <i>Scarus altipinnis</i> (1) <i>Chlorurus microrhinos</i> (1)	3	3	<i>N. unicornis</i> (2) <i>Lethrinus</i> sp. (1)
Te Ava Rua Pass	1	1	<i>Scarus</i> sp.	–	–	–
Rairua	5	9	<i>N. unicornis</i> (3) <i>Scarus</i> sp. (1) N/A (1)	–	–	–
Mahanatoa	4	4	<i>Scarus</i> sp. (3) <i>N. unicornis</i> (1)	–	–	–
Teruapupuhi Pass	1	1	<i>N. unicornis</i>	1	1	<i>N. unicornis</i>
Motu Mano	–	–	–	1	2	<i>N. unicornis</i>
Airport	1	1	<i>N. unicornis</i>	–	–	–
Vaiuru	3	3	<i>N. unicornis</i> (1) <i>C. microrhinos</i> (1) <i>Ctenochaetus striatus</i> (1)	2	5	<i>N. unicornis</i> (1) <i>Albula glossodonta</i> (1)
Total	18	23		7	11	

^a Total number of persons, in addition to the reporting patient, who have shared the same toxic meal.

^b N/A: undetermined.

and the Tuamotu. On an island basis, however, Raivavae had the highest incidence of reported ciguatera in 2002, with 464 cases per 10,000 people (Table 1). From 2002 to 2007, the annual CFP case numbers notified to the medical centre of Rairua (Raivavae) progressively dropped from 46 cases to half this level in 2004, and lower thereafter (4 and 1 cases in 2005 and 2006, respectively) (data not shown). However, following the reactivation of this surveillance program in 2007, a significant increase in reported CFP cases was observed in Raivavae, with 18 cases notified to the medical centre of Rairua in 2007 and 7 cases in 2008 (data not shown). It is noteworthy that 95% of the CFP cases registered in 2007 were notified after May 2007, i.e. following our first mission in the island. From these data, two possible sources of under-reporting were identified: firstly, the comparison between ILM records and PHD reports indicated that only 32% and 41% of the CFP cases reported annually in 2004 and 2005, respectively, were actually notified to ILM by public health physicians (Table 1). In the case of Rairua medical centre, the notification rate to ILM during this same period was 23% and 50%, respectively (data not shown). Secondly, based on the discrepancy often observed between the number of reporting patients and the number of guests who have shared the same toxic meal, it was estimated that an average 30% of intoxicated persons did not present for medical care at the Rairua medical centre for the period 2007–2008 (Table 2).

Finally, analysis of the 25 medical records collected over the same period, revealed that both parrotfish (*Scarus* spp., *Chlorurus microrhinos*) and unicornfish (*Naso unicornis*) were responsible for more than 80% of the poisonings that occurred in Raivavae in 2007–2008

(Table 2). The fishing areas most frequently involved were the Rairua–Mahanatoa and Vaiuru localities (Fig. 1B), although 25% of the reporting patients could not tell the origin of the toxic fish (Table 2). However, whereas the parrotfish represented 45% of the incriminated fish in 2007, they were not mentioned in the medical records collected in 2008. Similarly, the Rairua–Mahanatoa locality, which was cited in 50% of the CFP cases of 2007, was no longer reported in 2008 medical records. It is noteworthy that at least 30% of CFP incidents took place in the south part of the lagoon, i.e. in the so-called “healthy zone” according to the population.

3.2. Community interviews

Ciguatera was widely known in the community because of its high incidence. According to the population, the first flare-ups of ciguatera seemed to go back to the late 1990s and appeared to have started in the area of the main pass of Te Ava Rua, located north of the island following a round of successive cyclones which repeatedly hit the north pass. Among the indigenous population, only one fisherman was aware that ciguatera risk could arise from anthropogenic activities such as reef blasting, whereas several persons, especially among the oldest people, frequently associated ciguatera to the nuclear test explosions in Moruroa atoll. Many interviewed people also believed they were able to detect ciguatoxic fish by using a variety of simple home tests – feeding a piece of the fish to a dog or a cat, looking for the presence of a blood line at the tail of the dead fish, observing whether a piece of the fish repels ants or flies or whether the flesh stretches while hanging, etc. Additionally, most of these informants were not aware of the utility of reporting CFP

Table 3

Chronology of the natural and man-made disturbances undergone by the Raivavae lagoon.

Disturbances of natural origin (from the Atlas Climatologique de Météo France)	
Feb. 2nd–9th, 1976	FRANCES tropical cyclone (intensity: 970 hpal; winds of 90 km/h in the Australes).
Apr. 16th–23rd, 1977	ROBERT tropical cyclone (intensity: 955 hpal)
March 9th–13th, 1981	TAHMAR tropical cyclone (intensity: 990 hpal; winds of 100 km/h in the Australes)
Feb. 28th–March 3rd, 1988	CILLA tropical depression (intensity: 985 hpal; winds of 150 km/h in the Australes)
Dec. 5th–13th, 1991	WASA tropical cyclone (intensity: 955 hpal; winds of 148 km/h in the Australes))
Oct. 30th–Nov. 4th, 1997	Tail of MARTIN tropical cyclone (intensity: 955 hpal)
Nov. 21st–27th, 1997	Tail of OSEA tropical cyclone (intensity: 954 hpal)
Nov. 2003	Tail of tropical cyclone. Serious damages in Anatonu village
Disturbances of anthropogenic origin	
–	The island's first lime kilns used sheets of coral gathered from the edge of the lagoon
1967–1968	Excavations of coral slush in the area of the Motu de la Femme, for the building and upkeep of the island main road
Around 1967	Dynamite usage for the first time to enlarge the Te Ava Rua Pass in order to facilitate entry of construction material necessary for the Centre d'Experimentation du Pacifique (CEP) to build an observatoire for the survey of potential fall-outs of nuclear test explosions in Moruroa atoll
1982–1983	Embanking of the lagoon for the building of Raivavae town hall and extension of the old sports hall (carried out with rocks and soil, without the use of coral slush)
1987–1989	Second embanking of the lagoon to accommodate the current sports ground facility, and concreting works in order to build the Rairua landing quay
1989–1990	More dynamite usage for the Te Ava Rua Pass to maintain the current access of boats
1993	More dynamite usage on the north pass, but of which there is no official record
In the late 1990s	Dredging work in front of the village of Vaiuru for the setting up of a small embarkation launching channel, followed a few months later by fish poisoning incidents in the area of the fringing reef located in front of Vaiuru
1999–2002	Construction of an airport landing strip in the southern part of the island
2003–2005	Dredging work for the construction of a marina between the landing strip and the coast. Works were stopped at the end of 2005

poisonings to public health agents, and several of them mentioned the use of traditional remedies to treat ciguatera, which seems to be a common practice within the Raivavae community.

Data collated from anecdotal information from the population also provided us with the list and chronology of the incidents of significant environmental disturbances of both natural and anthropogenic origins undergone by the Raivavae lagoon, over the past decades. Table 3 summarizes this information.

According to the population, all infected sites were located in the north part of the lagoon, within an area comprised between Motu Pappararu and the village of Anatonu, as illustrated in Fig. 1B. Conversely, the south part of the lagoon, including the area where the airport landing strip is located, was regarded as safe by the population and, in fact, harboured three of the main fishing areas of the island, i.e. the Teruapupuhi Pass located north east of the island, the Motu Mano area where giant clams for the export are preferentially collected by the locals, and the Motu Piscine area currently used by pensions on the island for daily picnic tours during which giant clams and fish are often served to tourists.

Information provided by fishermen identified the filament-finned parrotfish “roro” and “haumeretue” (*Scarus altipinnis* ♀ and ♂), the steephead parrotfish “pae’e” (*C. microrhinos*), the brown unicornfish “ume” (*Naso unicornis*), the longnose emperor “oéo” (*Lethrinus olivaceus*) and the marbled grouper “hapuu” (*Epinephelus polyphkadion*) as the main species causing fish

poisonings among the local community. Other fish species also regarded as frequent poisoners by the population included the ember parrotfish “uhu mamaria” (*Scarus rubroviolaceus*) and the longnose parrotfish “rotea” (*Hipposcarus longiceps*). Surprisingly, carnivores were rarely mentioned in this list. Conversely, the following fish species were deemed edible by the locals: the highfin chub “nanue” (*Kyphosus cinerascens*), the dot-dash goatfish “ahuru” (*Parupeneus barberinus*), the clown coris “haupa” (*Coris aygula*) and the Tuamotu emperor “tamure” (*Lethrinus atkinsoni*). Overall, the fish species most popular with the population belonged predominantly to herbivores.

Finally, our attention was also called to an area located in front of the medical centre of Rairua known for its toxic giant clams. Underwater explorations revealed the presence of large brown cyanobacterial mats – further identified as *Oscillatoria bonnemaisonii* – in the surrounding of these potentially toxic giant clams. Substantial samples of these molluscs and associated benthic cyanobacterial mats were collected and are now currently analyzed for their toxicity in the laboratory. Preliminary results are in favour of the existence of a new eco-toxicological phenomenon in Raivavae related to the development of marine benthic cyanobacterial blooms and subsequent poisonings through the consumption of contaminated giant clams (Laurent, personal communication). As these results are out of the scope of this study, the outcomes of this research will be detailed further in another paper.

Table 4
Distribution and abundance of dinoflagellates in Raivavae lagoon.

Area	Site	Host algae	Gd ^a	OI ^a	Ov ^a	Pc ^a	
Te Ava Rua (A)	A-1	<i>Halimeda</i>	0	0	2	1	
	A-1	<i>Caulerpa</i>	0	0	0	0	
	A-2	Miscellaneous	0	0	0	0	
Rairua–Mahanatoa (B)	B-1	<i>Halimeda</i>	0	0	0	2	
	B-2	<i>Halimeda</i>	0	0	0	0	
	B-2	<i>Amphiroa</i>	0	11	0	0	
	B-3	<i>Halimeda</i>	0	0	0	0	
	B-4	<i>Halimeda</i>	0	0	0	0	
	B-5	<i>Halimeda</i>	1	0	0	0	
	B-6	<i>Jania</i>	0	0	0	0	
	B-6	<i>Amphiroa</i>	0	0	0	0	
	B-7	<i>Halimeda</i>	0	0	0	0	
	B-8	<i>Halimeda</i>	0	0	0	0	
	B-9	<i>Halimeda</i>	0	0	0	0	
Teruapupuhi (C)	C-1	<i>Halimeda</i>	0	0	0	0	
	C-1	<i>Amphiroa</i>	0	0	0	0	
	C-2	<i>Halimeda</i>	1	0	0	0	
	Motu de la Femme (D)	D	<i>Amphiroa</i> (April 2007)	11	0	4	0
		D	<i>Halimeda</i> (April 2007)	1023	0	0	0
D		<i>Jania</i> (April 2007)	1080	0	0	0	
D		<i>Jania</i> (May 2008)	141,890	0	0	0	
Niupapaa (E)	E-1	<i>Halimeda</i>	0	0	0	0	
	E-2	<i>Halimeda</i>	0	0	0	0	
Motu Piscine (F)	F-1	<i>Halimeda</i>	0	0	0	0	
	F-2	<i>Halimeda</i>	0	0	0	0	
Teaoa (G)	G	<i>Halimeda</i>	1	0	0	1	
Motu Mano (H)	H-1	<i>Halimeda</i>	0	0	1	7	
	H-2	Green algae	2	0	0	0	
	H-3	<i>Halimeda</i>	0	0	0	0	
Airport (I)	I-1	<i>Jania</i>	0	0	0	0	
	I-1	<i>Halimeda</i>	0	0	0	0	
	I-1	<i>Turbinaria</i>	0	0	0	0	
	I-2	<i>Amphiroa</i>	0	0	3	90	
	I-2	<i>Halimeda</i>	0	0	1	2	
	I-3	<i>Halimeda</i>	0	0	0	0	
	I-3	Red calcareous algae	0	0	4	6	
Vaiuru (J)	J	<i>Amphiroa</i>	0	0	0	0	
	J	<i>Halimeda</i>	0	0	0	0	
	J	Brown algae 1	0	0	0	0	
	J	Brown algae 2	0	0	0	0	
	J	Green algae 1	0	0	0	0	
J	Green algae 2	0	0	0	0		

^a Cell densities, expressed in cell g⁻¹ wet wt host algae: Gd = *Gambierdiscus* sp.; OI = *Ostreopsis lenticularis*; Ov = *Ostreopsis ovata*; Pc = *Prorocentrum* sp.

3.3. *Gambierdiscus* distribution, abundance and toxicity within Raivavae lagoon

Forty-four host-macroalgal samples were collected from 27 distinct sampling sites (Fig. 1B), and examined for the presence of *Gambierdiscus*, *Ostreopsis* (*O. lenticularis*, *O. ovata*) and *Prorocentrum* (Anderson and Lobel, 1987), three groups of dinoflagellates often associated with ciguatera. Most of the host algae collected were predominantly chlorophytes (*Halimeda*, $n = 25$) and rhodophytes (*Amphiroa*, $n = 6$; *Jania*, $n = 4$) (Table 4). The most commonly occurring dinoflagellate was, by far,

Gambierdiscus for which a maximum density of 173,000 cells g⁻¹ wet wt was even reported in May 2008. The most infected area in the island was the Motu de la Femme, where *Jania* and *Halimeda*, the most preferred host of *Gambierdiscus*, were found to harbour 1080–141,490 cells g⁻¹ wet wt and 1023 cell g⁻¹ wet wt, respectively (Table 4). *Gambierdiscus* was also predominantly found in Rairua–Mahanatoa locality, but at much lower densities (by 1–3 order of magnitude, as compared to Motu de la Femme). In addition, *Prorocentrum* was more prevalent in the area of the airport landing strip, while *Ostreopsis* was scarcely observed in the Raivavae lagoon.

Table 5

List and geographic origin of fish species sampled in Raivavae lagoon, size ranges and number of individuals tested per species, and results of toxicity analysis via RBA as compared to local knowledge.

Fish species	Common name	Local name	Sampling areas ^a	N	Size range ^b	Percentage of fish of each category				Local knowledge ^c	
						(–)	(+)	(++)	(+++)	Risky	Edible
<i>Scarus altipinnis</i> [1] ^d	Filament-finned parrotfish	haumeretue (♂), roro (♀)	A, B, C, E, F, G	22	(29–58)	18	–	18	64	✓	
<i>Chlorurus microrhinos</i> [1]	Steephead parrotfish	pae'e	A, B, E, F, H	19	(27–67)	74	11	5	11	✓	
<i>Chlorurus frontalis</i> [1]	Tan-faced parrotfish	oniho au	A, D	11	(34–52)	45	–	45	10		
<i>Hipposcarus longiceps</i> [1]	Longnose parrotfish	rotea	A, B, D	3	(38–48)	–	–	–	100	✓	
<i>Scarus schlegeli</i> [1]	Schlegel's parrotfish	oti moana	B, C, D, E, G	6	(35–44)	65	–	–	35		
<i>Scarus psittacus</i> [1]	Palenose parrotfish	pahoro	C, E	3	(32–34)	65	–	–	35		
<i>Kyphosus cinerascens</i> [1]	Highfin chub	nanue	A, B, D, H	21	(24–48)	48	5	29	19		✓
<i>Ctenochaetus striatus</i> [1]	Surgeon fish	maito	A, C	20	(18–29)	40	10	15	35		
<i>Naso unicornis</i> [1]	Brown unicornfish	ume	B, C, E, F, G, H	11	(21–57)	–	–	–	100	✓	
<i>Naso lituratus</i> [1]	Orangespine unicornfish	ume tarei	E	1	21	–	–	–	100		
<i>Siganus argenteus</i> [2]	Silvery rabbitfish	morava	D, E	3	(16–29)	35	–	65	–		
<i>Parupeneus barberinus</i> [3]	Dot-dash goatfish	ahuru	D, E, F	7	(29–38)	43	–	14	43		✓
<i>Plectropomus leopardus</i> [3]	Leopard coralgroup	tonu	C, F, H	6	(30–110)	85	–	15	–		
<i>Cephalopholis argus</i> [3]	Blue-spotted grouper	roi	B, F	2	(30–51)	50	–	–	50		
<i>Epinephelus polyphekadion</i> [3]	Marbled grouper	hapuu	B	1	60	–	–	–	100	✓	
<i>Epinephelus tauvina</i> [3]	Greasy grouper	faroa	A	1	82	–	–	–	100		
<i>Lethrinus atkinsoni</i> [3]	Tuamotu emperor	tamure	A, F	4	(31–52)	25	25	25	25		✓
<i>Lethrinus olivaceus</i> [3]	Longnose emperor	o'eo	B	1	37	–	–	–	100	✓	
<i>Gnathodentex aureolineatus</i> [3]	Goldline emperor	tara ihi	I	2	(23–24)	50	–	–	50		
<i>Monotaxis grandoculis</i> [3]	Bigeye emperor	mu	A	1	38	–	–	–	100		
<i>Lutjanus fulvus</i> [3]	Yellow-margined snapper	to'au	E, F	2	(24–36)	50	–	–	50		
<i>Caranx melampygus</i> [3]	Bluefin jack	naenae	H, I	2	(40–43)	–	–	100	–		
<i>Coris aygula</i> [3]	Clown coris	haupa	A, B, C, E	6	(39–63)	70	15	–	15		✓
<i>Cheilinus chlorourus</i> [3]	Maori wrasse	papae	E, F	2	(28–34)	–	–	–	100		
<i>Sargocentron spiniferum</i> [3]	Sabre squirrelfish	apa'i	E, F	3	(27–34)	–	–	–	100		

^a For a complete definition of the sampling areas code, see Section 2.^b Size range of fish individuals tested per species (minimum–maximum values, expressed in cm).^c Information acquired from the local population and ILM questionnaire during community interviews.^d Number in brackets represents the feeding type: [1]: mainly plants/detritus; [2]: plants/detritus + animals; [3]: mainly animals (from Fish Base).

Table 6

Ciguatera risk stratification within the Raivavae lagoon as inferred from toxicity analysis conducted on 160 fish specimens using the receptor-binding assay (RBA).

Sampling area	N ^a	Percentage of fish specimens of each category ^b				RBA values			Ciguatera risk ^d	Local knowledge ^e	
		(-)	(+)	(++)	(+++)	Min.	Max.	Species ^c		Toxic	Safe
Te Ava Rua Pass (A)	46	15	4	33	48	<0.31	5.58	<i>S. altipinnis</i>	2	✓	
Rairua–Mahanatoa locality (B)	21	5	28	5	62	<0.31	3.65	<i>C. aygula</i>	2	✓	
Teruapupuhi Pass (C)	18	78	–	5	17	<0.31	3.30	<i>N. unicornis</i>	0		✓
Motu de la Femme (D)	13	46	–	23	31	<0.31	1.91	<i>S. schegeli</i>	1		✓
Niupapaa (E)	24	50	–	12	38	<0.31	4.67	<i>N. unicornis</i>	1		✓
Motu Piscine (F)	20	45	15	10	30	<0.31	2.84	<i>S. spiniferum</i>	1		✓
Teaoa Pass (G)	4	25	–	–	75	<0.31	3.74	<i>N. unicornis</i>	2		✓
Motu Mano (H)	11	63	9	18	10	<0.31	2.09	<i>N. unicornis</i>	0		✓
Airport zone (I)	3	33	–	33	33	<0.31	0.93	<i>G. aureolineatus</i>	2		✓
Vaiuru locality (J)	–	–	–	–	–	–	–	–	–		✓

^a Total number of fish specimens sampled and tested in a given area.

^b Risk rank code for fish specimens: (-): RBA < 0.31; (+): RBA = [0.31–0.36]; (++) : RBA = [0.37–0.8]; (+++) : RBA > 0.8. RBA values are expressed in ng P-CTX-3C equivalent g⁻¹.

^c Fish species which has displayed the highest RBA value in a given area.

^d Risk range code for sampling area: 0: non-toxic or low risk zone; 1: medium-risk zone; 2: high-risk zone (for a complete definition, see Section 2).

^e Information acquired from the local population and ILM questionnaire during community interviews.

The three *Gambierdiscus* blooms detected at the Motu de la Femme were further analyzed for their (cigua)toxicity. DSF extracts of blooms harvested from *Jania* and *Halimeda* host alga displayed RBA values of 4.1 and 5.0 pg P-CTX-3C equivalent cell⁻¹, respectively.

Additionally, 11 clonal cultures of *Gambierdiscus* could be established in the laboratory from single-cell isolates collected in May 2008 from the Motu de la Femme. The taxonomic identification of these isolates and toxicity screening are currently underway, and so far, only one

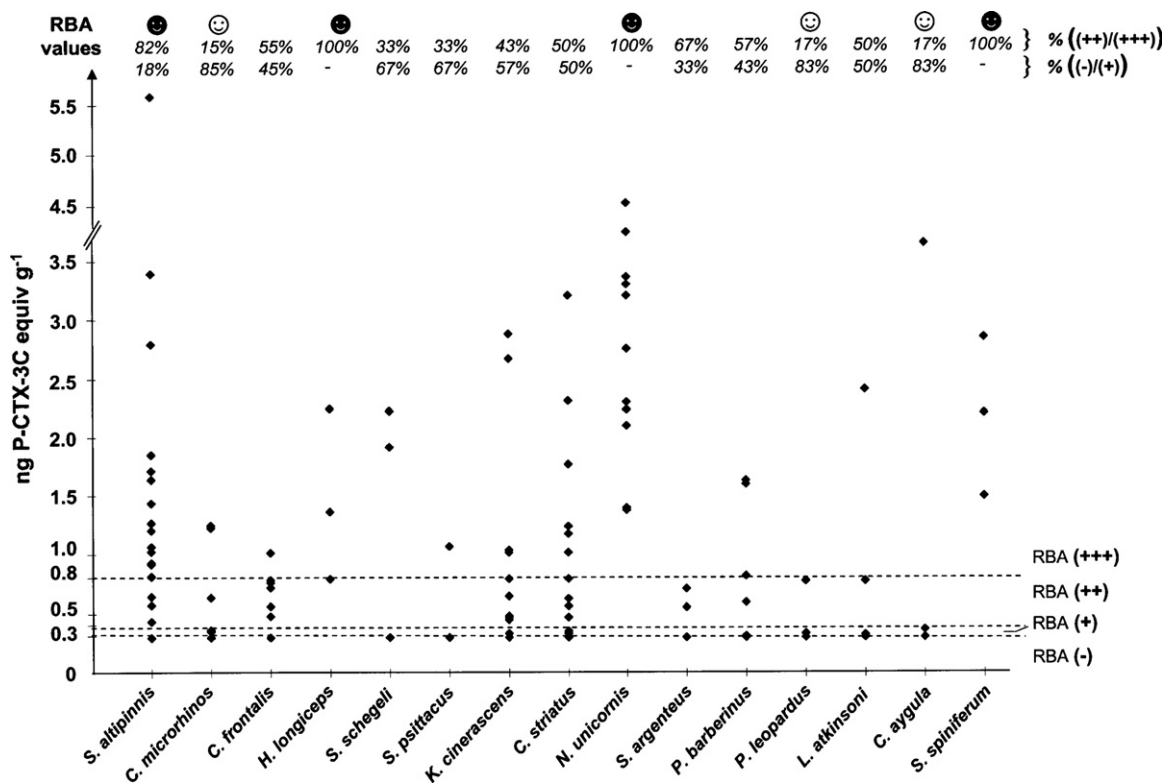


Fig. 2. Fish species sampled in Raivavae lagoon and variations in toxicity levels among specimens, as assessed by receptor-binding assays (RBAs). Only species groups for which $n \geq 3$ specimens were tested, are illustrated. For more details about the size (N) of each species group, see Table 5. (☺) corresponds to the fish species deemed edible by the population. (☹) indicates species regarded as high-risk species by the population. %((-)/(+) and %((+)/(+++)) correspond to the percentages of fish specimens rated (-) to (+), and (++) to (+++), respectively, within each of these species. For a complete definition of these risk categories, see Section 2.

clone (RAI-1), tentatively identified as a *G. polynesiensis* isolate, proved exceptionally toxic *in vitro* (Chinain et al., 2010).

3.4. Fish toxicity status and ciguatera risk stratification in Raivavae lagoon

A total of 160 specimens distributed among 25 distinct fish species were collected in Raivavae lagoon, mostly at the same sites as the algal samples, and tested for their toxicity via RBA (Fig. 1B and Table 5). Herbivores represented 73% ($n = 117$) of these specimens versus 25% ($n = 40$) of carnivores. For several of these species, however, the total number of individuals actually sampled was too small ($n < 3$) to be of real significance in subsequent analysis of toxicity data. This same remark also applies to 2 of the 9 fishing areas screened in this study, namely Teaoa Pass and the airport zone (Table 6). Fig. 2 presents the respective percentages of individuals per species that were rated (–) to (+) versus (++) to (+++), based on RBA values. The highest RBA values were 5.58 and 4.67 ng P-CTX-3C equivalent g^{-1} , and were reported in *S. altipinnis* and *N. unicornis*, respectively. Interestingly, for most of the herbivores, smaller-size specimens often displayed higher RBA values than their bigger congeners, most notably in the parrotfish, the surgeonfish and the highfin chubs (results not shown). The fish species most prone to CFP in the Raivavae lagoon were the brown unicornfish *N. unicornis* for which all 11 specimens tested were found highly toxic, the filament-finned parrotfish *S. altipinnis* ($n = 22$), and to a lesser extent, the longnose parrotfish *H. longiceps* ($n = 3$) and the sabre squirrelfish *Sargocentron spiniferum* ($n = 3$). Among the species rated as safe/infrequent poisoners, two belonged to carnivores: the blacksaddle coral grouper *Plectropomus leopardus* ($n = 6$) and the clown coris *C. aygula* ($n = 6$), along with the steephead parrotfish *C. microrhinos* ($n = 19$).

Table 6 indicates the overall distribution of the 160 specimens analyzed in Raivavae per sampling area and per risk category (as previously defined in Section 2). Except in Teaoa Pass (G), the airport zone (I) and Vaiuru locality (J), substantial numbers of specimens could be collected from all other areas. From these data, the proposed ciguatera risk stratification within the Raivavae lagoon was the following: Te Ava Rua Pass and the Rairua–Mahanatoa locality remained the most susceptible areas to CFP incidents (risk 2) with 81% and 67% of fish rated as frequent poisoners/high-risk specimen, respectively, as opposed to Teruapupuhi Pass and Motu Mano which appeared as low risk zones (risk 0) with 78% and 72% of fish rated as safe/infrequent poisoners, respectively, and no more than 20% in the (+++) range. Of note, all *N. unicornis* individuals were found consistently toxic whatever their sampling area. This species also displayed the highest RBA values in 4 of the 9 sampling areas concerned by this study (results not shown).

Since most of our samplings took place preferentially during the hot season when populations of *Gambierdiscus*

tend to reach maximum abundance, the seasonality of ciguatera risk was not addressed in this study.

3.5. Community education and outreach

The objectives and outcomes of this research were presented to the population and local authorities of Raivavae through several restitution meetings conducted in April 2007 and September 2007 in Rairua and Anatonu villages. On these occasions, several recommendations were made with regard to a more sustained effort in reporting CFP incidents to public health facilities, and the avoidance of specific species and fishing areas, to minimize the risk of ciguatera. In particular, the population was advised to avoid eating two fish species, the “roro” or “haumeretue” and the “ume”, and to avoid consuming fish from the Te Ava Rua and Rairua–Mahanatoa fishing areas. Specific recommendations as to where to collect giant clams intended for local trade were also given. In addition, posters and brochures about ciguatera were made available in the waiting room of the Rairua Health centre. Finally, educational modules stressing on the relations between a poor-quality environment and ciguatera, and targeted at the youth of Mahanatoa Primary School, were also proposed during each mission.

4. Discussion

This study conducted in Raivavae is a good example of integrated research that could be widely generalized in ciguatera endemic areas. Indeed, it has allowed the collation of a multiple set of data regarding the epidemiological, eco-toxicological and sociological aspects of this disease, which need to be considered while managing the ciguatera risk in a given area.

4.1. CFP reporting program

Despite the extensive long-term survey program conducted by both ILM and the PHD since the late 1960s, it is likely that the true incidence of CFP in French Polynesia far exceeds the numbers reported (Bagnis et al., 1985a, 1992; Chateau-Degat et al., 2007). The significant differences frequently observed in data derived from community household surveys versus health department and medical facilities records are indicative of the considerable bias actually affecting such surveys (Bagnis, 1969; Morris et al., 1982; Lawrence et al., 1992). Still, analysis of 2007–2008 data indicates that CFP incidence in the Tuamotu–Gambier and the Australes archipelagos remains extremely high, as compared to other islands of the Pacific (Bruslé, 1997; Lehane and Lewis, 2000). In particular, Raivavae (Australes) may be regarded as a hot spot of ciguatera in French Polynesia, with an average 140 cases per 10,000 population for the same period, even though the true annual incidence of this disease may be five times higher in this island, based on information collated from medical records and comparison of PHD and ILM incidence figures. In Raivavae, as in many remote islands of French Polynesia, at least two causes

of under-reporting could be identified: (i) the workload of public physicians, and their interest for this survey programme that vary from one health centre to another, which have a direct impact on their notification rate to ILM, and (ii) the frequent resort to homemade traditional remedies by local residents who, therefore do not feel the need to seek for medical care from public health facilities until their state really requires medical assistance. This suggests that most of the cases that are actually reported correspond to the most severe ones, with mild cases often going non- or under-reported (Chateau-Degat et al., 2007). The fact that incidence figures in Raivavae are likely to represent only 20% of actual cases is in accordance with previous reports by Lewis (1986) for the South Pacific.

Comparison of Raivavae incidence figures from 2000 to 2008 has revealed an increase of almost fivefold in reported CFP cases between 2005 and 2007, followed by a rapid decrease to half the level of 2007 the following year. As previously suggested by Bruslé (1997), it is likely that this increase is more attributable to an improvement in data collection following the reactivation of this survey program rather than a rise in the occurrence of ciguatera. Indeed, an increased awareness by the local population of the importance of reporting CFP illness, combined with a more active participation in case notification by the medical staff of Rairua health centre was among some of the most important products of our research in this island. Conversely, the decrease observed in incidence figures as early as 2008 might be the result of the prevention actions conducted in 2007 within the local population with regard to fish avoidance and selection of specific fishing areas. Observation that the Rairua–Mahanatoa fishing area and parrotfish, both presented as unsuitable for the subsistence fishing in the island, no longer figured in 2008 medical records tends to support this statement.

4.2. *Gambierdiscus* distribution, abundance and toxicity

Most of the 27 sampling sites that were monitored for the presence of toxigenic benthic dinoflagellates showed either absence or low cell counts, except for the Motu de la Femme where exceptionally high densities of *Gambierdiscus* were reported (at least on one occasion). In this regard, the maximum *Gambierdiscus* abundance recorded in this study ($\cong 173,000$ cells g^{-1} wet wt host alga) would be in the top densities ever recorded as summarized by Cruz-Rivera and Villareal (2006). On average, densities of *Gambierdiscus* encountered in 2007 in the most infected area of the lagoon were in the same range as values previously reported in other parts of French Polynesia (Bagnis et al., 1985b; Chinain et al., 1999), but higher than what was currently observed in the Carribean (Belize: ~ 450 cells g^{-1} , Morton and Faust, 1997; West Indies: approximately 100–300 cells g^{-1} , Ballantine et al., 1985), Australia (~ 140 cells g^{-1} ; Gillespie et al., 1985), Hawaii (~ 127 cells g^{-1} ; Parsons and Preskitt, 2007) or the Indian Ocean (~ 120 cells g^{-1} ; Quod and Turquet, 1996). The peak cell densities

observed in May 2008 were more in the range of what was reported in 1974 by Bagnis et al. (1985b) and Yasumoto et al. (1980) in the Gambier ($\sim 400,000$ cells g^{-1}) which so far remains the highest densities ever observed at a global scale. This suggests that *Gambierdiscus* may indeed be regarded as the primary producer of the CTXs that further accumulate in the piscivorous food web of Raivavae lagoon. Coherent with this hypothesis are the levels of toxicity evidenced in these wild *Gambierdiscus* ($\cong 5.0$ pg P-CTX-3C equivalent cell $^{-1}$ or a corresponding lethality in mice of 2.5×10^{-5} MU cell $^{-1}$), very similar to the ones currently monitored in the Society and Marquesas archipelagos (Chinain et al., 1999; Darius et al., 2007).

Our observations in the area of Motu de la Femme also confirmed the highly patchy distribution of this dinoflagellate (Ballantine et al., 1985), even on spatial scales of a few meters; as proof, the high variability observed in cell counts among *Amphiroa* versus *Jania* and *Halimeda* samples, collected close to one another in April 2007.

Many studies have examined the relationship between *Gambierdiscus* and its algal hosts (for a review, see Cruz-Rivera and Villareal, 2006) and suggestions of host selectivity based on form and structure (Parsons and Preskitt, 2007), or biochemical factors (e.g. stimulatory exudates) have been largely evoked (Bomber et al., 1989; Morton and Faust, 1997). Nevertheless, an understanding of the reasons for these host preferences is far from complete. In Raivavae lagoon, *Gambierdiscus* was found to exhibit an apparent preference for *Jania* and *Halimeda*, in decreasing order, which is in accordance with previous findings by Yasumoto et al. (1979), Bomber et al. (1988) and Chinain et al. (1999), but this algal host preference of *Gambierdiscus* may also vary according to islands as seen in Darius et al. (2007).

Usually, identifying local areas with a high density of ciguatoxic organisms is of some help in reducing the risk of ciguatera. In this regard, the prevalence of *Gambierdiscus* as well as the levels of toxin contents evidenced in both wild and cultured cells isolated from the Motu de la Femme (see Chinain et al., 2010) showed a good correlation with the risk range further associated with this area based on RBA values in fish. However, in this study, it was not possible to substantiate a clear relationship between the prevalence of toxic herbivorous fish and the dynamics of *Gambierdiscus* populations in the remaining sampling areas. This was particularly obvious in the areas of Te Ava Rua Pass and Rairua–Mahanatoa locality where highly toxic fish were prevalent, whereas very low densities of *Gambierdiscus* were observed. To explain this apparent discrepancy, Bagnis et al. (1985b) have shown that the presence of toxic herbivorous fish in a given area may be the result of the presence, 6–12 months before, of *Gambierdiscus* blooms in the surrounding reef ecosystem. More recently, Laurent et al. (2008) have also emphasized the potential contribution of benthic cyanobacteria of the Oscillatoriales group in the toxic reservoir accumulated in herbivores living nearby large mats of these cyanobacteria.

Alternatively, Anderson and Lobel (1987) and Cruz-Rivera and Villareal (2006) argued that the persistence of macroalgae with (high) densities of epiphytic *Gambierdiscus* may only be an indication of what is *not* being eaten by herbivores. All these observations attest to the high degree of complexity in the routes by which ciguatoxins enter food webs.

4.3. Fish toxicity and risk stratification within Raivavae lagoon

The ciguatera risk in Raivavae lagoon was assessed based on the toxicity levels of 160 fish specimens collected from various sampling areas. Among the variety of techniques that have been established to detect and quantify CTXs in fish, the receptor-binding assay was selected for this study as it has proven to be a very valuable, suitable and sensitive tool in similar monitoring programs conducted in other ciguatoxic sites of French Polynesia (Darius et al., 2007). Herbivores represented >70% of the fish that were sampled and tested, suggesting a higher local availability of this trophic group in Raivavae lagoon, both in terms of species and individuals, as compared to data from similar risk assessment campaign conducted in Fakarava island, in the Tuamotu archipelago (data unpublished). This seems to be a common characteristic of the Australes lagoons, as the same observation also applies to Tubuai, the neighbouring island (Darius et al., 2007). Based on RBA data, we found that fish species most prone to CFP in Raivavae belonged predominantly to herbivorous Acanthurids (*N. unicornis*) and corallivorous Scarids (*S. altipinnis* and *H. longiceps*), whereas two out of the three species rated as safe/infrequent poisoners belonged to carnivores (*P. leopardus* and *C. aygula*). Furthermore, in toxic species, no clear relationship was observed between RBA values and the size and weight of the individuals tested, as previously emphasized by Darius et al. (2007) in other ciguatoxic sites of French Polynesia. Our findings support previous statement that surgeonfish and parrotfish are considered key vectors of ciguatera in the Pacific (Bruslé, 1997), but are in marked contrast with observations by Pottier et al. (2001) that herbivores are not normally associated with ciguatera in the Carribean. These results also seem to contradict the popular notions that (i) predatory (carnivores) fish are most likely to be affected by ciguatera, and (ii) the larger, the greater the risk (Tosteson et al., 1988; Pottier et al., 2001).

From our data and those of Darius et al. (2007), it appears that *S. altipinnis* is consistently rated as a high-risk species in the Australes, with >80% of the samples testing positive for RBA and individuals displaying the highest RBA values both in Raivavae and Tubuai. There is general agreement on the fact that CTXs are not metabolized and stored identically in each species (Tosteson et al., 1988). Differences in digestive strategies and feeding habits among herbivores may help explain why certain species are found consistently more toxic than others. For instance, herbivorous fish are generally classed as “grazers” or “browsers” (Jones, 1968), with the grazers being most frequently implicated in ciguatera poisonings and

browsers being of lower risk (Anderson and Lobel, 1987). In the present study, both *S. altipinnis* and *N. unicornis*, which were rated as highly toxic species in Raivavae, actually belonged to grazers.

Our findings were generally congruent with the local knowledge of the population regarding risky species and areas. In contrast, most of the species regarded as generally edible by the population, with the exception of *C. aygula*, displayed varying levels of toxicity from non-toxic (–) to (+), (++) or highly toxic (+++) specimens within the same species group. For example, *K. cinerascens* ($n = 21$) gave 10, 2, 5, and 4 specimens, respectively, in each category. Differences in the feeding history of each fish contribute to the variations observed in toxin content within a same species, and can be explained if blooms of ciguatoxin-producing *Gambierdiscus* are highly patchy within the Raivavae lagoon. The fact that 90% of the *Ctenochaetus striatus* tested were toxic in Te Ava Rua Pass (rated as a toxic zone) whereas 90% of the same fish were found non-toxic in Teruapupuhi Pass (rated as a non-toxic to low risk area) is consistent with this hypothesis.

Similarly, many of the areas generally regarded as safe by the population were found to actually harbour a non-negligible number of fish sporadically toxic, most notably in Motu de la Femme and the airport areas, where bio-ecological indicators such as signs of coral distress, presence of dinoflagellate populations, toxicity levels in fish and dinoflagellate samples, etc., are cause of concern, not to mention the presence of large mats of potentially toxic cyanobacteria in Motu de la Femme (Laurent, personal communication). It is important to note that these two locations were also areas where past and/or recent environmental disturbances have occurred.

4.4. Natural and man-made disturbances in Raivavae

As previously observed in the neighbouring island of Tubuai by Darius et al. (2007), the area with highest CFP risk in Raivavae was found on the windward side of the island where hurricane or tropical depression damages to reef systems were the greatest. Indeed, according to the population, CFP outbreaks were first experienced in Te Ava Rua Pass, following a round of successive tropical depressions which repeatedly hit the north parts of both Tubuai and Raivavae Islands. Recent data from Tubuai, however, suggest that the north pass of Te Ava Moana, formerly a highly toxic site, is now evolving towards a non-ciguatoxic status (Darius et al., 2007), while in Raivavae, the continuous damages to coral reef ecosystems that persistently occurred in the north pass of Te Ava Rua and Rairua locality may have contributed to the emergence of chronic ciguatera poisonings in these areas. However, data from scientific baseline studies or long-term monitoring programs are lacking to substantiate the relationship between these anthropogenic activities and fish poisoning incidents, locally.

Numerous reports have emphasized the role of transitory ecological disturbances on increased risk of

ciguatera (for a review, see Bruslé, 1997 and references therein). According to Bagnis et al. (1985b), the resulting CFP outbreaks may range from one year to more than 15 years. All these observations support the idea that ciguatera is indeed a sensitive indicator of environmental disturbances in tropical marine ecosystems, as previously suggested by Hales et al. (1999). Given the rate at which these systems are modified in developing islands of French Polynesia, and the importance of seafood resources for these island communities, there is an urgent need to increase our capability for the prediction of CFP risk and the prevention of human illness in these areas.

4.5. Local knowledge and management of ciguatera

CFP literature appears generally poor in data addressing the issue of traditional knowledge widely used by island communities to manage the risk of ciguatera (Lewis, 1983; Alvarez et al., 1992; Morrison et al., 2008). In this regard, the present study may be regarded as an attempt in that area as it has provided information about the risk perception of ciguatera among the local residents (i.e. which fish species they think are infected and what are the areas where they mostly occur). Our results suggest that, overall, the traditional information held in Raivavae may not be scientifically complete but is functionally correct, at least regarding the fishing areas most popular with the population (Te Ava Rua Pass, Rairua–Mahanatoa locality, Teruapupuhi Pass, Motu Piscine and Motu Mano). As a comparison for example, the CFP incidence rate in 2007 in Fakarava Island (an atoll in the Tuamotu archipelago as populated as Raivavae) was almost 3 times higher than the one reported in Raivavae. As for the discrepancy observed between our toxicity data and the population perception of the CFP risk in Teaoa Pass and the airport zone, this result needs to be confirmed with regard to the insufficient number of fish specimens sampled and tested for these areas. Additionally, this study has also provided a better insight into the risk-taking behaviour among the local residents. In Raivavae, as in many remote islands of French Polynesia whose populations rely heavily on fish resources, local consumers generally hold a fatalistic attitude with regard to ciguatera, and occasional poisoning seems to be accepted. Comparison of 2007 versus 2008 medical records from Rairua Health centre, however, suggests a self-regulating behaviour of the local residents towards fish species consumption (i.e. avoidance of parrotfish) and fishing areas (i.e. avoidance of the Rairua–Mahanatoa area), which we believe is related to the public meetings and general recommendations made to the population in 2007 following restitution of field results, and the wide diffusion among fishermen of the map regarding the ciguatera risk stratification within their lagoon. The high proportion of CFP incidents still involving *N. unicornis* in 2008 is surprising, though, and may reflect local fish preferences, as the “ume” is often regarded as a gourmet fish meal in many atolls and islands of French Polynesia.

4.6. Future research prospects

Aside from the avoidance of specific fish, one important aspect of CFP management resides in the availability of simple, cost-effective individual tests to detect ciguatera toxins in fish, which can be used by fishers or consumers. In the absence of such tests, local residents in endemic areas, including Raivavae, often rely on a variety of local tests based on experience and folklore, but none of those have been scientifically validated. Hence, future research prospects in Raivavae should include the inventory of traditional tests for ciguatoxic fish and traditional remedies to treat ciguatera currently on hand locally, and the verification of their reliability using laboratory tests.

Among the other issues that remained to be addressed in Raivavae, are as follows:

- (i) The monitoring on a longer term of critical areas such as the one in the surrounding of the airport, in an attempt to evaluate the impact of such infrastructure on the local coral reef ecosystems.
- (ii) The clinical, epidemiological and eco-toxicological survey of Rairua locality where a new form of seafood poisoning involving benthic cyanobacteria and giant clams has been evidenced (Laurent et al., 2008).
- (iii) The gradual transition from traditional to occidental dietary habits already proposed for other Pacific islands (Coyne, 1984; Lewis and Ruff, 1993 and references therein), and which is currently witnessed in the Australes (Counil et al., 2009; Ferland et al., 2009).

In the process of such studies, closer interactions between sociologists, physicians, epidemiologists, biologists and other laboratory specialists will be needed, to develop better control and prevention programs for a reduced risk of seafood poisonings among island countries populations.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

Appendix I.



Institut Louis Malardé

Epidemiological surveillance of ciguatera
and other types of marine biotoxin food poisoning in French Polynesia

PATIENT INFORMATION

Surname: _____ Name: _____
Age: _____ Sex: _____ Female Male

CLINICAL DATA

	YES	NO
Gastrointestinal symptoms		
Nausea/vomiting (circle)	<input type="checkbox"/>	<input type="checkbox"/>
Diarrhoea	<input type="checkbox"/>	<input type="checkbox"/>
Neurological and systemic symptoms		
Tingling of extremities	<input type="checkbox"/>	<input type="checkbox"/>
Disrupted sense of touch	<input type="checkbox"/>	<input type="checkbox"/>
Problems when in contact with cold	<input type="checkbox"/>	<input type="checkbox"/>
Itching/skin symptom except for wounds from scratching (circle)	<input type="checkbox"/>	<input type="checkbox"/>
Dizziness/Headaches	<input type="checkbox"/>	<input type="checkbox"/>
Vision problems	<input type="checkbox"/>	<input type="checkbox"/>
Muscular pains/cramps/muscular weakness (circle)	<input type="checkbox"/>	<input type="checkbox"/>
Joint pains	<input type="checkbox"/>	<input type="checkbox"/>
Chills/hypothermia	<input type="checkbox"/>	<input type="checkbox"/>
Behavioral disorders (agitated, disorientated)	<input type="checkbox"/>	<input type="checkbox"/>
Language/balance/coordination/walking problems (circle)	<input type="checkbox"/>	<input type="checkbox"/>
Burning sensation (throat, mouth)/"metallic" taste (circle)	<input type="checkbox"/>	<input type="checkbox"/>
Urogenital burning	<input type="checkbox"/>	<input type="checkbox"/>
Others (specify): _____		
Cardiovascular symptoms		
Pulse (/mn): _____		
Blood pressure: _____		
Other symptoms (specify): _____		
Time elapsed between meal and appearance of symptoms: _____		
Number or previous episodes of food poisoning: _____		
Number of people affected by this food poisonings, in addition to the patient: _____		

DATA CONCERNING THE FISH

Date eaten: _____
Local name of fish or seafood responsible for the poisoning (giant clam, crab, etc): _____
Parts eaten: **Flesh** **Head** **Viscera**

Precise fishing area - Specify with x mark on attached map

Island: _____ Island group: _____

HEALTH FACILITY INFORMATION

Island group: _____ Island: _____
Date of patient visit: _____
Health facility name: _____

Please return to: CIE - Institut Louis Malardé, BP 30 - 98713 PAPEETE, TAHITI

Tél: (689) 41.64.53 - Fax: (689) 41.64.06

The original version of this questionnaire was in French. The English translation was done by the Secretariat of the Pacific Community

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