

Ciguatera Fish Poisoning in the Gulf and Caribbean: What Do We Really Know?

Intoxicación por Ciguatera Peces en el Golfo y el Caribe: ¿Qué es lo que Realmente Sabemos?

Ciguatera dans le Golfe et des Caraïbes: Que Savons-nous Vraiment?

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ABSTRACT

Globally, ciguatera fish poisoning (CFP) is the principal cause of non-bacterial illness associated with seafood consumption. The toxins (ciguatoxins) responsible for CFP are produced by dinoflagellates in the genus *Gambierdiscus*, which are endemic to tropical and sub-tropical areas. Ciguatoxins are lipophilic and bioaccumulate in marine food webs, typically reaching their highest concentrations in large, carnivorous fish. While rarely fatal, CFP can cause a multitude of gastrointestinal, neurological and cardiovascular symptoms that can persist for days, months or years. There is no cure for CFP, however promising research that may provide treatment options is underway. Currently all CFP treatments are simply supportive therapy for relieving the symptoms. CFP is prevalent throughout the Caribbean, although reports of incidences are scattered. The fact that CFP reporting has been inconsistent in many areas makes it difficult to accurately analyze the spatial and temporal variation in CFP occurrence. In general, CFP in the Caribbean has been managed through traditional knowledge of local fishers and residents. However, because *Gambierdiscus* abundance and water temperatures are positively correlated, there is concern that increasing seawater temperatures may increase the range of *Gambierdiscus* and incidences of CFP. With this concern and the resurgence of interest in CFP in the Caribbean and Gulf of Mexico prompted by the potential of commercial harvests of invasive lionfish, it is imperative that we understand the capabilities of new detection and monitoring techniques as well as the food handling and liability issues.

KEY WORDS: Ciguatera fish poisoning, lionfish, management, detection

OVERVIEW

It is difficult to get an accurate picture of the spatial variation of ciguatera fish poisoning (CFP) incidences throughout the Gulf and Caribbean because CFP is difficult to diagnose, it is generally managed locally, and reporting CFP is not mandatory in all areas. Anecdotal information about ciguatera fish poisoning (CFP) and its effects are widespread. For this reason our working group made a commitment to a series of studies on the ecology, taxonomy and physiology of *Gambierdiscus* to provide monitoring capabilities for CFP and inform management decisions. The first effort was an active survey to uniformly query local public health professionals and fishery managers requesting information on CFP for the 11-year time period 1996 through 2006. At the same time we completed an intensive literature review for CFP in the Caribbean, including cases caused by fish eaten in Caribbean and reported elsewhere. By incorporating the information reported by the 24 island nations and territories, as well as the 9 mainland countries along the Caribbean Sea, we mapped the occurrences of CFP in the Caribbean and compared those data with the published literature (Figure 1) (Tester et al. 2009, 2010).

In the next study we examined relationships between sea surface temperatures (SSTs) and CFP incidence rates in the Caribbean (Tester et al. 2010). This was done in tandem with a series of experiments designed to determine the effects of temperature on the growth rate of organisms responsible for CFP. Growth rates of five species of *Gambierdiscus* isolated from the Caribbean and the U.S. South Atlantic Bight were determined at temperatures between 18°C and 33°C (Kibler et al. 2012) (Figure 3). The thermal growth optima for *Gambierdiscus* species were compared with seasonal sea surface temperatures derived from thermal. The highest rates of CFP, between 34 and 59 cases per 10,000 population per year for the period from 1996 through 2006, were identified in the Lesser Antilles in the easternmost part of the Caribbean (Figures 2a,b). These high CFP incidence rates co-occur with some of the warmest water temperatures in the Caribbean and where annual temperatures are least variable (Figures 2A,B). Not surprisingly the data from Kibler et al. (2012) show temperatures of 27-32°C are optimal for the growth of most Caribbean *Gambierdiscus* species (Figure 3).

Significant strides have been made in the taxonomy and the ability to detect *Gambierdiscus* species from the Caribbean. Starting with a revision of the genus *Gambierdiscus* and the description of four new species from the Caribbean (Litaker et al. 2009) this project culminated in the development of species-specific quantitative polymerase chain reaction (qPCR) assays that could detect as few as 10 *Gambierdiscus* cells per sample (Vandersea et al. 2013). The taxonomic revisions were especially important because it appears that not all *Gambierdiscus* species are toxic and others range in toxicity. Before monitoring protocols can be developed, it is vital to know the target species. The species-specific qPCR assays are suitable for a broad spectrum of species identification and quantification applications to support research. The can also serves as a tool to develop risk assessments for CFP local reefs ecosystems or even basin wide risk assessments could be completed with international collaborations.



Figure 1. Ciguatera fish poisoning occurrence in the Caribbean reported by country from 1996 - 2006.

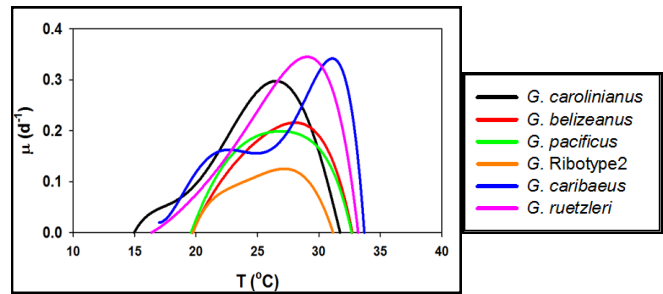
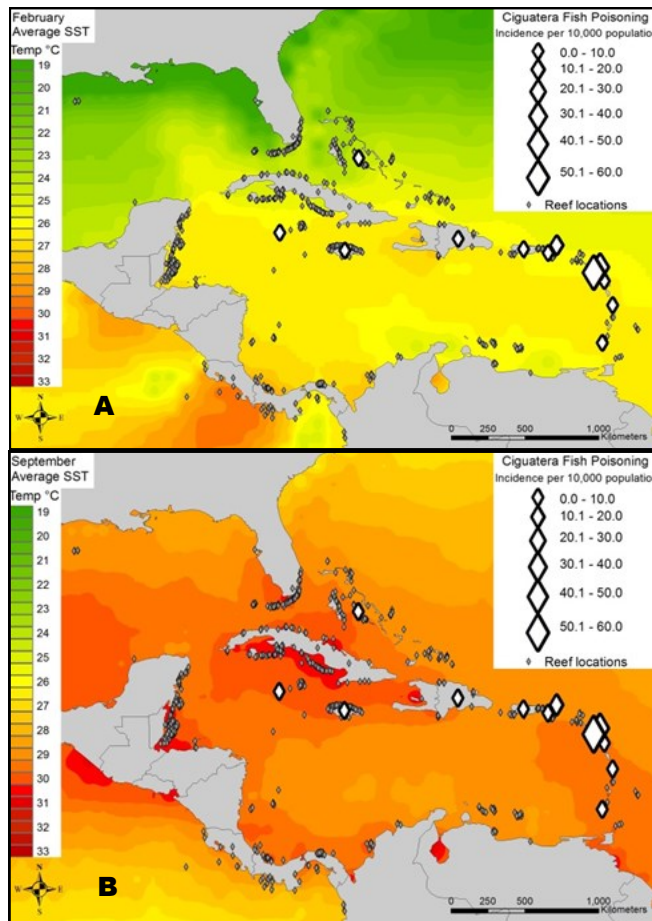


Figure 3. Growth rate versus temperature for six species of *Gambierdiscus* from 15 to 34°C. Summarized from Kibler et al. (2012).



Figures 2A & 2B. A. Average ciguatera fish poisoning incidence rates per 10,000 population per year from 1996–2006 across the Caribbean, plotted with temperature contours ($^{\circ}C$) from annual average sea surface temperatures for February, the coldest month of the year from 2002–2007. B. Average ciguatera fish poisoning incidence rates per 10,000 population per year from 1996 to 2006 across the Caribbean, plotted with temperature contours ($^{\circ}C$) from annual average sea surface temperatures for September, the warmest month of the year from 2002 to 2007.

While much progress has been made in the taxonomy, ecology and physiology of *Gambierdiscus* in the last decade our nemesis is toxic detection and quantification. One of several methods currently used to detect ciguatoxins is the receptor binding assay (RBA) that operates on a competitive basis (Caillaud et al. 2010). RBAs are functional assays and the assumption is that the toxicity is proportional to the amount of binding activity. Ciguatoxins in the sample compete with labeled toxins for a specific binding site on sodium channels. The competing toxin is generally labeled with a radioactive compound that can be quantified. There is significant progress with the development of a competitive fluorescence-based synaptosome binding assay for brevetoxins (McCall et al. 2012) that is currently being adapted for use in measuring ciguatoxins in fish. A fluorescence-based assay could be especially useful and widely applied because no radioactive standard is required and this method would not generate hazardous waste. Reducing the costs and eliminating the need for a (radioisotope) certified laboratory would greatly facilitate wider use of this screening assay.

Cytotoxicity assays are also functional assays used for ciguatoxin testing. They typically use tissue culture cells which are incubated with a fluorescent vital stain. After samples are extracted, the extract is serially diluted. Aliquots from the dilution series are added to the cultured cells and incubated. Samples are read and the amount of cell death caused by the extract is quantified by the change in fluorescent signal compared with the control. While functional assays are accepted measures for CTX activity, confirmation of samples by liquid chromatography coupled with mass spectrometry (LC-MS) is the preferred method. LC-MS provides very high sensitivity and selectivity and is used for the identification of CTX in the presence of other compounds. This method requires sophisticated instruments as well as experienced operators for satisfactory results.

Perhaps the greatest impediment to toxin detection is the lack of toxic standards for the ciguatoxins. There is no commercial source for these standards. A summary of the toxicity information for the Caribbean *Gambierdiscus* species shows us that ciguatoxicity has only been tested for

three species (Table 1). Chinain et al. (2010) tested one species, *G. belizeanus*, using the receptor binding assay (RBC) but no separation with liquid chromatography and subsequent mass spectrometry analysis was performed on the extract to confirm the identity of the toxin. Two other Caribbean *Gambierdiscus* species were tested by Lartigue et al. (2010) using cytotoxicity assays and both *G. caribaeus* and *Gambierdiscus* ribotype 2 expressed toxicity as well. The water soluble toxin, maitotoxin, as measured by lysis of human erythrocytes, has been confirmed in five *Gambierdiscus* species and *Gambierdiscus* ribotype 2 (Holland et al. 2013). *Gambierdiscus* ribotype 2 and *G. ruetzleri* had the highest lytic activity while *G. carolinianus* had the lowest. There was no difference in lytic activity associated with different growth temperatures of the *Gambierdiscus* isolates. While it is generally thought maitoxins are not implicated in human intoxication, this is being reexamined since maitoxin has recently been found in snapper tissue Kolhi et al. 2012).

Pottier et al. (2001) summarized ciguatera fish poisoning in the Caribbean and Western Atlantic and during the last decade or more there has been a resurgence of interest in this topic. While we generally know which fish can be ciguatoxic there are no regionally, species-specific data to inform decisions about harvesting or consuming Caribbean fish. The researchers in the Guadeloupe area have been the most active and there are toxicity studies currently being funded by the National Oceanic and Atmospheric Administration's program on the Ecology and Oceanography of Harmful Algal Blooms. NOAA's studies are limited to the U.S. Virgin Islands. These and other efforts are not yet mature enough or comprehensive enough to provide the assessment to determine which fish species are toxic and in what locations. With the promise of new screening methods it is urgent there be ecological studies that include the food webs that support higher trophic level fisheries across the Caribbean and western Atlantic (Morris and Akins 2008).

Currently, there is no legally accepted level for the human health effects of ciguatera fish poisoning. However, a recent study by Dickey and Plakas (2010) examined analytical information from case and outbreak investigations to determine Caribbean ciguatoxin threshold contami-

nation rates for adverse effects in seafood consumers. From this study has come the basis for industry and consumer advisory levels of 0.10ppb C-CTX-1 equivalent toxicity in fish from the tropical Atlantic, Gulf of Mexico and Caribbean. This threshold level includes a 10-fold safety factor to address individual human risk factors, uncertainty in the amount of fish consumed and analytical accuracy (Dickey and Plakas 2010). In March 2013, the U.S. Food and Drug Administration requested comment on Draft Guidance for Industry: Purchasing Reef Fish Species Associated with the Hazard of Ciguatera Fish Poisoning (<http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Seafood/ucm344980.htm>). It notes:

"Illnesses due to unsafe concentrations of CFP toxins have been linked to commercially caught fish such as: barracuda (Sphyraena spp.), grouper (including Epinephelus spp., gag (Mycteroperca microlepis), scamp (Mycteroperca phenax), and amberjack (Seriola dumerili). Other reef fish associated with unsafe concentrations of CFP toxins include, but are not limited to: grouper (Family Serranidae), snapper (Family Lutjanidae and Symphorus nematophorus), jacks and trevally (Family Carangidae), wrasse (Cheilinus undulatus), mackerel (Scomberomorus spp.), tang (Family Acanthuridae), moray eels (Family Muraenidae), and parrotfish (Scarus spp.). In addition, we have also found CFP toxins in lionfish (Pterois volitans and Pterois miles) collected in waters surrounding the U.S. Virgin Islands. However, as of January 2013, there have been no reports of CFP illnesses associated with the consumption of lionfish."

Nellis and Barnard (1986) provided a legal and social review of ciguatera fish poisoning in the Caribbean and examines the applicability of the Uniform Commercial Code adopted in the Virgin Islands in 1965. They remark that:

"The courts have focused on the consumer's knowledge of the ciguatoxic potential of the fish and have considered whether fish poisoning was within the reasonable expectation of the consumer or whether the consumer knowingly assumed the risk."

Further they reviewed case law and determined:

"If the consumer is fully aware of the danger and nevertheless proceeds voluntarily to make use of the product and is injured by it he is barred from recovery." Biaggi (1992) summarized the legal aspects of ciguatera fish poisoning in Puerto Rico, including two test cases brought before the

Table 1. Summary of known and unknown toxicity of 5 *Gambierdiscus* species and two undescribed ribotypes, all known to occur in the Caribbean. No cultures of *Gambierdiscus* ribotype 1 exist. *Gambierdiscus* ribotype 2 is the CCMP 1665 isolate from the Provasoli-Guillard National Center for Marine Algae and Microbiota.

<i>Gambierdiscus</i> Species	Various Assays		LC-MS Confirmation		Reference
	CTX	MTX	CTX	MTX	
<i>G. belizeanus</i>	RBA	HA	UK	UK	Chinain et al. 2010; Holland et al. 2013; Lartigue et al. 2009,
<i>G. caribaeus</i>	CYTO	HA	UK	UK	Holland et al. 2013
<i>G. carolinianus</i>	UK	HA	UK	UK	Holland et al. 2013
<i>G. carpenteri</i>	UK	HA	UK	UK	Holland et al. 2013
<i>G. ruetzleri</i>	UK	HA	UK	UK	Holland et al. 2013
<i>Gambierdiscus</i> ribotype 1	UK	UK	UK	UK	
<i>Gambierdiscus</i> ribotype 2	CYTO	HA	UK	UK	Lartigue et al. 2009

courts to establish liability. Biaggi concludes by saying, "In summary, ciguatera cases represent fortuitous events which do not generate responsibility. As long as the product (fish) is well handled and preserved, fishermen, wholesalers, distributors, restaurant owners and all persons involved in the fishing industry can rest assured that they will not be held legally responsible for their product happens to have eaten the wrong kind of dinoflagellate."

With renewed interest in CFP in the Caribbean, Gulf of Mexico and western Atlantic prompted by the potential of commercial harvests of invasive lionfish, it is imperative that we initiate comprehensive, species-specific studies of food webs that support higher tropic level fisheries on an area by area basis. Local and traditional knowledge should to be compiled and evaluated for guidance on identifying ciguatera "hot spots" or areas of no concern. Serious consideration needs to be given to a two tier monitoring protocol to monitor *Gambierdiscus* cell abundance and ciguatoxins. A cell-based monitoring protocol using quantitative polymerase chain reaction assays developed for the Caribbean and Gulf of Mexico *Gambierdiscus* species allows rapid detection and quantification of environmental samples. Reef systems can be mapped and monitored for "hot spots" of high *Gambierdiscus* abundance, especially of the known toxic cells. These data can inform risk assessments regionally and provide early warning of CFP events. This allows time to mitigate the consequences of food web toxin transfer in endemic areas and focus toxin monitoring efforts. The second tier of monitoring would employ functional assays and LC-MS confirmation of toxicity of samples exceeding the guidance level for Caribbean ciguatoxins.

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