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Ciguatera Poisoning in French Polynesia: a review of the distribution and toxicity of *Gambierdiscus* spp., and related impacts on food web components and human health

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Abstract :

Ciguatera Poisoning (CP) is a seafood poisoning highly prevalent in French Polynesia. This illness results from the consumption of seafood contaminated with ciguatoxins (CTXs) produced by Gambierdiscus, a benthic dinoflagellate. Ciguatera significantly degrades the health and economic well-being of local communities largely dependent on reef fisheries for their subsistence. French Polynesia has been the site of rich and active CP research since the 1960's. The environmental, toxicological, and epidemiological data obtained in the frame of large-scale field surveys and a country-wide CP case reporting program conducted over the past three decades in the five island groups of French Polynesia are reviewed. Results show toxin production in Gambierdiscus in the natural environment may vary considerably at a temporal and spatial scale, and that several locales clearly represent Gambierdiscus spp. "biodiversity hotspots". Current data also suggest the "hot" species G. polynesiensis could be the primary source of CTXs in local ciguateric biotopes, pending formal confirmation. The prevalence of ciguatoxic fish and the CTX levels observed in several locales were remarkably high, with herbivores and omnivores often as toxic as carnivores. Results also confirm the strong local influence of Gambierdiscus spp. on the CTX toxin profiles characterized across multiple food web components including in CP-prone marine invertebrates. The statistics, obtained in the frame of a long-term epidemiological surveillance program established in 2007, point towards an apparent decline in the number of CP cases in French Polynesia as a whole; however, incidence rates remain dangerously high in some islands. Several of the challenges and opportunities, most notably those linked to the strong cultural ramifications of CP among local communities, that need to be considered to define effective risk management strategies are addressed.

Highlights

► French Polynesia is a biodiversity hotspot for *Gambierdiscus* species ► *Gambierdiscus polynesiensis* is the main source of ciguatoxins in French Polynesia ► Herbivores and omnivores can be as toxic as carnivores in French Polynesia ► French Polynesia has a long-term epidemiological surveillance program on CP ► Cultural ramifications of CP have strong implications for risk management strategies

Keywords : Ciguatera poisoning, French Polynesia, Gambierdiscus, ciguatoxins, toxin profiles, risk management

1. Introduction

Ciguatera Poisoning (CP) is a non-bacterial seafood poisoning highly prevalent in French Polynesia. This poisoning results from the bioaccumulation in marine food webs of toxins known as ciguatoxins (CTXs), originating from benthic dinoflagellates in the genera *Gambierdiscus* and *Fukuyoa*. Natural and man-made disturbances are some of the factors likely to exacerbate the proliferation of these microalgae thus leading to the emergence of CP risk in coral reef environments (Chinain et al., 2010b). The mode of transmission of CP to humans is well documented: CTXs are readily transferred from algae to herbivores by grazing, then to carnivoresby predation, and ultimately to humans following the consumption of these tainted marine products. Due to the wide distribution of CTX physiological targets, CP is characterized by a proteiform syndrome that may present with digestive, cardiovascular, musculoskeletal, and/or neurological symptoms including exaggerated nociception (for a review, see Friedman et al., 2017). The effective management of patients is significantly hampered by the occurrence of atypical forms and/or chronic sequelae in some patients, as well as the lack of confirmatory diagnosis tests and specific antidotes. Figure 1 depicts the biogenesis of CP in coral reef environments, the subsequent transfer of CTXs in the food web, and the health impacts on human consumers. In light of the heavy reliance of local populations on lagoon resources, this underdiagnosed, inadequately treated, and underreported disease currently constitutes a major public health issue and a threat to food sustainability in CP-endemic areas such as in French Polynesia.

The earliest written account of CP in French Polynesia dates back to the 18th century and was provided by James Morrison, the boatswain's mate on board the *HMS Bounty* while mooring in the Society archipelago (Jaunez, 1966). Interestingly, in his logbook transcription, Morrison also reported the following: "As the natives are unable to tell the good (fish) from the bad, they hesitate to throw them away and risk eating them…". This suggests that oral histories of ciguatera may have circulated among native communities of French Polynesian long before Morrison's written account. Later, in the 19th century, accounts of the presence of poisonous fish in the Tuamotu Islands were provided as early as 1829 by the explorer Jacques-Antoine Moerenhout (Moerenhout, 1837), in the Gambier Islands by Father Honoré Laval (1807-1880) in 1834 (Laval, 1969), and the Marquesas by Father Pierre in 1848. In his writings, Father Pierre mentioned the "discomfort" caused by the ingestion of fish caught in some bays at certain seasons of the year.

Following the food chain theory proposed by Randall whereby the toxin involved was presumed to be produced by a benthic microorganism first ingested by herbivorous fish, then transferred to larger carnivores (Randall, 1958), French Polynesia quickly became the site of rich and active CP research in the ensuing decades. In 1964, a mass-poisoning outbreak that affected 33 people and resulted in three fatalities occurred on Bora Bora Island following the consumption of giant clams (Bagnis, 1967). In addition to the classical combination of cardiovascular (bradycardia), digestive (diarrhea, nausea, vomiting) and neurological disorders (dysesthesia, paresthesia, itching, myalgia, arthralgia, etc.), atypical signs were also reported in the victims such as a rapid onset of symptoms (< 30 mn), severe anorexia and psychiatric disorders as well as the unusual severity of the poisoning, which resulted in the coma and death of some patients. At the request of local authorities, this toxic event prompted field investigations conducted jointly by two experts from the University of Hawaii, Banner and Helfrich, with the help of Bagnis, a French military physician then stationed in the Tuamotu Gambier. Further, in 1967, a dedicated CP research unit was established at the Institut

Louis Malardé (ILM) to investigate the etiology of this yet enigmatic poisoning, which led to the identification of the causative organism - a benthic dinoflagellate - almost a decade later (Yasumoto et al., 1977). Tentatively identified as *Diplopsalis* sp., this organism was later placed in a new genus and named *Gambierdiscus toxicus* according to the Gambier Island where it was first described (Adachi and Fukuyo, 1979). This breakthrough further paved the way for several decades of active research punctuated by major advances in CP knowledge, such as the isolation and elucidation of the chemical characteristics and structure of the reference ciguatoxin CTX1B (Legrand et al., 1989; Murata et al., 1990), and the further description of novel *Gambierdiscus* species, including *G. polynesiensis*, a species currently regarded as the most toxic known to date (Chinain et al., 1999a; Chinain et al., 2010a).

Despite concerted research efforts over the past 50 years, our understanding of this complex eco-toxicological phenomenon as well as our capacities to efficiently manage the health and socioeconomic impacts of CP remain incomplete. Meanwhile, the globalization of CP has prompted a resurgence of interest within the scientific community and regulatory agencies. As a result, the Inter-Agency Global Ciguatera Strategy (GCS) was developed in 2015 which identified three priority fields of improvement:(i) the causative organism detection, monitoring, and risk forecasting, (ii) toxin detection in algal cells and fish, and (iii) epidemiological data collection, reporting and assessments (IOC-IPHAB, 2015). In particular, some elements of this strategy highlight the need for the development of a global, distribution and abundance database for *Gambierdiscus* spp.*,* refined with species-specific toxicity data to better inform risk assessment models. Moreover, surveillance and monitoring programs to determine the amount of CTX contamination in fish and associated toxin profiles per region and the creation of temporal and geographic risk maps are strongly encouraged to allow the definition of effective and integrated risk management options. In this regard, the integrated approach to the study of CP established in French Polynesia since 2007 (see section 4.2.1, and Chinain et al., 2020a) aligns well with several of the GCS recommendations, in that it combines a country-wide epidemiological surveillance program of CP cases with algal and toxin-based field monitoring programs useful to develop risk maps in CP-prone areas.

The present paper provides an overview of the main advances achieved over the past two decades (1999-2021) in CP knowledge and understanding based on the wealth of data obtained in the frame of environmental and toxic surveys conducted in various islands/atolls of French Polynesia between 1999 and 2018 and collection of epidemiological data between 2007 and 2021. In the first section of the results, the current knowledge on the genetic diversity, distribution, and toxicity of *Gambierdiscus* spp. is summarized, with a focus on the species-specific variations evidenced in the toxin potential of ≈ 120 *Gambierdiscus* strains isolated from the five island groups that compose French Polynesia. In a second section, data on the CTX accumulation in fish and toxin profiles characterized across multiple food web components are presented, along with two case studies in long-standing CP hotspots of French Polynesia. A third section examines CP statistics and the clinical characteristics of CP in French Polynesia. The last chapter addresses the current challenges and opportunities identified in the implementation of CP management programs in French Polynesia, with a focus on the strong socio-cultural ramifications of CP leading to high risk-taking behaviors among local populations, while promoting the development of adaptive strategies useful to minimize the poisoning risk. In the concluding section, several areas for future research efforts are briefly discussed.

2. Material and methods

Since the data presented in this paper encompass two decades of CP research in French Polynesia, diverse protocols/methods were used for the sampling, extraction, and detection of *Gambierdiscus* spp. and CTXs, which reflect the methodological and technical advances achieved in this research field during this period.

2.1. Study site

Data presented herein were obtained in the frame of field studies conducted in various locations of French Polynesia (278,786 inhabitants, 2022 census (ISPF, 2022)) and its five island groups, namely the Australes, the Marquesas, the Gambier, the Society, and the Tuamotu archipelagoes, which include 121 high islands and atolls spread over a surface as large as Europe stretching from 134°W to 155°W and 8°S to 28°S (Figure 2).

2.2. Biological material

2.2.1. Gambierdiscus samples and taxonomic identification

Abundance and toxicity data on wild *Gambierdiscus* populations were obtained from samples collected on various macrophytes selected among the most abundant and widely distributed species in red, brown, and green algae (Table 1), following the method described in Chinain et al. (1999b). These samples were obtained in the frame of opportunistic samplings conducted during risk assessment campaigns in Nuku Hiva, Raivavae, and Rapa and a 37 month-long survey in Tahiti consisting of weekly samplings in Teavaraa Pass located in the municipality of Papara (Table 1). First, *Gambierdiscus* cells dislodged from macroalgal substrates by manual shaking were concentrated by centrifugation at 2000 xg for

20 min. Cell abundance expressed in cells per gram of macroalgal wet weight (cells gww^{-1}) was assessed microscopically. Cell pellets were stored at -20 °C until further extraction and toxicity analyses.

The 123 *Gambierdiscus* clones of the ILM culture collection were isolated from either macroalgal samples (Chinain et al., 2010b) or artificial substrates (i.e., window screens, WS) following the method described by Tester et al. (2014). *Gambierdiscus* clonal cultures were established and routinely maintained in the laboratory as previously described by Chinain et al. (2010a). All strains are part of the culture collection of the Laboratory of Marine Biotoxins at ILM, where they are deposited. The species composition and relative cell abundance of *Gambierdiscus* species in WS samples were assessed by means of semi-quantitative qPCR assays targeted at the following species: *G. polynesiensis*, *G. toxicus*, *G. pacificus*, *G. australes*, *G. caribaeus*, *G. carpenteri*, *G. belizeanus*, *G. carolinianus*, *G. ruetzleri*, and *G. honu*. These assays were performed using the primer sets and protocol previously described by Vandersea et al. (2012) and Darius et al. (2018b). The same procedure was followed for the taxonomic identification at the species level of *Gambierdiscus* clones isolated from field samples, except for *G. honu* strains which were identified at Cawthron Institute according to the method of Stuart et al. (2022).

2.2.2. *Fish samples*

Fish and marine invertebrate samples were collected in the frame of random toxic surveys conducted by ILM between 2003 and 2018 in several islands of French Polynesia (Supplementary Material S1) to evaluate CTX accumulation across multiple food web components, except for fish sourced from Moruroa atoll (Tuamotu Archipelago) which were provided by the French Army. Fish were selected among species commonly consumed by local populations or well-known for their susceptibility to CTXs. Fish specimens were collected by spearfishing, and identified at the species level using the reference guidebook of Bacchet et al. (2017). Their total length and weight were measured to the nearest centimeter and gram, respectively. A total of 1893 fish (963 herbivores, 260 omnivores, and 670 carnivores) originating from eight distinct 2islands/atolls featuring 20 families and 92 species were sampled. Fish flesh was conditioned in the form of fillets, kept at -20°C until extracted (see section 2.3), and tested for their CTX-like toxicity (see section 2.4).

Fish remnants implicated in poisoning incidents were provided by CP victims, either as frozen meat fillets or as prepared meal leftovers, and stored at -20°C until further extraction and toxicity analyses.

2.2.3. Ciguatoxin standards

A large stock of CTX3C and CTX1B standards were established at ILM in the 1990s, following the major purification work conducted on Gambierdiscus cells and livers of moray eels using nuclear magnetic resonance (NMR) and fast-atom bombardment tandem mass, as described in previous studies (Legrand et al., 1989; Murata et al., 1990; Yasumoto et al., 2000). The successive aliquots of CTX3C and CTX1B needed for the calibration of the different assays conducted in the present study (from 2003 to 2018) were prepared from this material.

2.3. Extraction protocols

Gambierdicus wild samples and cultured cells harvested at the early stationary phase were extracted following the protocols previously described by Chinain et al. (1999b; 2010a), respectively. Briefly, samples were extracted under sonication with methanol (MeOH), followed by aqueous methanol ($MeOH/H_2O$) 50/50 (*v/v).* The resulting dried crude extracts (supernatants) were combine and further partitioned between dichloromethane (CH_2Cl_2) and $MeOH/H_2O$ 60/40 to allow the separation of CTXs from hydrosoluble compounds. The CH_2Cl_2 phases in which

CTXs are preferentially recovered were further dried under vacuum and stored at 4°C until tested for their toxicity using two functional detection methods (see sections 2.4.1 and 2.4.2, respectively).

Fish samples were extracted following two different protocols as previously described by Darius et al. (2021). Fish samples further analyzed using the receptor binding assay (RBA) were first extracted under sonication with MeOH. After centrifugation, the supernatants were directly deposited on Sep-Pak® C_{18} cartridges for rapid CTX purification. The resulting MeOH/H_2O 90/10 liposoluble fractions (LF90/10) were dried in a SpeedVac concentrator and stored at +4°C until toxicity analyses. Fish samples further analyzed using the neuroblastoma cell-based assay (CBA-N2a) were extracted following the same procedure as the one used for *Gambierdiscus* samples until the recovery of dried CH_2Cl_2 phase. These fractions were then further defatted by a second partition step using cyclohexane and MeOH/H_2O 80/20. The resulting methanolic phases were recovered and purified using Sep-Pak® C_{18} cartridges. Finally, the LF90/10, likely to contain the majority of CTXs, were recovered and dried in a SpeedVac concentrator and stored at +4°C until toxicity analyses.

2.4. Detection methods

2.4.1. Receptor binding assay (RBA)

This functional method is based on the ability of CTXs to bind to site 5 of the α-subunit of the voltage-gated sodium channels (VGSCs) (Poli et al., 1986; Lombet et al., 1987). Classically, the competitive binding on receptor sites between CTXs and labeled brevetoxins (PbTxs) results in a dose-dependent decrease in radioactivity (rRBA, [^{3}H]PbTx-3) or fluorescence (fRBA, bodipy ® PbTx-2) signal as CTX contents increase (Poli et al., 1986; Lombet et al., 1987; McCall et al., 2014). The composite binding activity of CTXs present in a biological sample is further compared to the binding activity of a purified CTX allowing the determination of CTX content. For *Gambierdiscus* and fish samples, the presence and quantification of CTXs were evaluated in parallel with pure CTX standards (e.g., CTX3C and CTX1B) following the protocol previously described in Darius et al. (2007) and Darius et al. (2021) by testing the LF90/10. The receptors were prepared according to the protocol described in Dechraoui et al. (1999) from rat brain membranes. The composite binding affinity of CTXs present in *Gambierdiscus* and fish samples is expressed in pg CTX3C eq. cell^{-1} and ng CTX3C eq. g^{-1} of flesh tissue, respectively, with limits of detection (LOD) and quantification (LOQ) estimated at 0.015 and 0.31 pg CTX3C eq. cell^{-1} for *Gambierdiscus* samples and 0.155 and 0.31 ng CTX3C eq. g^{-1} or 0.07 ± 0.02 and 0.13 ± 0.01 ng CTX1B eq. g^{-1} of flesh tissue for fish samples (Darius et al., 2007; Darius et al., 2021). In total, 65.6% (i.e., n=1242) of the fish analyzed in the frame of random toxic surveys conducted between 2003 and 2018 in French Polynesia were tested using the RBA.

2.4.2. Neuroblastoma cell-based assay (CBA-N2a)

This functional assay is based on the exposure of mouse neuroblastoma cells (N2a) to the cytotoxic effects of toxins acting as VGSC activators, such as CTXs and PbTxs (Catterall and Nirenberg, 1973; Manger et al., 1993). Of note, proper detection of these two toxin groups requires the addition of ouabain (O), a sodium/potassium ATPase pump blocker, and veratridine (V), a sodium channel activator (Nicolas et al., 2014). The N2a cell line (CCL-131) was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). In the presence of a non-destructive ouabain/veratridine treatment (OV+ condition), CTXs typically induce a dose-dependent decrease of N2a cell viability whereas no cytotoxic effects of CTXs are detected on N2a cells alone (OV- condition). Next, the composite cytotoxic activity of CTXs present in a biological sample is compared to the cytotoxic activity of a pure CTX standard (e.g., CTX3C in the present paper) to allow determination of

CTX content. Practically, for *Gambierdiscus* and fish samples, the detection and quantification of CTXs are achieved following the protocol previously described in Viallon et al. (2020) and Darius et al. (2022), by testing the LF90/10. The resulting composite cytotoxicities in *Gambierdiscus* and fish samples are expressed in pg CTX3C eq. cell^{-1} and ng CTX3C eq. g^{-1} of flesh tissue, respectively, with LOD and LOQ values estimated at 0.33 ± 0.03 and 0.79 ± 0.12 fg CTX3C eq. cell^{-1}, and 0.03 ± 0.01 and 0.06 ± 0.01 ng CTX3C eq. g^{-1} of flesh tissue, respectively (Viallon et al., 2020; Darius et al., 2022). The same LOD and LOQ values for fish samples can also be expressed in CTX1B eq. g^{-1} as the EC_{50} values of CTX3C and CTX1B were found similar (Darius et al., 2018b). In total, 34.4% (i.e., n=651) of the fish analyzed in the frame of random toxic surveys conducted between 2003 and 2018 in French Polynesia were tested using the CBA-N2a.

2.5. CP case reporting system

Ciguatera poisoning is not a notifiable disease in French Polynesia. Reporting CP incidents thus remains optional and is based on the goodwill of public health staff assigned to the 61 health care facilities disseminated throughout the country, including outlying hospitals, infirmaries, dispensaries, and first-aid posts (currently, reporting by private practitioners remains anecdotal). For each reporting patient, a standardized clinical form is completed by the medical staff (Supplementary Material S4). Information is gathered on the island of residence, age, gender, clinical symptoms experienced by the patient, details on the toxic meal (marine species involved, part(s) eaten, and fishing area), number of previous CP, number of affected consumerss, etc. All notification forms are further centralized at ILM, which issues an annual report widely disseminated via the Public Health Directorate network. Since 2014, CP statistics can be downloaded from a website (www.ciguatera.pf) which allows the on-line reporting of CP incidents by private physicians and the general public through a dedicated application. Since 2022, an improved CP reporting system has been developed and made available through an e-platform for training and data sharing on CP (https://ciguawatch.ilm.pf).

3. Results and discussion

3.1. Genetic diversity, distribution, and toxicity of *Gambierdiscus* spp. from French Polynesia

Gaining insights into the genetic diversity and suite of toxins produced by *Gambierdiscus* populations/strains in a given area is critical to ascertain the real threat posed to surrounding marine organisms and local populations (Holmes and Lewis, 2023). Figure 3 depicts our current state of knowledge on the genetic diversity and distribution of *Gambierdiscus* spp. in the five island groups of French Polynesia, overlaid with toxicity data obtained from clonal cultures successfully established in the laboratory from field material (i.e., macroalgal and/or WS samples). At least seven out of the 19 *Gambierdiscus* species known to date (Nguyen-Ngoc et al., 2023, and references therein) are found in French Polynesia, namely *G. toxicus, G. australes, G. pacificus, G. polynesiensis, G. honu, G. caribaeus* and *G. carpenteri* (Chinain et al., 1999a; Litaker et al., 2009; Rhodes et al., 2017), while no occurrence data are currently available for the four species in the genus *Fukuyoa* (Gómez et al., 2015; Li et al., 2021). However, given the bias and/or limits inherent to the methods used in this study (e.g., cell isolation and culturing, PCR with sets of primers targeted at a limited number of species), this list is far from being exhaustive. It is highly likely more species will be uncovered in the near future as sampling efforts increase throughout the five island groups of French Polynesia, and the use of recently developed approaches for molecular analysis is more likely to uncover the true extent of *Gambierdiscus* biodiversity, such as those based on high-throughput sequencing (i.e., metabarcoding) (Smith et al., 2017), or fluorescent *in situ* hybridization (FISH) probes (Pitz et al., 2021). Interestingly, the species *G.*

polynesiensis was present in all five island groups. The species *G. australes* and *G. carpenteri* also appear to be widely distributed in French Polynesia, consistent with their ubiquitous occurrence worldwide whereas the distribution range of *G. polynesiensis* and *G. honu* seems to be restricted to the Pacific region (Chinain et al., 2020a, for a review). Based on current data, several locales in French Polynesia could be regarded as “biodiversity hotspots” for *Gambierdiscus*: so far, five distinct species have been reported from Tubuai (Australes) and Nuku Hiva (Marquesas), and six species from Moorea (Society) and Mangareva (Gambier). Additionally, despite lower sampling efforts in the Society archipelago as compared to other sites in the Gambier (Mangareva) or the Marquesas (Nuku Hiva), up to seven species are already recognized in Tahiti Island (Figure 3). In any case, a revisit of these data obtained with PCR primer sets designed in the 2010’s now appears necessary as several new species have since been described worldwide over this past decade (Chinain et al., 2021, for a review and references therein; Nguyen-Ngoc et al., 2023). These apparent disparities in *Gambierdiscus* species’ richness across island groups, if confirmed, could be attributable to different biotic and abiotic factors or a combination of both (Chinain et al., 2020b, for a review), but it is also likely additional species will be characterized in French Polynesia as monitoring efforts accelerate in islands/atolls not yet explored.

It should be noted that *Gambierdiscus* populations are now reported as far south as Rapa (Iti) Island located in the Australes archipelago (Figure 3). This finding was made in the frame of field investigations conducted in 2010 following a fatal mass poisoning outbreak (Chinain et al., 2020c). Symptoms reported in patients were evocative of CP, consistent with the results of the large-scale toxic survey conducted on 251 fish caught around the island. *Gambierdiscus* wild samples collected from various macrophyte substrates in CP-prone fishing sites also tested positive for CTXs. Unfortunately, the exact genetic composition of *Gambierdiscus* assemblages in Rapa is yet to be determined as all subsequent attempts to establish clonal cultures failed. Using temperature time-series data, the authors then examined the potential link between climate change and the recent emergence of CP in this temperate-like locale of French Polynesia. Results were indicative of a global warming trend in the Rapa area, which has likely contributed to create favorable conditions for the migration and settlement of *Gambierdiscus* spp. in this area.

These findings are consistent with novel occurrence reports of *Gambierdiscus/Fukuyo*a spp. in temperate-like areas of the globe including the northern Gulf of Mexico (Tester et al., 2013), Korea (Jang et al., 2018), Japan (Funaki et al., 2022), the Mediterranean Sea (Gaiani et al., 2022), or nearer, in the temperate waters of New South Wales (Australia) (Larsson et al., 2018; Larsson et al., 2019). The presence of tropical species such as *Gambierdiscus* and *Fukuyoa* in temperate regions accords with suggestions of several researchers regarding the impact of climate change on the geographical expansion of tropical microalgae (for review, Tester et al., 2020, and references therein). In Australia, it has been suggested that blooms of *G. carpenteri* populations stretching as far as 37°S south of Sidney (New South Wales) may be explained by currents bringing cells from tropical Queensland to the warming New South Wales waters (Kohli et al., 2014).

A review of the literature shows that few field studies have examined the toxicity of *Gambierdiscus* spp. populations in the natural environment, as compared to the wealth of data derived from laboratory-based studies. Table 1 and Figure 4 summarize the toxicity data of *Gambierdiscus* populations collected in the frame of a long-term field study in Tahiti as well as opportunistic samplings in Nuku Hiva, Raivavae, and Rapa. They show toxin production in *Gambierdiscus* in the natural environment may vary considerably both at a temporal and spatial scale. For example, time-series data available from the Teavaraa Pass study site in Tahiti showed a 19-fold variation in the CTX-like toxicity of *Gambierdiscus* spp. assemblages over a 37 months' period as assessed by rRBA (with the highest value estimated at 18.96 pg CTX3C eq. cell^{-1}) in a sample characterized by a low cell abundance (i.e., 47 cells gww^{-1}) (Figure 4, Table 1). Peak cell densities were recorded indifferently during the hot (e.g., 7593 and 13,965 cells gww^{-1} in November 2000 and November 2002, respectively) or the cool season (e.g., 7009 cells gww^{-1} and 6791 cells gww^{-1} in July and October 2002, respectively). Of note, no correlation was observed between cell biomass and the amounts of toxins detected in blooms: for instance, two blooms (*) very similar in biomass (≈ 1682 vs. 1626 cells gww^{-1}) showed a 3.6-fold variation in toxicity levels, whereas two other blooms (**)differed in cell densities by 3.8-fold yet displayed similar toxin amounts (i.e., ≈ 0.52 vs. 0.53 pg CTX3C eq. cell^{-1}) (Figure 4).

Long-term field ecological surveys are essential to clarify the periodicity and annual cycles (if any) of *Gambierdiscus* populations and identify the conditions conducive to their proliferation and subsequent CP outbreaks. Yet, long-term studies consisting of a follow-up of *Gambierdiscus* blooms and related CTX-like toxicity are poorly documented in the literature. Scarce information compiled from historical data, as reviewed by Litaker et al. (2010), only provide toxicity data based on the mouse bioassay not directly comparable with the rRBA values presented herein. This was the case in the four years' study previously conducted between 1993 and 1997 in this same study site of Papara concomitant with a severe coral bleaching episode (Chinain et al., 1999b). The authors reported a seasonal trend in *Gambierdiscus* spp. Abundance (i.e., *Gambierdiscus* populations appeared to reach maximum abundance at the beginning and end of the hot season), but toxicity did not follow a similar pattern. While the 1999-2002 results presented herein appear to be contradictory, as blooms of *Gambierdiscus* were observed indifferently during the cool or hot season (Figure 4), they may reflect the local influence of the anthropogenic disturbances (i.e., major construction works that took place on the coastline area of the sampling site during the study period). In contrast, the present results were consistent with previous observations by Chinain et al. (1999b) that there was a poor relationship between *Gambierdiscus* abundance and measured toxicity.

It has been hypothesized that variations in *Gambierdiscus* populations' toxicity in the natural environment are mainly driven by the presence of more toxic isolates or species whose relative abundance varies temporally. In other words, in areas with high *Gambierdiscus* species diversity, low-toxicity species contribute only a small portion of the toxin flux into the food chain compared to the highly toxic species. This concept is now well established and documented in the literature (Litaker et al., 2010; Darius et al., 2018b; Díaz-Asencio et al., 2019; Bravo et al., 2020; Tester et al., 2020; Tudó et al., 2020). It suggests it is therefore possible to identify areas where CP risk is greatest by monitoring only species regarded as high CTX producers. In this context, studies aiming at quantifying the differences in species-specific toxicity in the laboratory could help in developing an effective cell-based risk assessment strategy for CP. Table 2 summarizes the results of the extensive toxicity screening conducted on the cultures of the 123 *Gambierdiscus* spp. clones of ILM culture collection using the CBA-N2a. Toxicity estimates in this genus range from very low to relatively high values, indicating not all *Gambierdiscus* species/strains reported in French Polynesia are high CTX producers: for instance, cultures of *G. australes, G. caribaeus*, *G. carpenteri*, *G. honu*, *G. pacificus,* and *G. toxicus* actually exhibited very low toxicity of the order of femtograms (from 1.4 up to 92 fg CTX3C eq. cell^{-1}) (Table 2). In contrast, the majority of the 20 *G. polynesiensis* strains tested for their toxicity using CBA-N2a proved significantly more toxic than the other species, producing pg amounts of CTXs (from 1.03 up to 7.85 pg CTX3C eq. cell^{-1}), except for two strains originating from the Gambier Islands, one from the Tuamotu, and one from the Australes whose mean toxicities were within the range of 50 to 487 fg CTX3C eq. cell^{-1} (Table 2). Regarding *G. polynesiensis* strains analyzed from the Australes and the Marquesas, one was isolated from Motu de la Femme (Raivavae, Australes) and four from Anaho Bay (Nuku Hiva, Marquesas). Interestingly, all five strains display CTX-like toxicity exceeding 3 pg CTX3C eq. cell^{-1}. Given the good correlation demonstrated between RBA and CBA-N2a when applied to the quantification of CTXs in *Gambierdiscus* matrices (Darius et al., 2022), the remarkably high toxicity assessed in *Gambierdiscus* blooms sampled from these two areas in 2004 and 2008 (Table 1) strongly suggest *G. polynesiensis* represented a significant part of these wild assemblages. Unfortunately, information on the species composition and relative abundance of *Gambierdiscus* spp. in these field samples are not available, as these data were generated at a time when *Gambierdiscus* taxonomy was still unresolved and qPCR assays were not yet available. Moreover, following atypical sea urchin and gastropod poisoning outbreaks in Nuku Hiva Island (Marquesas archipelago), Darius et al. (2018b) conducted a comparative study on *Gambierdiscus* spp. cell abundances and food web toxicity in three similar bays, and provided clear evidence that the highest toxicity occurred in Anaho Bay where the most toxic *Gambierdiscus* species, *G. polynesiensis*, dominated. Such observations support the hypothesis that *G. polynesiensis* could be the dominant producer of CTXs in French Polynesian ciguateric biotopes and that this species could serve as a CP risk biomarker in regions where this species is present. Consequently, the current approach advocated in risk assessment programs conducted in French Polynesia is the extensive monitoring of areas with occurrence reports of *G. polynesiensis* using species-specific molecular tools (Chinain et al., 2020a). However, the overall contribution to toxin flux of frequently occurring *Gambierdiscus* species with reduced toxicity should not be overlooked as their long-term presence in a fishing area might also lead to the progressive bioaccumulation of CTXs in fish, which can reach dangerous CTX levels over time (Xu et al., 2021).

All these findings highlight the significant variations observed in CTX production across species, but also between strains from different geographic origins (intra-specific variance). Even more, for a given strain, CTX production is also likely to vary according to the age of the strain, the growth stage of cultures at the time of harvest, and culture conditions (for reviews, see Parsons et al., 2012; Chinain et al., 2020b, and references therein; Longo et al., 2020). Furthermore, a recent study by Wang et al. (2018) also provided evidence that the growth and toxin production of *Gambierdiscus* spp. could be regulated by quorum-sensing bacteria. Taken together, all these observations point out the complexity of the toxinogenesis in *Gambierdiscus* spp., and suggest that this functional trait may be under the control of a combination of factors, including genetic (Kohli et al., 2017; Van Dolah et al., 2020; Wu et al., 2020), physiological (Bomber, 1989; Chinain et al., 2010a; Longo et al., 2019), environmental (Sperr and Doucette, 1996; Lartigue et al., 2009; Vacarizas et al., 2018; Longo et al., 2020), and microbial drivers (Wang et al., 2018; Wu et al., 2022).

3.2. Environmental impacts of ciguatera

3.2.1. CTX accumulation in fish based on random toxic surveys

Programs aiming at defining high-risk fish species and toxic locations by region can generate important information useful in defining effective CP risk management strategies, keeping in mind large regional differences can sometimes be observed (FAO-WHO, 2020). Paired with CP statistics, such data can be useful to health authorities to issue timely alerts going from precautionary measures to fishing bans as conditions dictate. They can also be of use to local populations to update their traditional knowledge of CP or refine avoidance strategies widespread among island communities (see section 4.2.2). Here, data relating to CTX accumulation and toxin profiles in a wide array of marine organisms across multiple food web components are presented, along with two case studies (Box 1 and 2) describing novel CP vectors in French Polynesia..

Table 3 details the prevalence of toxic fish assessed in several iconic CP hotspots of French Polynesia following large-scale toxic surveys that evaluated CTX accumulation in a wide array of herbivorous, omnivorous, and carnivorous fish species (Supplementary Material S1). The highest percentage of toxic fish was reported from Fakarava and Moruroa atolls (87%, with n=157 and n=217, respectively) while the lowest percentage was found in the Gambier Islands (59%, n=368). In the other CP hotspots, results were in the range of 66% to 77% (Table 3). While the high prevalence of toxic fish in Moruroa was not surprising since this atoll was the site of intense military activities for three decades (Ruff, 1989), the inconsistency noted in the proportion of toxic fish in Fakarava as compared to the Gambier Islands was unexpected: indeed, Fakarava, a Biosphere Reserve of Unesco, is considered a well-preserved atoll well-known to scuba divers for its iconic underwater landscapes, whereas the majority of the lagoon area in the Gambier Islands has been the site of intense black pearl farming activities for decades (André et al., 2022). The high prevalence of toxic fish in Nuku Hiva (77%) also contrasts with the relatively low incidence rate (IR) (57 cases/10,000 inhab.) reported from this island. This apparent inconsistency could be partly due to the bias linked to the sampling efforts in Anaho Bay (Nuku Hiva) where multiple field samplings have been conducted since 2015 following atypical CP cases involving marine invertebrates (see Box 1).

Comparison with data from similar toxic surveys conducted in historical CP endemic areas such as the Republic of Kiribati and Cuba, showed toxic fish prevalence in these regions also varied from 65% to 80% (Chan et al., 2011; Díaz-Asencio et al., 2019).

Figure 5 summarizes the CTX contents found in 13 fish species distributed among five herbivorous, two omnivorous, and six carnivorous fish species for which sampling efforts involved more than 20 individuals, regardless of their geographic origin (see also Supplementary Material S2). The results show that CTX levels exceeding 10 ng CTX3C eq. g^{-1} were detected among specimens in five of the seven species classified as herbivores and omnivores (e.g., *Naso lituratus* (maximum CTX level of 12.73 ng CTX3C eq. g^{-1} found in a sample from Moruroa), *Chlorurus microrhinos* (10.72 ng CTX3C eq. g^{-1} in a sample from Rapa), *Scarus rubroviolaceus* (18.04 ng CTX3C eq. g^{-1} in a sample from Nuku Hiva), *Ctenochaetus striatus* (12.65 ng CTX3C eq. g^{-1} in a sample from Nuku Hiva), and *Crenimugil crenilabis* (21.02 ng CTX3C eq. g^{-1} in a sample from Nuku Hiva)) (Figure 5, Supplementary Material S2). Of note, the very high CTX level found in a specimen of *C. crenilabis* collected from Anaho Bay (Nuku Hiva) was similar to the ones determined in trochus and sea urchin samples collected nearby (Darius et al., 2018a; Darius et al., 2018b), thus confirming the high CP risk status associated with this area (see Box 1). In contrast, the maximum levels reached in carnivorous species ranged from 3.47 to 8.47 ng CTX3C eq. g^{-1}, with the exception of a specimen of *Caranx melampygus* collected in Moruroa which displayed a maximum value of 14.28 ng CTX3C eq. g^{-1} (Figure 5, Supplementary Material S2). Interestingly, 79% (n=28) of *Epinephelus polyphekadion* and 54% (n=26) of *Cephalopholis argus* samples were negative (Supplementary Material S2), while the highest percentage of negative individuals among herbivores never exceeded 28% and concerned the *C. microrhinos* species group. Actually, 25% (i.e., 34 individuals/136) of the *C. microrhinos* specimens analyzed were found to contain CTX contents higher than 5.04 ng CTX3C eq. g^{-1} (Supplementary Material S2). Too, while *Naso unicornis* individuals displayed a median CTX content of 3.30 ng CTX3C eq. g^{-1}, this value never exceeded 1.61 ng CTX3C eq. g^{-1} among the carnivores, as determined in *C. melampygus* (Supplementary Material S2).

Despite the uneven sampling efforts noted across fish species and trophic stages, the present data rise interesting comments:

(i) in French Polynesia, herbivores and omnivores can bioaccumulate CTX contents as high as carnivores, and sometimes even more. These observations are consistent with previous findings by Darius et al. (2007), Chinain et al. (2010b), Gaboriau et al. (2014), and Chinain et al. (2020c), and explain why herbivores represent a significant portion of the fish species frequently involved in CP incidents in French Polynesia (Chinain et al., 2010b; Chinain et al., 2020c) and more widely in the Pacific region (Rongo and van Woesik, 2011). Based on these findings, the former Randall hypothesis suggesting that the preys of carnivorous fish (i.e., herbivorous and detritus feeding fishes or invertebrates) may be poisonous but not containing enough toxin to elicit symptoms if eaten by someone should be reconsidered at least for the Pacific region. This contrasts with other CP-endemic regions such as Japan, Hong Kong, the Caribbean, La Réunion, Macaronesia, etc., where herbivorous fish are not normally associated with ciguatera (Oshiro et al., 2010; Chan, 2014; Bravo et al., 2015; Boucaud-Maitre et al., 2018; Núñez-Vázquez et al., 2019; Habibi et al., 2021).

(ii) the high CTX contents generally found in fish from French Polynesian ciguateric biotopes are consistent with published data from other historical CP-endemic regions where similar toxic surveys (i.e., across multiple food web components) were conducted, such as Kiribati (Chan et al., 2011; Mak et al., 2013; Zhu et al., 2022) and Cuba (Díaz-Asencio et al., 2019). In other regions, a review of the literature (Supplementary Material S3) shows a majority of studies primarily focused on carnivores and, in some instances, a single fish species. Worthy of note, CTX levels assessed in carnivores from Fiji, Australia, Japan, Bahamas, Guadeloupe, U.S. Virgin Islands, and the newly affected Macaronesia region (i.e., Canary Islands) were relatively low compared to the ones in the present study, with values up to 0.79 ng C-CTX1 g^{-1} using LC-MS/MS or up to 2.175 ng CTX1B eq. g^{-1} using CBA-N2a (Oshiro et al., 2021a; Oshiro et al., 2021b; Loeffler et al., 2022a; Estevez et al., 2023; Ramos-Sosa et al., 2023 ; Supplementary Material S3 and references therein). The only exception concerns groupers, snappers, and greater amberjacks from Hawaii, Kiribati, Vietnam, Cuba, and Madeira and Selvagens Islands in which toxicity ranged from 6.231 to 81.84 ng CTX3C eq. g^{-1} (Supplementary Material S3 and references therein).

A popular notion about CP is that the risk of finding CTXs is higher in larger, aged fishes (Lehane and Lewis, 2000). Hence, in some countries, current risk management practices include bans on the sale of fish over a certain size or weight (Darius et al., 2022, and references therein). Even more, in the Canary Islands, data derived from large-scale toxic surveys were tentatively used to establish a predictive score of ciguatoxicity in fish (Bravo et al., 2015; Sanchez-Henao et al., 2019). In the case of French Polynesia, imposing size restrictions on some high-risk species in an attempt to prevent or limit CP does not seem a relevant strategy. As a matter of fact, a recent study examined the age and growth relationship to ciguatoxicity in five CP-prone coral reef fish species from French Polynesia based on otolithometry (Darius et al., 2022). The authors concluded that size, weight, age, and growth were not reliable determinants of fish ciguatoxicity which appears to be rather species and/or site-specific, although larger fish posed an increased risk of poisoning. This latter observation was supported by similar findings in carnivores from Kiribati waters (Chan et al., 2011; Mak et al., 2013) and the Atlantic region (Sanchez-Henao et al., 2019).

3.2.2 CTX toxin profiles across multiple food web components

Quoting CP experts, "*effective and integrated risk management options would require the definition of toxin profiles in each region, both in algal strains and in seafood to define risk evaluation protocols*" (FAO-WHO, 2020). Indeed, such data are useful to inform CTX flux models, as illustrated in the recent study by Holmes and Lewis (2023) on the origin of ciguatoxicity in the grouper *Plectropomus leopardus*. Figure 6 summarizes the toxin profiles characterized so far in various food web components in French Polynesian ciguateric biotopes, namely *G. polynesiensis* (strain RIK7, (Longo et al., 2019)), *Tridacna maxima* (giant-clam, filter-feeding bivalve, (Roué et al., 2016)), *Tectus niloticus* (trochus, detritivore, (Darius et al., 2018b)), *Tripneustes gratilla* (sea urchin, herbivore, (Darius et al., 2018a)), *Chlorurus microrhinos* (parrotfish, herbivore, (Sibat et al., 2018)), *Gymonothorax javanicus* (moray eel, carnivore, (FAO-WHO, 2020)) and *Eumegistus illustris* (deep sea fish, carnivore, (Darius et al., 2021)).

In *G. polynesiensis*, the high cytotoxicity detected by CBA-N2a in clonal cultures results from the composite bioactivity of at least seven CTX analogs, with CTX3B (49-*epi*-CTX3C) and CTX3C (two algal CTXs in the Pacific CTX3C group) representing 56 to 91 % of the total CTXs produced, as described in Longo et al. (2019) and Darius et al. (2022). These analogs further bioaccumulate throughout the trophic chain, from primary consumers such as giant clams, sea urchins, and trochus in which they remain the dominant analogs (64% to 100% of the overall toxin profile), up to carnivores such as moray eels in which CTX3C still represents 6.2% of the total CTXs detected (Figure 6). Algal CTXs in the Pacific CTX1B group (i.e., CTX4A and CTX4B) are also found in the early stages of the trophic chain, representing up to 64% of the total CTX content in a *C. microrhinos* specimen caught from the Gambier Islands. In carnivores, these algal congeners are further metabolized and biotransformed into more oxidized analogs such as CTX1B, 54-deoxyCTX1B, or 52-*epi*-54-deoxyCTX1B (Ikehara et al., 2017) which represent between 31.3% to 100 % of the toxin profile characterized in moray-eels and deep-sea fish, respectively (Figure 6). These data on the diversification of toxin profiles during CTX transfer in the food chain are congruent with what has been reported in herbivores, omnivores, and carnivores from other Pacific Island Countries and Territories (PICTs) (Mak et al., 2013; Oshiro et al., 2021b; Zhu et al., 2022), Hawaii (Yogi et al., 2014; Loeffler et al., 2022a), Japan (Yogi et al., 2011; Yogi et al., 2014; Oshiro et al., 2021a; Oshiro et al., 2022; Oshiro et al., 2023), Vietnam (Loeffler et al., 2022b), and India (Spielmeyer et al., 2022).

All together, these findings strongly support the hypothesis of the local influence of *Gambierdiscus* species (i.e., genetic composition of *Gambierdiscus* populations, and type of CTXs they produce) on the CTX-like content and toxin profiles in fish (Yogi et al., 2011; Yogi et al., 2014; Soliño and Costa, 2018), at least in primary consumers such as herbivorous fish and marine invertebrates (Mak et al., 2013; Silva et al., 2015; Darius et al., 2018a; Darius et al., 2018b; Sibat et al., 2018).

Box 1: Case study of Anaho Bay (Nuku Hiva Island, Marquesas archipelago)

In 2014-2015, atypical CP outbreaks following the consumption of marine invertebrates, namely *T. niloticus* (gastropod) and *T. gratilla* (sea urchin), were reported in a secluded area of Nuku Hiva Island, Anaho Bay (Darius et al., 2018a; Darius et al., 2018b; Gatti et al., 2018) (Figure 7A-C). The unusual severity of these toxic incidents prompted field and toxicological investigations, which confirmed the presence of multiple CTX analogs in these organisms (Figure 6). The combined use of WS artificial substrates and qPCR further allowed the reliable identification and enumeration of *Gambierdiscus* species in the area. Data revealed the "hot" species *G. polynesiensis* predominated in the area and was likely the source of the CTX analogs detected in surrounding toxic marine invertebrates (Darius et al., 2018a; Darius et al., 2018b). Concurrently, using a multi-toxin screening approach, the deployment of Solid Phase Adsorption Toxin Tracking (SPATT) devices allowed not only the detection of algal CTXs in the environment but also of other toxin classes (i.e., okadaic acid and dinophisystoxin-1) currently responsible for emerging phycotoxin risks in other parts of the world (Roué et al., 2020). In light of the high CP risk associated with this area, a fishing ban was issued in Anaho Bay in 2018. These pilot studies not only highlight the need to extend current monitoring efforts to the surveillance of these novel CP vectors often prized by South Pacific communities but also provide the demonstration of the potential interest of valuable field-monitoring tools such as WS, qPCR, and SPATT technologies for the development of Early Warning Systems (EWS) for CP (FAO-IOC-IAEA, 2023).

Box 2: Case study of Mangareva Island (Gambier)

In 2003, following the report of unusual CP incidents in Mangareva Island (Gambier archipelago), 22 locally-caught specimens (representing five species) of deep-water fish were screened for their toxicity employing the mouse biological assay (MBA) and rRBA. Almost two decades later, CBA-N2a and liquid chromatography tandem mass spectrometry (LC-MS/MS analyses performed on archive samples kept at -20°C confirmed the presence of significant levels of CTXs in seven of these specimens (i.e., *Eumegistus illustris* (brilliant pomfret, n=1), *Etelis coruscans* (longtail red snappers, n=2), *Pristipomoides filamentosus* (Crimson jobfish, n=2), *Epinephelus tuamotuensis* (reticulate grouper, n=1), and *Saloptia powelli* (golden grouper, n=1)) (Darius et al., 2021). In the most toxic specimen of *E. illustris*, three distinct CTX analogs were formally identified, namely CTX1B, 52-*epi*-54-deoxyCTX1B, and 54-deoxyCTX1B (Figure 6). Of note, CTX1B was also detected in two other specimens of *E. tuamotuensis* and *P. filamentosus*. Such findings emphasize the importance of a systematic monitoring of CTXs in all exploited fish species in CP hotspots, including deep-water fish which constitute a significant portion of the commercial deep-sea fisheries in many Asian-Pacific countries (Darius et al., 2021).

In conclusion, acquiring comprehensive data on the toxin profiles characterizing *Gambierdiscus* spp. populations in a given region can provide baseline knowledge on the factors likely to influence ciguatoxicity in subsequent food web components (i.e., locally caught fish and marine invertebrates) (Yogi et al., 2011; Yogi et al., 2014; Ikehara et al., 2017). While the accumulated knowledge on Pacific CTXs is extensive, much work remains to be conducted for CTXs specific to the Caribbean and the Indian Ocean regions (FAO-WHO, 2020). The recent characterization of an algal precursor of the C-CTXs dominant in ciguatoxic fish from the Caribbean in some *G. caribaeus* and *G. silvae* strains and a putative I-CTX in a *G. balechii* strain from Indonesia should accelerate the momentum (Mudge et al., 2023; Tartaglione et al., 2023).

Another important consideration with regards to CTX transfer in marine food webs with strong implications in terms of CP risk management programs is the expected timeline between an environmental perturbation or trigger and subsequent transfer of CTXs from toxic cells to herbivorous fish, or between *Gambierdiscus* peak cell densities and increasing CP cases in humans. Results of a study consisting of a 14-month field effort with weekly samplings and toxin analysis of 270 *Gambierdiscus* samples and 465 specimens of *Ctenochaetus striatus* suggest a lag time of one month occurs between increased *Gambierdiscus* cell abundances and the detection of toxin in herbivorous fish (FAO-IOC-IAEA, 2023), while a three-month lag time is seen between peak *Gambierdiscus* densities and a peak in CP cases (Chateau-Degat et al., 2005).

3.3. Ciguatera impacts on human health

3.3.1. Ciguatera prevalence in French Polynesia

In French Polynesia, CP is the main, if not only, cause of seafood poisoning (Gatti et al., 2008). The first epidemiological studies on CP were initiated in the early 70s (Bagnis et al., 1979), but it was not until 2007 that a country-wide CP epidemiological surveillance program was established as part of a joint effort between the Public Health Directorate and ILM (see section 2.5). Based on CP records received between 2007 and 2021, the general trend observed in French Polynesia (as a whole) points towards an apparent decline of CP, from a peak of 615 cases in 2009 to 190 cases in 2021. However, it is unclear whether this trend is the result of a true decrease of CP risk in the environment and/or better adjustment to CP by local communities, or if these statistics merely reflect a progressive drop in the contribution of health structures to the CP reporting program.

Groupers (19.2%), snappers (16.3%), emperors (8.8%), jacks (7.8%), wrasses (6.7%), barracudas (4.2%), and triggerfish (3.5%) were among the fish groups most commonly involved in CP incidents in French Polynesia (extracted from the epidemiological surveillance network database). However, the contribution of parrotfish and surgeonfish/unicorn fish to local CP cases should not be overlooked (12.6% and 9.3%, respectively) (see also section 3.2). These findings are in marked contrast with observations from the Caribbean, Macaronesia, and East and Southeast Asia, where CP incidents mostly involve carnivorous species (for a review, see Chinain et al., 2021).

Data also show no island group is spared by CP although IRs can differ widely from one year to another with mean IRs ranging from 0 to 1805 cases per 10,000 inhabitants depending on the island. In French Polynesia, as in the vast majority of CP-endemic countries, these statistics also suffer from significant under-reporting by both health care workers and those affected. For instance, official records for 2021 show that in the Society Archipelago, up to 44% of cases following the collective consumption of a single contaminated fish were not declared, either because none of the affected guests took time to consult, or because the medical staff only reported one case for the whole group by lack of time. An on-line survey conducted in 2018 among 88 local healthcare workers (HCWs) revealed that although a majority of respondents acknowledged the public health importance of the epidemiological surveillance program currently in place, more than half of the HCWs failed to report CP cases for varying reasons: they did not know how to report CP cases, they simply omit to do so or lacked time (unpublished results). As for the generalized reticence to consult among CP victims, distance to the nearest health care facilities does not appear as the main cause of poor reporting. Actually, due to the very casual attitude towards CP often observed in these local communities, mild cases are rarely reported. Too, many CP victims tend to rely on self medication most notably on traditional remedies due to the lack of effective treatment options. Additional reasons for under-reporting also include the cost of consultation, and the very limited resources of health facilities in remote areas in terms of drug supply and/or supportive care capacity.

Basically, IR data not only depend on the level of case reporting specific to each island/region, but are also largely influenced by multiple drivers, such as the extent of environmental and anthropogenic disturbances, differences in fish bioavailability and/or dietary habits, and even the level of CP ecological knowledge among local communities (see section 3.4.2.b). To illustrate this point, IRs reported from several CP hotspots of French Polynesia were tentatively examined according to the percentages of toxic fish determined in these respective islands through random toxic surveys. Table 3 shows that the IRs reported from Fakarava and the Gambier Islands (i.e., 389 and 208 cases/10,000 inhabitants, respectively) were consistent with the respective prevalence of toxic fish determined in these two sites (i.e., 87% and 59%, respectively). No IR data was available from Moruroa Atoll, one of the several uninhabited islands in French Polynesia. Additionally, despite comparable toxic fish prevalence and similar sampling efforts in Rapa, Nuku Hiva, and Raivavae (i.e., 77% (n=251), 76% (n=262), and 70% (n=274), respectively) contrasting IRs were observed in these three islands with a 6.5-fold difference between Rapa and Raivavae (i.e., 857 vs. 132 cases/10,000 inhabitants, respectively), and a 15-fold variation between Rapa and Nuku Hiva (57 cases/10,000 inhabitants). There are several explanations for this disparity in IRs:

(i) in Raivavae and Nuku Hiva, two islands confronted with CP risk for decades, local populations have developed thorough ecological knowledge about CP (Chinain et al., 2010b), whereas in Rapa the majority of residents were likely naïve to CP until the 2009 mass-poisoning outbreak and therefore, could not rely on the traditional avoidance strategies highly popular among Raivavae and Nuku Hiva islanders (Chinain et al., 2020c).

(ii) in Raivavae, IR was twice as high as in Nuku Hiva, a likely consequence of the marked risk-taking behavior noticed among Raivavae residents despite the benefit procured by accurate CP ecological knowledge (Chinain et al., 2010b).

3.3.2 Clinical features of Ciguatera poisoning in French Polynesia

The general clinical features of CP illness in French Polynesia are in accordance with those reviewed by Friedman et al. (2017). Hereafter are described some of the characteristics of CP illness in French Polynesia based on the analysis of 1004 CP records collected between 2018 and 2021.

In more than 80% of cases, the clinical signs of the disease manifested within the first 12 hours after ingestion of the toxic meal. Neurological symptoms predominated including: paresthesia (80%), cold allodynia (64%), muscular disorder (55%), headache (50%), oral dysesthesia (49%), itching (47%), asthenia (42%), dysgeusia (33%), and hypothermia (28%). They presented concurrently with gastrointestinal symptoms (i.e., diarrhea (75%) and nausea 45%)), arthralgia (53%), and cardiovascular disorders (i.e., hypotension (27%) and bradycardia (22%)). Less commonly, burning sensation/tingling of urogenital parts (18%), orofacial pains (11%), hallucinations (4%), and disorientation (2%) were also observed. On average, isolated CP cases (as opposed to collective poisonings) represented 50% of total cases, and approximately half of the newly affected CP victims declared they had previous CP history (e.g., up to 20 CP in some individuals), a feature commonly observed among populations that rely heavily on marine resources for their subsistence. Men in their 40s were generally the most affected, as they often tasted suspect fish first before giving it to the rest of the household, consumed larger portions of fish, and were more prone to risk-taking behaviors, such as the consumption of the head and viscera of fish known to contain higher contents of CTXs (Oshiro et al., 2021a). Pediatric cases (i.e., in patients ≤ 15 years old) remain anecdotal, although they may represent up to 8% of the total CP cases reported annually. Moreover, since lagoon fish are introduced into babies' diets at the early stage of food diversification, CP cases in infants under one-year old have been previously reported by medical staff (Chateau-Degat et al., 2007). Too, in light of the isolated reports suggesting transmission of CP from a nursing mother to an infant, in French Polynesia nursing mothers with CP are recommended to suspend breastfeeding for a period of one month as a precautionary measure. No lethal case has been reported over the past 15 years in French Polynesia, but the chronicity of CP in some victims who may experience symptom recurrence months or years after the initial exposure has significant impacts on the quality of life of patients. Chronic CP cases (i.e., symptoms persisting over three months after the initial illness) are reported in at least 20% of patients (FAO-WHO, 2020, and references therein; Gatti et al., 2021) and persist mostly under the traits of neurological and psychiatric disorders (i.e., tenacious fatigue, paresthesia, dysesthesia, diffuse pruritus, difficulty concentrating, anxiety, and even depression). They may occur continuously or through transitory recurrences and are triggered by factors such as the consumption of various marine products (fish, seafood), alcohol, nuts, animal/plant proteins, under high stress situation, or following intense physical activity. A recent exploratory study was conducted among 49 CP patients hospitalized in the Taaone General Hospital of Tahiti in an attempt to screen for potential predictors of chronic CP (Gatti et al., 2021). Among the 37 variables studied, five significant predictors of having symptoms lasting over three months were identified: age, tobacco consumption, acute bradycardia, laboratory measures of urea, and neutrophils. Such findings warrant further investigations.

3.3.3. CTX contents in fish remnants involved in local CP toxic incidents

Detection of CTXs in implicated fish represents the current “gold standard” diagnosis of CP (Friedman et al., 2017). Moreover, establishing a comprehensive database on the CTX levels consistently measured in toxic meal remnants is useful to estimate the lowest observable adverse effects level (LOAEL), and define a code of practice for CP control by region. The CTXs are a group of toxins that are not yet regulated in Europe nor at the international level. Currently, the guidance safety level for CTXs (i.e., amount of CTXs expected not to exert effects in sensitive individuals when consuming a single fish meal) recommended by the US Food and Drug Administration (US-FDA) and the European

Food Safety Authority (EFSA) is estimated at 0.01 ppb Pacific CTX1B, taking into account a 10-fold safety margin (EFSA, 2010; US-FDA, 2022). Table 4 summarizes the results of the toxicological analyses performed by rRBA and CBA-N2a on flesh of fish remnants responsible for 16 CP events involving individuals who developed signs of the poisoning. Fourteen samples were associated with carnivorous species (Carangidae, Labridae, Lethrinidae, Lutjanidae, Serranidae, and Muraenidae), and two samples with herbivores (Acanthuridae and Scaridae). CTX values ranged from between LOD-LOQ values (i.e., 0.03-0.06) up to 6.62 ng CTX1B eq. g^{-1} (i.e., 662 times the safety level recommended by the US-FDA). These analyses were carried out exclusively on fish flesh, as other parts, such as the head and viscera, were generally not or no longer available. Hence, CP patients who preferentially consumed these parts rich in CTXs (i.e., CP events #1, #3-5, #9, and #14, Table 4) were likely exposed to higher CTX levels. In addition, the lack of information on the amount of food ingested by patients (e.g., if they had multiple servings of the same fish meal), limits constructive interpretation on toxin intakes. Despite these limits, interesting observations can be highlighted:

(i) data provided in Table 4 confirm the current underestimation of CP cases in French Polynesia which exceeded 50% in this particular context (i.e., 24 declarants out of 48 affected guests). This high under-reporting rate is consistent with statistics available from CP endemic regions (for a review, see Chinain et al., 2021).

(ii) the patient #10, a man in his 40s, nurse by profession, and without a known history of CP, allergy, or auto-immune/chronic disease, ate the cooked flesh of a parrotfish purchased in a supermarket and developed a particularly severe CP-characteristic combination of cardiovascular signs (hypotension and bradycardia), digestive signs (diarrhea, abdominal pain, mild nausea, vomiting) and neurological signs (paresthesia, dysesthesia, pruritus, cold allodynia, dizziness, muscular disorders, arthralgia, balance/walk/coordination disorders, dysgeusia, headache, urogenital burning/ pain, etc.) He also presented with hallucinations and severe anorexia. Surprisingly, the CTX content estimated by CBA-N2a in fish remnants provided by the patient was comprised between 0.03 and 0.06 ng CTX1B eq g^{-1}, consistent with the conservative guidance level of the US-FDA. Interestingly, similarly low values were also reported by (Farrell et al., 2017) in fish remnants implicated in CP incidents in Eastern Australia, another CP-endemic area.

(iii) except for event #12, exposure to highly toxic fish contaminated with more than 1 ng CTX1B eq. g^{-1} was consistent with a rapid onset of the symptoms which generally manifest within 2h to 11h of fish consumption.

(iv) the consumption of head and viscera (i.e., in events #1, #3, #4, #5, #9, and #14) did not always lead to severe CP cases (i.e., the diversity of symptoms experienced, and patient's hospitalization are used as severity indicators).

(v) the severity of the poisoning is not necessarily linked to a high CTX content in fish as, in the case of patients from events #4 and #8 who were exposed to contrasted CTX levels (0.41 and 3.51 ng CTX1B eq. g^{-1}, respectively), only patients from event #4 required hospitalization whereas the victim from event #8 necessitated only ambulatory care (Table 4). Likewise, in event #10, the consumption of a weakly contaminated herbivorous fish (CTX level comprised between 0.03 and 0.06 ng CTX1B eq. g^{-1} (i.e., the LOD and LOQ of the CBA-N2a)) was enough to trigger a wide array of digestive, cardiac, and neurologic symptoms in the affected patient, whereas the patient from event #14 only experienced neurologic signs despite the consumption of a carnivorous fish (*Lutjanus gibbus)* contaminated with up to 6.2 ng CTX1B eq. g^{-1}. The case of patient #12 with a history of 4-6 previous CP, who had onset of symptoms within 48h after ingesting a *Caranx ignobilis* specimen dosed at 1.9 ng CTX1B eq. g^{-1}, is even more puzzling (Table 4).

Overall, comparison with the limited data available from other CP endemic regions confirms CTX levels measured in fish remnants implicated in toxic incidents in Hawaii (Pacific), Guadeloupe (Caribbean), and India (Indian Ocean) may vary considerably, ranging from 0.02 up to 2.6 ng CTX1B eq. g^{-1} (for a review, see Chinain et al., 2021). All these observations suggest the setting off and severity of CP illness may be a function of multiple factors, including individual susceptibility (e.g., age, previous CP history, co-morbidities, etc.), dose of toxins ingested, and type of fish consumed.

4. Ciguatera risk management in French Polynesia: current challenges and opportunities

The strong cultural attachment of island communities to marine resources makes them particularly vulnerable to CP risk. According to Skinner et al. (2011), taking into account under-reporting, an estimated 500,000 Pacific Islanders might have suffered from CP between 1973 and 2008. Despite sustained efforts to guarantee consumers' protection in French Polynesia, the implementation of efficient CP risk management programs is hampered by several well-identified challenges (e.g., the lack of updated regulatory texts or marked risk-taking behaviors among local communities) which need to be considered for the design of tailored management strategies. Fortunately, building on some of the opportunities also specific to this country, in particular those linked to the socio-cultural ramifications of CP, could prove a relevant, effective approach. Both aspects will be briefly addressed in the following section.

4.1 Current challenges

4.1.1 Heavy reliance of local communities on fish products

Fish contribute substantially to the livelihoods and household incomes of local populations in PICTs. French Polynesia is no exception to the rule: in a survey conducted on Moorea Island (Society archipelago), over 50% of households interviewed declared they consume fish six to seven times a week, and 76% had at least one member of the household actively involved in reef fishing activity (Morin et al., 2016). Overall, fish products represent 46% of the self-produced food consumed annually in French Polynesia, with 82% of the fish caught by the household (ISPF, 2020). Consequently, fish consumption rates reported in several islands are among the highest in the world (i.e., an average annual fish consumption rate of up to 167 kg capita^{-1} year^{-1} (DIREN, 2015) as compared to a global rate of 18.8 kg capita^{-1} year^{-1} (FAO, 2012)). Fish also represent a major source of income for local communities. Unfortunately, CP often frustrates the intention of establishing prolific commercial lagoon fisheries in many atolls regarded as CP hotspots. In addition, the well-known reputation of French Polynesia as a country at high risk of CP also represents a major impediment to the development of reef fish export trade towards European markets.

While well-recognized, the true implications of CP on local economies in terms of public health, fish trade, and tourism are difficult to quantify. Few studies are available for the Pacific region, such as in French Polynesia (Glaziou and Legrand, 1994; Morin et al., 2016) and the Cook Islands (Rongo and van Woesik, 2012), but the lack of consistent and organized economic data - a common situation for small island nations - generally places a wide-error margin around current estimates.

4.1.2 Lack of updated regulatory texts

Despite the alarming, yet under-estimated statistics of CP, a limited number of texts regulating fish trade in relation to CP risk currently exists in French Polynesia. In total, 10 deliberations, decrees, orders, or country laws, most of which should be regarded as obsolete, are found, such as the municipal decree of November 8th, 1939 prohibiting the sale of eight reef fish species in the Papeete municipal market. Likewise, article n° 19 of the municipal deliberation n° 68-51 of August 18th, 1968 regulating the sale of fish on local public stalls, states: "*it is forbidden (...) inside the market, to sell fish deemed toxic* ". But in the absence of specific fish control programs, such a ban remains difficult to enforce. At the EU level, several directives exist, such as the Order n° 1183 CM of December 20th, 2005 prohibiting the export towards EU countries of "*poisonous fish in the Tetraodontidae, Molidae, Diodontidae, and Canthigasteridae families, and of fishery products containing biotoxins such as ciguatera or other toxins deleterious to human health*". No regulatory limits for CTXs are currently in place, and a duly validated reference test is still lacking. All these observations highlight the urgent need for establishing a more appropriate legislation in French Polynesia to minimize the risk of placing contaminated fish on markets.

4.1.3 Poor traceability of fish products

Another major challenge in the implementation of effective CP management programs is the poor traceability of lagoon and reef fish products from fishing sites to retail outlets. In 2020, it was estimated that around 770 tons of the lagoon fish caught in French Polynesia (i.e., 18% of the overall production) were transferred from other archipelagoes to the main island of Tahiti, by far the most populated (ISPF, 2020). The majority of these products originates from atolls of the western Tuamotu, most notably Rangiroa, Arutua, and Kaukura, which alone accounted for approximately 30% of the lagoon fish exported to Tahiti (DRM, 2020). This means a significant proportion of potentially toxic fish are sold in markets far away from their source. In Tahiti, consumers have access to fish products through a variety of distribution channels, including public markets, supermarkets, stores, restaurants, family gifts, and even streetside vendors. Tracing a fish back to its home waters in the event of an outbreak is thus often complex if not impossible due to the multiplicity of resellers and the lack of a ticketing system. Since June 2022, however, a country law was issued in French Polynesia to allow some form of traceback: it requires that any fish vendor (e.g., wholesalers, retailers, resellers, processors, etc.) selling fish products to local companies or restaurants and/or public administrations including canteens, must be duly registered. Establishing an invoice giving clear indication of the geographical origin of fish has also become mandatory (DRM, 2022).

4.1.4 Marked risk-taking behavior among local populations

A common observation in CP-endemic regions is that exposure to CP does not act as a complete deterrent to eating fish, or even fish that are known to be ciguatoxic, and this appears to be an accepted risk amongst Oceanian peoples (Baumann et al., 2010; Rongo and van Woesik, 2011). Data collected through the country-wide epidemiological surveillance program currently in place in French Polynesia show that in 2018 approximately 52% of CP victims have actually experienced previous CP at least once in their lifetime, and even up to 10 or 20 times for some of them (extracted from the epidemiological surveillance network database). This is particularly obvious in communities living in remote islands where food availability and diversity are more limited than in Tahiti and the main islands. This somewhat fatalistic attitude towards CP translates into high risk-taking behaviors in terms of consumption habits. In a recent questionnaire survey conducted among French Polynesian fishing communities, 29% of informants with previous history of CP declared they were fully aware they were taking a risk while consuming the toxic fish meal. Among the many reasons given for this high risk-taking behavior were: (i) the pleasure of eating the fish (even if suspected toxic) was more compelling than the potential discomfort associated with the disease; (ii) although they suspected the fish was toxic, they deliberately consumed it to prevent other family members from poisoning themselves; (iii) they have a strong confidence in the efficacy of traditional remedies that are easily accessible and widely used throughout PICTs (Chinain et al., 2020b). Another specific risky consumption habit often reported among French Polynesian communities is the deliberate consumption of fish parts rich in CTXs such as the head, roe, and viscera, which are regarded as delicacies in some islands. By way of example, during the fatal mass-poisoning outbreak that affected Rapa Island between 2009 and 2010, approximately half of the 114 victims who reported their illness declared they had consumed the viscera and/or head of the fish (Chinain et al., 2020c). These observations emphasize the importance of sustained educational and preventive management efforts among local populations (see section 3.4.2c).

4.2. Current opportunities

4.2.1 French Polynesia, the ground of active CP research since the 1960's

Despite the very high incidence rates consistently reported from PICTs, few countries in the Pacific region currently have a defined action plan in response to CP threat (Chinain et al., 2021). In this regard, French Polynesia stands as an exception as it is the only nation among PICTs having a long-term monitoring program and a small unit entirely devoted to CP research based at ILM. Since 2007, the strategy developed by ILM in CP control programs is an integrated approach which comprises four main elements: (i) a country-wide epidemiological surveillance program (see section 5.1), (ii) a technology watch aiming at improving the field-monitoring and detection capabilities of CP organisms and toxins in the laboratory, (iii) field campaigns in sensitive French Polynesian lagoons to investigate the etiology of novel, atypical poisonings, and/or provide local populations with risk maps of fishing areas and species to avoid based on the random evaluation of CTX accumulation in a wide array of marine organisms highly prized by local populations, and (iv) community outreach interventions targeted at healthcare workers, fishermen, and the general public (Chinain et al., 2020a).

Owing to this context of active CP research, an action plan currently exists in French Polynesia in response to CP incidents, which involves four main entities: ILM, the Health Monitoring Office and Observatory (BVSO), and the Environmental Health Office (BSE) placed under the authority of the Ministry of Health, and the Marine Resources Directorate (DRM) under the umbrella of the Ministry of Marine Resources. Each entity has respective mandates in relation to seafood safety, monitoring, or management of CP. More precisely, the Laboratory of Marine Biotoxins of ILM centralizes the CP case declaration forms received from public health structures, and issues monthly and annual reports. In the event of a major outbreak, or if poisoning cases specifically involve fish purchased in public retail outlets or consumed in restaurants, ILM sends alerts to both the BVSO and BSE, to allow timely public health actions from the BVSO (i.e., confirmatory epidemiological investigations, and liaison with public health structures for the dissemination of prevention messages among local populations). The BSE issues requests for confirmatory (CTX) analyses on suspect fish batches by ILM, conducts trace-back investigations with the help of DRM among supermarkets and restaurant owners to formally identify the source of the toxic lots (e.g., identity of wholesaler or retailers, harvest area of the incriminated marine products, etc.), and, if needs dictate, takes all appropriate measures to remove toxic fish from retail outlets. Finally, in an outbreak context, ILM also has the responsibility of conducting field-monitoring campaigns in affected islands.

Toxin-producing cultures of “hot” strains of *Gambierdiscus* represent a significant source of algal CTXs (Longo et al., 2020). Preliminary studies by Ikehara et al. (2017) also opened promising prospects as to the preparation of piscivorous reference toxins from algal toxins by way of enzymatic oxidation experiments. In this regard, the active research conducted at ILM over the past two decades can greatly benefit current efforts to establish a sustainable source of CTX standards whose shortage is severely limiting the implementation of food safety surveillance programs globally. Practically, our researches not only confirmed the ability of French Polynesian strains of *G. polynesiensis* to produce a wide array of Pacific CTX analogs (Chinain et al., 2010a; Longo et al., 2019) in relatively high amounts, they also suggest toxin production (types of Pacific CTX analogs produced, in addition to amounts) can be maximized by way of *in vitro* manipulation of environmental variables (Longo et al., 2020). In parallel, the significant sampling efforts deployed by the laboratory in the frame of the numerous field campaigns conducted throughout French Polynesian islands over the past two decades have allowed the establishment of a unique bank of biological materials (representing approximately 2750 fish samples) duly characterized in terms of their ciguateric status. Current efforts at ILM aim at capitalizing on these major research outcomes to accelerate the production of algal and fish reference material (including CTX standards) for easier commercial access by the scientific community. As a result, the Phyconesia website was recently put online (www.phyconesia.pf*)*

4.2.2 Benefits derived from traditional knowledge

The famous Cuban naturalist Felipe Poey's observation that regulation will not substitute for people having sufficient knowledge about CP to protect themselves is as valid today as it was in the mid-1800s (Morrison et al., 2008). Observations suggest many fishing communities living in CP-endemic areas have developed a remarkable local ecological knowledge of specific areas, fish species, and/or seasons to avoid when fishing, as highlighted in the studies by Chinain et al. (2010b) and Raab et al. (2021) in Raivavae Island (French Polynesia) and Puerto Rico, respectively. This likely explains why CP risk is often trivialized among these communities.

Toxic fish are not distinguishable from edible ones as CTXs do not seem to affect fish physiology and behavior, fish aspect (skin color variation), smell, or taste (Lehane and Lewis, 2000). Due to the persistent lack of field tests currently unavailable to local communities, subsistence and recreational fishers have developed a number of traditional practices and adaptive strategies which hopefully contribute to compensate for the high risk taking behaviors highlighted above.

Local residents in endemic areas often rely on a variety of folk tests to detect toxic fish that are quick to perform on their daily fish catches. The most widespread test is a "folk bioassay" that consists in feeding a piece of the suspect fish (usually the liver) to ants, flies, dogs, or cats, and watching for signs of poisoning in the animal (Lewis, 1986; Darius et al., 2013). However, few of these folk tests are scientifically validated. Of note, a study aiming at evaluating the effectiveness of two homemade tests based on the physical characteristics of fish, namely the *rigor mortis* test (RMT) and the hemorrhagic test (HT), was conducted in Raivavae Island (Australes archipelago). According to RMT and HT, flabby fish and the presence of hemorrhagic signs at the cut-tail of fish, respectively, are the signs that these fish are ciguatoxic (Darius et al., 2013). For this purpose, a total of 107 fish were collected by spear-fishing at various fishing grounds around the island and subjected to the evaluation of five local testers by means of these two tests, and answers were further compared to rRBA toxicity data obtained in the laboratory. Results showed the performance of RMT and HT varied from one tester to another, depending mainly on the tester's skill and/or whether he had a long practice of the test. The best scores obtained using RMT and HT were 55% and 70%, respectively, with the best agreement between testers also obtained with HT. The authors concluded that the use of these two traditional tests combined with a good knowledge of the "hot" fishing sites and fish species may help minimize the poisoning risk (Darius et al., 2013).

Complementary to these folk detection tests, local populations have also developed a number of adaptive strategies to avoid or minimize the risk of contracting CP, such as avoiding fishing in CP-prone areas and rejecting risky fish species, discarding liver and head before cooking, favoring the consumption of fish specimens of smaller size, space out fish meals, etc. (Lewis, 1983; Lewis, 1986).

Another cultural trait characteristic of French Polynesian natives is the great confidence they have in the efficacy of traditional remedies over Western medicine (Chassagne et al., 2022), which undoubtedly influences their perception of CP risk, and may contribute to exacerbate risk taking behaviors in some individuals (see section 3.4.d). Indeed, in CP-endemic areas, phytotherapeutics are often prescribed (Kumar-Roiné et al., 2011). Roots, leaves, barks, or fruits are prepared by decoction, infusion, or maceration, in different proportions and to a proper dosage, based on "recipes" handed down through generations. In PICTs, nearly 100 plants listed in traditional medicine are used to treat CP (Chinain et al., 2020a, and references therein). However, their efficacy has not yet been systematically studied in clinical trials. Interestingly, a survey conducted in French Polynesia concluded that 49% of public health professionals willingly recommend the use of traditional medicine to CP patients (ILM, unpublished data), most notably a decoction prepared from the leaves of *Heliotropium foertherianum.* Its main active ingredient, rosmarinic acid, isolated from the plant's leaves, has demonstrated striking neuroprotective effects against CTXs *in vitro* (Rossi et al., 2012; Braidy et al., 2014). Such findings not only corroborate the relevance of the widespread use of this plant among local populations, but also open promising prospects in studies investigating treatments to improve patients' care. It is suggested that in communities highly exposed to CP risk, the use of some of the above traditional practices combined with an accurate local ecological knowledge may contribute to maintaining poisoning incidents at an acceptable level.

4.2.3 Benefits of information, education, and outreach for primary prevention

Since timely diagnosis and treatment often are not available to sick people, developing a prevention approach is of outmost importance to help reduce the still very high CP incidence rates in PICTs. In French Polynesia, primary prevention relies heavily on community outreach and education. Examples of information useful to recreational or subsistence fishermen and the communities they serve are risk maps identifying local reef areas with a high density of ciguatoxic *Gambierdiscus* spp. populations and fish, a list of the most commonly ciguatoxic fish species specific to each island, or general information on the disease and treatment options including dietary recommendations to limit the risk of CP relapses. The dedicated CP websites of ILM (www.ciguatera.pf; https://ciguawatch.ilm.pf/) provide a wide range of downloadable information on CP, such as educational brochures and posters available in multiple languages, CP interactive maps, and statistics on CP epidemiology in French Polynesian islands including ciguatoxic fish species involved in CP outbreaks. Culturally-tailored outreach interventions held during public meetings organized on the sidelines of field campaigns as well as educational modules presented during school interventions in the frame of “week of science” or citizen science events complement this communication strategy. An information kit targeted specifically at the medical sector to improve CP case reporting by healthcare workers is also available. Experience shows that outreach interventions can help reduce poisoning cases in a significant way, as attested by the five-fold reduction in CP incidence rate observed in Rapa between 2009 and 2012. In this particular case, educating local communities about the origin of CP and preventive measures appeared to create self-regulating behavior among local residents, as illustrated by the noticeable changes in both fishing habits (i.e., avoidance of CP-prone species and fishing sites) and consumption habits (e.g., significant drop in the number of patients who declared they consumed fish viscera and/or head, or individuals who clearly avoided sharing meals of potentially toxic fish with multiple guests to limit poisoning risk (Chinain et al., 2020c)).

5. Lessons learnt and future directions

Much progress has been made in the understanding and management of CP risk over the past decades, thanks in part to sustained monitoring efforts and the increasing availability of refined techniques. In this regard, significant breakthroughs from international research programs were critical in advancing CP knowledge and assisting in its management in French Polynesia. By way of example, the widespread use of cheap, easy-to-use artificial substrates combined with species-specific assays now allow a systematic and more reliable assessment of the biodiversity and dynamics of CP organism communities. As a result, a more cost-effective approach to identify areas where CP risk is greatest can be considered, consisting in the regular monitoring of only species of concern in the genera *Gambierdiscus* and *Fukuyoa*. Moreover, the recent introduction of even more powerful approaches in molecular analysis, such as metabarcoding, will help to uncover the true extent of the diversity of other potentially toxic HAB species (e.g., *Ostreopsis* spp. and *Prorocentrum* spp., which often co-occur with *Gambierdiscus* in ciguateric biotopes), and provide insights into the likelihood of emerging toxin risks in French Polynesian waters. Concurrently, the significant advances achieved by international groups in the last few years in the development of analytical methods able to evaluate and characterize the different toxic analogs involved in poisoning incidents now provide health authorities and the scientific community with essential tools towards a more reliable diagnosis and effective management of these toxin risks.

Lessons learnt over the course of this continuous history of CP research in French Polynesia that could benefit other nations currently confronted with CP problem mainly relate to management options: (i) the necessity to collate adequate CP epidemiological data (e.g. symptomatology and CP incidence rates) while providing local populations with updated information on the locations at high risk of ciguatera based on thorough environmental surveys of *Gambierdiscus* populations and CP-prone fish species; and (ii) the importance of extending such surveys to other organisms most representative of tropical food webs, in particular several species of marine invertebrates which represent a potential risk for human consumption. The combined knowledge derived from these efforts will help rationalize the monitoring and management economic costs of CP in the long run, a limiting factor for many developing countries in endemic areas. Indeed, although many of these countries have identified CP as a national priority in terms of seafood safety, food security, and nutrition, very few of them actually have ongoing monitoring programs due mainly to a lack of resources and competing health priorities. Regarding prevention of CP, several practical information drawn from the French Polynesian experience must be considered: in many instances, poisonings are not necessarily and systematically associated with the consumption of large reef fish, as the consumption of small individuals (including among herbivores) can also lead to severe CP incidents. Moreover, in countries where local communities are chronically exposed to marine products tainted with infra-toxic doses of CTXs, consumers will likely develop CP symptoms at lower CTX levels than those living in non-endemic areas, thus stressing the need to set up regulatory/guidance limits tailored to each country. In CP endemic areas, the strong cultural ramifications of CP largely influence risk perception among local populations and often translate into high risk-taking behaviors in terms of consumption habits. Hence, quoting (Morrison et al., 2008), "decisions about the management of ciguatera cannot be separated from its broader socioeconomic and ecological context, which implicitly requires a multidisciplinary approach, including contributions from social sciences". This latter approach could be key in elaborating tailored prevention messages.

Despite the major advances mentioned above, CP outbreaks are still difficult to predict and avoid due to the uncertainty of *Gambierdiscus* blooms, and the variability of CTX accumulation in fish, highlighting the need for intensified field work globally to ground-truth hypotheses generated in the laboratory. These field data will be useful to inform spatial risk maps and/or predictive models of the abundance and seasonality of *Gambierdiscus* spp. populations, and related severity of CP outbreaks. At a local level, research areas deserving particular attention have been identified in order to capitalize on the expertise developed over the past several years. First, little is known about the effects of CP-related toxins in marine organisms. A limited number of biological models have been tested in recent papers. It will be interesting to adapt such studies to the local context by assessing the direct toxicity of *Gambierdiscus* spp. on economically valuable seafood species to help with the management of fishery resources in French Polynesia. Regarding CP victims, the current lack of specific biomarkers to confirm human exposure to CP toxins explains the persistent failure by health professionals to achieve proper diagnosis of CP and provide timely medical care to CP victims. In this context, developing methods capable of detecting CTXs in patients' blood, urine, or feces could assist in identifying cases consistently. In addition, research efforts towards the identification of effective treatment options remains a priority. The noticeable lack of traceability of fish products harvested in areas far away from their sale points constitutes another crucial issue in French Polynesia, but significant progress towards the implementation of an effective trace-back system is to be expected as ongoing work for increased coordination builds upon the current dynamics and communication between agencies presently involved in the response to CP incidents. Lastly, despite the very high IRs reported from PICTs, CP remains dangerously under-recognized by most national governments in the Pacific region. In order to implement a more proactive surveillance of this disease at a regional scale, ILM has recently developed an online platform (https://ciguawatch.ilm.pf/) to provide PICTs with practical tools, comprehensive tutorials, and technical and/or expertise support for improved surveillance and mitigation capacities in the Pacific region. If successful, the combined efforts generated in the frame of this regional initiative should allow developing a shared regional database on CP that includes not only epidemiological and environmental data, but also awareness material and traditional knowledge gathered from a wide array of stakeholders. This, in turn, could benefit current international initiatives which focus on data collation and sharing on the distribution of HAB species and in particular *Gambierdiscus* and records of CP events wih human impacts, through the OBIS (Ocean Biodiversity Information) and HAEDAT (Harmful Algal Event) databases, respectively (Hallegraeff et al., 2021). To advance in all these research fields, improved networking at a regional and international level aroused by the renewed interest in CP will hopefully assist in the definition of a global response to a global problem.

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Table 1. CTX-like toxicity in wild *Gambierdiscus* spp. collected from macroalgal samples in various locales of French Polynesia.

Archipelago	**Island**	**Sites**	**Year**	**n***	**Macrophyte sampled**	**Abundance Cells g^{-1} algae**	**CTX levels** (pg CTX3C eq. cell^{-1})	**References**
Society	Tahiti	Teavaraa Pass **	1999-2002	144	*Jania* and *Amphiroa* algal turf	[0 – 13,965]	[< LOD – 18.96]	this study

Marquesas	Nuku-Hiva	Anaho Bay	2004	2	*Amphiroa fragilissima, Chlorodesmis fastigiata, Halimeda distorta, Chnoospora minima*	[3 - 20,000]	[0.85 - 3.9]	(Darius et al., 2007)
Australes	Raivavae	Motu de la Femme	2008	2	*Halimeda, Jania, Amphiroa, Turbinaria,*	[11 – 141,890]	[4.1 - 5.0]	(Chinain et al., 2010b)
	Rapa	Akatamiro Bay, Iripau Bay, Motu Tarakoi, Cape Komire	2010	4	*Lobophora variegata, Dictyota dichotoma*	[2,250 – 10,350]	[0.5 - 13.5]	(Pawlowiez et al., 2013)

*n= total number of wild *Gambierdiscus* samples analyzed in each sampling site. ** GPS coordinates of Teavaraa Pass: 17°46’95’’S - 149°28’34’’W.
CTX levels: data correspond to the lowest and highest CTX-like toxicity values measured in wild samples using rRBA.
LOD of rRBA established at 0.0015 pg CTX3C eq. cell^{-1}.

Table 2. CTX-like toxicity of the 123 *Gambierdiscus* spp. clones currently available in the ILM culture collection, as assessed by CBA-N2a.

Species	**Gambier**	**Australes**	**Society**	**Marquesas**	**Tuamotu**	**NT****
G. polynesiensis	[0.050 ± 0.008 - 7.85 ± 1.14] n* = 9	[1.03 ± 0.12 - 4.17 ± 0.62] n = 2	7.75 ± 2.60 n = 1	[3.25 ± 0.76 - 6.84 ± 1.27] n = 5	[0.487 ± 0.074 - 5.19 ± 1.38] n = 3	20
G. pacificus	[< LOD - 0.008] n =2	< LOD n=4	[< LOD - 0.002] n=4	[< LOD - 0.0022] n=6	< LOD n=6	22
G. australes	[< LOD - 0.020 ± 0.003] n =10	[< LOD - 0.092 ± 0.015] n=12	[< LOD - 0.04 ± 0.009] n=5	-	< LOD n=2	29
G. toxicus	[< LOD - 0.0058] n =13	-	[< LOD - 0.004] n=3	< LOD n=1	-	17
G. carpenteri	-	< LOD n=3	< LOD n=4	[< LOD - 0.025 ± 0.005] n=13	-	20
G. honu	< LOD n=1	[< LOD - 0.0014] n=4	-	-	-	5

G. caribaeus	[< LOD - 0.019 ± 0.002] n =10	-	-	-	-	10

* n= number of strains tested per species and island group. **NT = total number of strains per species.
The composite CTX-like activity, expressed in pg CTX3C eq. cell^{-1}, was assessed by CBA-N2a in cell extracts of *Gambierdiscus* spp. clonal cultures. Toxicity data correspond to the lowest and highest values measured in the (n) strains tested within each species. Clones with low toxicity (< 0.008 pg CTX3C eq. cell^{-1}) were tested once whereas the remaining clones were tested in three independent CBA-N2a assays. Mean ± SD values were calculated from three independent experiments.

Table 3: Prevalence of toxic fish in CP hotspots of French Polynesia.

Island	Years of sampling	Number of fish tested	% toxic fish	Mean IR* (/10,000 inhab.)
Fakarava	2008	157	87	389
Moruroa	2003, 2004, 2005, 2006, 2007, 2008	217	87	**
Rapa	2010	251	77	857
Nuku Hiva	2004, 2015, 2016, 2018	262	76	57
Raivavae	2007, 2008, 2009	324	66	132
Gambier islds	2012, 2013	368	59	208

* Mean IRs rates were calculated from CP records received at ILM during the year(s) of sampling.

** Uninhabitated island.

Table 4. *Toxicity analyses on fish remnants involved in CP events reported in French Polynesia between 2006 and 2020, and case-related information*

Event	Fish species	Nb affected people /Nb of guests	Nb declared case /Nb affected people	Age	Sex	Part(s) consummed	Nb previous CP	Onset of symptoms	Symptoms			Toxicity in fish flesh **	
									D	C	N	rRBA	CBA-N2a
#1	*Caranx melampygus*	2/2	1/2	45	F	Head	0	ns	X		X	0.69	
#2	*Cheilinus undulatus*	4/4	1/4	40	F	Flesh	0	5h	X		X	1.13	
#3	*Lethrinus sp*	3/3	3/3	12	M	Flesh	0	8h	X	X	X	1.92	
				51	M	Flesh	2	5h	X		X		
				49	F	Flesh + head	1	2h	X		X		
#4	*Lethrinus obsoletus*	2/3	2/2	ns	M*	ns	ns	<12h			X	0.41	
				ns	F	Head	1	<12h	X		X		
#5	*Lethrinus obsoletus*	1/1	1/1	60	M	Flesh +viscera	1	24h			X	0.46	
#6	*Plectropomus laevis*	7/7	1/7	42	M	Flesh	0	4h		X	X	1.02	
#7	*Epinephelus polyphekadion*	3/3	3/3	51	F	Flesh (8 fish nuggets)	1	3-4h	X	X	X	0.57	
				24	M	Flesh (6 fish nuggets)	0	12h	X		X		
				20	F	Flesh (2 fish nuggets)	1	15h			X		
#8	*Lutjanus bohar*	5/5	1/5	50	F	Flesh	0	≤2h	X	X	X	3.51	
#9	*Acanthurus xanthopterus*	2/2	1/2	52	F	Head	0	6h	X	X	X	0.24	
#10	*Scaridae*	1/1	1/1	40	M	Flesh	0	ns	X	X	X		<LOD-LOQ>***
#11	*Gymnothorax* sp.	4/4	1/4	47	F	Flesh	1	4h	X	X	X		1.1
#12	*Caranx ignobilis*	7/7	1/7	61	M	Flesh	4-6	48h	X		X		1.9
#13	*Carangidae*	2/2	2/2	32	M	Flesh	0	>12h	X		X		0.87
				34	F	Flesh	0	>12h			X		
#14	*Lutjanus gibbus*	1/1	1/1	51	M	Flesh +Head	3	11h			X		6.2
#15	*Lutjanus bohar*	3/3	3/3	53	M*	ns	ns	<3h	X	X	X		6.62
				48	F*	ns	ns	<3h	X	X	X		
				26	F*	ns	ns	<3h	X	X	X		
#16	*Lethrinus olivaceus*	1/1	1/1	61	F	Flesh	0	<2h	X	X	X		5.31

Symptoms: (D) digestive – (C) cardiovascular – (N) neurological. ns: not specified. * patients who required hospitalization

** Toxicity analysis processed on fish remnant (flesh only) from the individual that was consumed and responsible for symptoms.

Toxicity data as assessed by rRBA (Chinain et al., 2020) and CB-N2a are expressed in ng CTX1B eq g^{-1}. *** CTX levels measured in this fish was between the CBA-N2a limit of detection (LOD) and limit of quantification (LOQ) established at 0.03 ng CTX1B eq g^{-1} and 0.06 ng CTX1B eq g^{-1}, respectively.

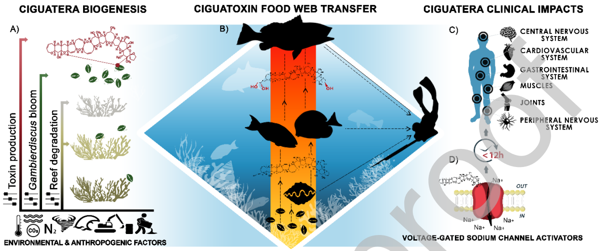

Figure 1: *Ciguatera Poisoning. A) biogenesis in coral reef ecosystems; B) ciguatoxin (CTX) transfer in marine food webs; C) health impacts of CTXs in consumers; D) voltage-gated sodium channels as biological targets of CTXs.* © Institut Louis Malardé

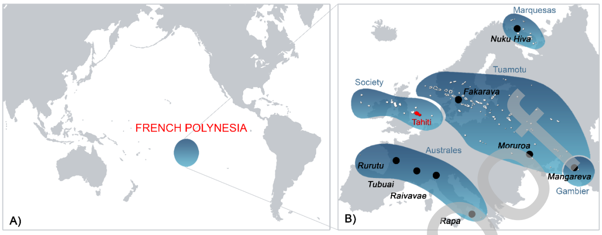

Figure 2: *Maps of* A) *French Polynesia (South Pacific) and B) its five island groups (from North to South, Marquesas, Society, Tuamotu, Australes and Gambier archipelagoes) overlaying the map of Europe. The islands and atolls where environmental and toxic surveys were conducted are also specified.*

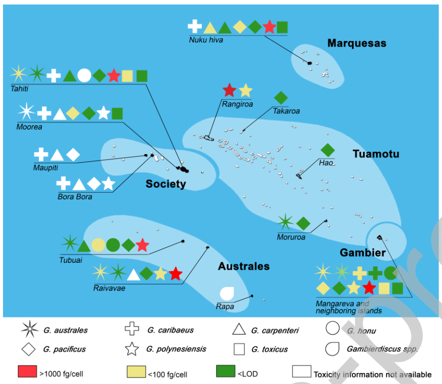

Figure 3 : Current state of knowledge on the genetic diversity and distribution of Gambierdiscus spp. in the five island groups of French Polynesia. Species diversity data combined the results of qPCR assays on both WS samples and clones isolated from field material. Toxicity data, available only for Gambierdiscus clones successfully established in the laboratory, are also shown and were obtained using the CBA-N2a.

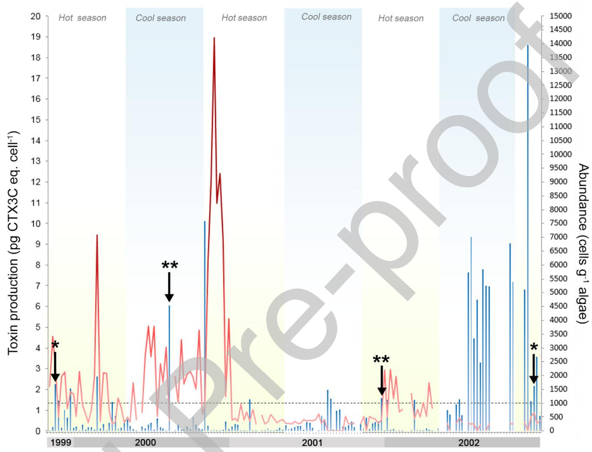

Figure 4. *Follow-up of the abundance (in blue) and related toxicity (in red) of Gambierdiscus spp. populations sampled weekly on macroalgal hosts in the study site of Teavaraa Pass in Papara (Tahiti, French Polynesia). During the study period, the hot and cool seasons lasted from November to April, and from May to October, respectively. Cell densities, expressed in cells gww^{-1}, were assessed microscopically. The horizontal dashed line indicates the cell density threshold corresponding to a bloom (set arbitrarily at 1000 cells gww^{-1}). Toxicity data, expressed in pg CTX3C eq. cell^{-1}, were obtained using rRBA.*

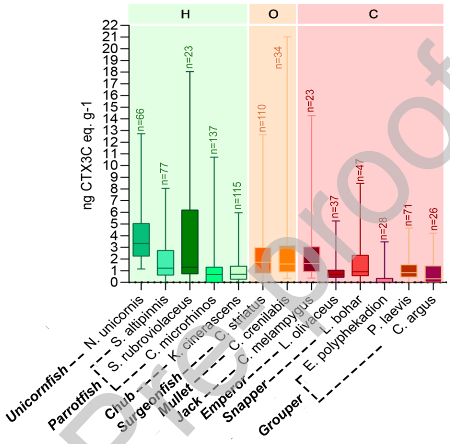

Figure 5*: Distribution of CTX levels in 13 fish species for which n > 20. Fish were collected in the frame of toxic surveys conducted between 2003 to 2011 in Fakarava and Moruroa (Tuamotu archipelago), Tubuai, Raivavae, Rurutu, and Rapa (Australes archipelago), and Nuku Hiva (Marquesas archipelago).*

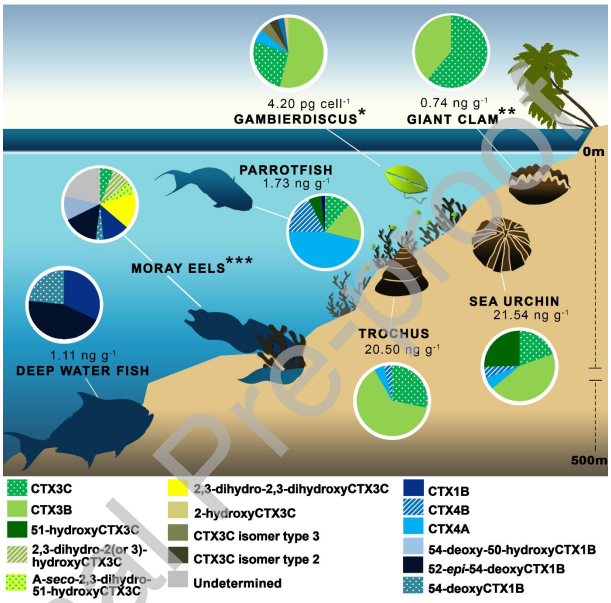

Figure 6. *LC-MS/MS determination of CTX toxin profiles in various organisms across multiple food web components of French Polynesia. LC-MS/MS protocols followed those described in Sibat et al. (2018). The number associated with each pie chart corresponds to the overall CTX content estimated by LC-MS/MS in each type-organism (expressed in pg CTX3C eq. cell^{-1} and ng CTX3C eq. g^{-1} in Gambierdiscus and marine organisms, respectively). All toxin profiles were characterized from wild specimens (Darius et al., 2018a;*

*Darius et al., 2018b; Sibat et al., 2018; Darius et al., 2022) except for *Gambierdiscus (cells of a clonal culture of RIK7 G. polynesiensis harvested at stationary phase, (Longo et al., 2019)), **giant clams (Tridacna maxima individuals fed G. polynesiensis cells analyzed in the frame of ex situ contamination experiments, (Roué et al., 2016)), and *** moray-eels (data derived from historical studies by Yasumoto and coworkers, (FAO-WHO, 2020).*

Figure 7. A) *Anaho Bay study site in Nuku Hiva Island (Marquesas archipelago, French Polynesia). Underwater pictures of B) Tectus niloticus and C) Tripneustes gratilla.* © UMR EIO

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: