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AN OVERVIEW OF CIGUATERA FISH POISONING IN THE BAHAMAS

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ABSTRACT

Ciguatera fish poisoning (CFP) is the most common form of non-bacterial seafood poisoning in the world, sickening tens of thousands of people annually. It most frequently occurs in coral reef environments, including the South Pacific, Caribbean, and Bahamas. CFP has a long history in the Bahamas, yet it has been little studied in this archipelago. The purpose of this study, therefore, was to conduct a literature review of CFP in the Bahamas, as well as new surveys on the distribution of *Gambierdiscus* in the eastern Bahamas (San Salvador), including the first record of *G. carolinianus* in the Bahamas. Lastly, reported cases of CFP were examined to determine if any trends in time were present; none were found, but an average of 153 cases of CFP were reported between 1995 and 2011.

INTRODUCTION

Ciguatera fish poisoning (CFP) is a type of (sub)tropical seafood poisoning caused by ciguatoxins, lipophilic polyether compounds produced by members of the benthic dinoflagellate genus, *Gambierdiscus*. These dinoflagellates are epiphytic, and are (inadvertently) consumed by herbivores and detritivores feeding on macrophytes and detritus in coral reef environments. As ciguatoxins are lipophilic, they can bioaccumulate in the herbivores and biomagnify in higher trophic levels (e.g., carnivores). Humans are then exposed to the toxin when we consume fish containing these compounds. If ciguatoxin concentrations are high enough (guidance levels set at 0.1 ppb for the Caribbean in the Fish and Fishery Products Hazards and Controls Guidance, April 2011), the exposure can lead to CFP.

Over 175 symptoms have been associated with CFP (Becker and Sanders 1991), including (but not limited to) nausea, gastrointestinal distress, brachycardia, paresthesia (tingling of mouth and extremities), and dysesthesia (temperature reversal). Symptoms can onset within hours of consuming toxic fish and last for days, weeks, or even months, depending on the patient and toxin dose (Dickey and Plakas 2010). CFP is the most common non-bacterial form of seafood poisoning in the world, with an estimated 50,000 cases per year. Yet, CFP is often under-reported (only an estimated 1 out of 10 cases are reported) and misdiagnosed. Additionally, there is no high-throughput, inexpensive screening mechanism to safeguard against CFP, and there is no known cure.

A Brief History of Ciguatera Research

Ciguatera is a naturally occurring disease with a long historical record. Halstead (1965) cites reports from the T'ang Dynasty (A. D. 618-907) suggesting ciguatoxicity in amberjack. The first documented case of ciguatera in the Pacific was in 1606, recorded by the Portuguese explorer Pedro Fernandez de Queiros in the New Hebrides. A suspected outbreak of ciguatera was also recorded by Captain Cook, whose crew was sickened

after eating three red fish resembling *Sparus pagrus* or *Sparus erithrynus*, caught off the coast of Vanuatu during his second voyage in 1774 (Doherty 2005).

Despite such historical reports, ciguatera research primarily began during World War II and intensified in the 1950s and 1960s (Scheuer 1994). The major discoveries in ciguatera research were summarized by Lehane and Lewis (2000) as follows. Randall (1958) suggested that the toxins causing ciguatera were biomagnified through the food web and not produced by the fish themselves. Rather, he proposed that herbivorous fish took up the toxins through ingesting algal material that colonized new or denuded surfaces of dead corals. Helfrich and Banner (1963) demonstrated that the toxin could be transferred from ciguateric fish flesh into non-toxic fish. Scheuer et al. (1967) isolated and named the toxin responsible for ciguatera (ciguatoxin). Yasumoto et al. (1977) confirmed Randall's (1958) hypothesis that the source of the toxin was indeed an alga, specifically the benthic, epiphytic dinoflagellate, *Gambierdiscus toxicus* Adachi et Fukuyo, and that another toxin produced by *Gambierdiscus* (maitotoxin) did not accumulate in fish tissue and therefore likely did not play a role in ciguatera. Murata et al. (1989) identified the structure of ciguatoxin. A hypothesis linking ciguatera outbreaks with intense bursts of ciguatoxin production was proposed by Helfrich and Banner (1968) and expanded upon by Holmes et al. (1991) and Legrand (1998) suggesting that "super-producing" strains of *Gambierdiscus* were needed to create such high toxin levels.

While *Gambierdiscus* can be found on nearly all algal substrates, highest abundance is found on highly foliose reds and browns (Parsons et al. 2012). Typical abundance values range from 10-1000's of cells per gram wet weight of host algae, but may reach 10^5 cells per gram of host (Yasumoto et al. 1980). Extremely high local variability has been noted; abundance per gram of host substrate can vary by an order of magnitude at sites only a few meters apart (Ballantine et al. 1985).

Disturbance apparently plays a role in fostering ciguatera outbreaks but the mechanistic linkages are unclear and difficult to experimentally verify Kaly and Jones (1994). Randall (1958) postulated that coral death created open habitat for toxic algae (presumed at that time to be a cyanobacterium). Yasumoto et al. (1980) noted that Gambier Islands inhabitants associated ciguatera outbreaks with increased lagoon construction, and postulated that reef disturbance created new habitats for attachment of host macroalgae. Algal cover can also increase as a direct result of stresses to coral reefs such as eutrophication, sedimentation, and herbivore reduction due to overfishing (Hughes 1994, Lapointe 1989, 1997, Pastorok and Bilyard 1985). Shipwrecks, wartime activities, peacetime military activities, dynamiting coral reefs, and hurricanes (Randall 1958, Ruff 1989, Taylor 1984) have all been implicated in the increased incidence of ciguatera. Most recently, ciguatera has been associated with elevated water temperatures (Hales et al. 1999), indicating that climate change may play a growing role in ciguatera (Chateau-Degat et al. 2005).

Ciguatera in the Bahamas

Ciguatera is a well-known malady in the Bahamas. Chisholm (1808) reported that poisonous fish (particularly barracuda) have been known to be present in Bahamian Seas "since the days of Catesby". Poey (1866) reported the presence of toxic fish caught from the Bahamas banks. Mowbray (1916) interviewed Bahamian fisherman who believed that fish were more toxic on one side of an island versus another. Gudger (1930) found evidence of CFP in writings by Locke in 1675, and suspected earlier reports in early Spanish chronicles of the West Indies, though they were not examined. Randall (1958) cites E. Forsythe, who reported that fish caught off Fresh Creek, Andros Island became toxic following a severe storm in 1908. The magnitude of CFP was such that the area was closed for fishing for several years.

More recently, O'Toole et al. (2012) recorded that local fisherman on Eleuthera Island believed fish were more toxic on the west side of the island, and that bigger fish and/or those caught from deeper reef areas were more toxic (particu-

larly barracuda). Interestingly, these views were corroborated by the telemetry and toxicity data collected in this study, which demonstrated that barracuda were more toxic on the west side of the island. Barracuda seem to be the most often implicated fish in cases of CFP (Olsen et al. 1984). There was an average of 5.8 cases of CFP reported per 10,000 people between 1996 and 2006 (Tester et al. 2010), and an average of 140 cases of CFP were reported in the Bahamas between 2007 and 2010 (CAREC 2010).

Bomber (1987) conducted the first survey of *Gambierdiscus* distributions in the Bahamas and found that it was present in waters of Anguila Cay, Deep Water Cay, Gingerbread Grounds, Matinilla Reef, Grand Bahama (SW Point), Stranger's Cay, and West Little Abaco. *Gambierdiscus* was not seen at Grand Cay, Orange Cay, Mackie Bank, Matinilla Shoal, Sebastian Inlet, or Walker's Cay. While he called his isolates "*G. toxicus*", subsequent molecular research by Richlen et al. (2008) and taxonomic revisions by Litaker et al. (2009) indicated that many "cryptic" species actually exist, resulting in the need to re-examine distributions of the various *Gambierdiscus* species globally, including the Bahamas.

It should be noted that a *Gambierdiscus* culture (B775) reported to have been isolated in Mullet Bay, Bahamas in Richlen et al. (2008) and Litaker et al. (2010) was actually isolated from Mullet Bay, Bermuda (S. Morton, pers. comm.), so the report of *G. caribaeus* in the Bahamas (Litaker et al. 2010) is not valid. Therefore, there are no known reliable records of which *Gambierdiscus* species are found in Bahamian waters.

In addition to their survey work discussed above, Bomber (1987) and Bomber et al. (1989) also examined the toxicity of *Gambierdiscus* clones and found that clones exhibited a wide range of toxicity (Table 1), which they suggested was due to genetic differences (i.e., it is likely that multiple species are present in Bahamian waters).

Knowledge Gaps

The above literature review demonstrates that: 1) CFP has been endemic to the Bahamas for centuries; 2) *Gambierdiscus* populations are present in Bahamian waters (although surveys are limited to the northern Bahamas); 3) results of early toxicity tests suggest that multiple species/strains might be present; and 4) CFP is an ongoing problem in the Bahamas, although little data exist on which fish species (besides barracuda) are most implicated in cases of CFP, and which regions in the Bahamas are possible "hot spots" from which the majority of CFP-causing fish are being caught. The objectives of this study, therefore, were to: 1) conduct additional surveys to better determine the distribution of *Gambierdiscus* in the Bahamas; 2) determine which *Gambierdiscus* species are present in Bahamian waters; and 3) determine if cases of CFP have increased over time in the Bahamas (i.e., possibly due to climate change or environmental pressures).

Table 1. Toxicity (mouse units = MU) of several Gambierdiscus strains tested by Bomber et al. (1989). All strains were isolated from Gingerbread Grounds, Bahamas. Higher cells MU^{-1} values are inversely related to cell toxicity.

Clone	toxicity (cells MU^{-1})
B6	16,392
B30	5093
162	2170
163	504
170	522
171	1804
172	2605

METHODS

Field Surveys

Field surveys were conducted around the island of San Salvador in June 2013 (Figure 1). We snorkeled at various patch reefs including Rocky Point (June 13), French Bay and Lindsay's Reef (June 15), and Bamboo Point and East Beach (June 16). We collected 4 macrophyte samples (<30 g each) in 50 ml centrifuge tubes at each site. Genera targeted included *Halimeda* (a calcareous green algae), *Galaxaura* (a calcareous red algae),

Laurencia (a red algae), *Dictyota* (a brown algae), *Thalassia* (seagrass), and *Turbinaria* (a brown algae). Once back onshore, we shook the tubes containing the macrophytes to dislodge epiphytes, and filtered the samples consecutively through 200 µm and 20 µm sieves, repeating this procedure five times for each tube (to ensure all epiphytes were dislodged). The material collected on the 20 µm sieve was rinsed into 15 ml centrifuge tubes for transport back to the laboratory and stored on a window sill with loosened tops (to allow for gas exchange and photosynthesis). Samples were then brought back to Florida Gulf Coast University (FGCU) for further microscopic examination.

Molecular Analysis

Study area. Samples for culture establishment were collected from Graham's Harbor, located on the northern side of the island of San Salvador in the Bahamas (24° 07' 10.79" N, 74° 27' 56.44" E).

Sample collection. Field samples for culture establishment were collected on March 29, 2013. Samples of two types of algal assemblages were collected from a depth of approximately 0.5-1 m. The first assemblage comprised a mixture of *Sargassum* sp. and a mix of intertidal red algae. The second sample was collected from a seagrass bed, and comprised a mixture of *Halimeda*, *Penicillus*, *Udotea*, and *Thalassia*, and their associated epiphytes.

For sample processing, macroalgae were vigorously shaken for at least one minute to loosen the dinoflagellates, which were then sieved using a 20 µm sieve, manually excluding visible macroalgal fragments. The fraction retained on the 20 µm sieve was rinsed into a tissue culture flask, and brought to 25 mL with filtered seawater and approximately 1 mL of modified K medium (Morton and Norris, 1990). Subsequent cell isolation, culture establishment, and laboratory analysis were performed at the Woods Hole Oceanographic Institution, MA, USA.

Enumeration and culture establishment. For culture establishment, individual *Gambierdiscus* cells were isolated by micropipetting at 100x magnification, rinsed in sterile seawater, and established in 25% modified K medium (Morton and Norris 1990) and 75% sterile seawater. Isolates were subsequently transferred into tissue culture flasks and maintained in 100% modified K medium at 23°C, 32 practical salinity unit (psu), 100 $\mu mol \cdot m^{-2}\ s^{-1}$ of light, and 12:12h light:dark photoperiod. A total of 19 non-axenic, monoclonal cultures of *Gambierdiscus* spp. were established.

DNA sequencing. A subset of ten isolates was selected for species identification using DNA sequencing. DNA was extracted from ~1mL of each culture using the MoBIO PowerSoil DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) following the manufacturer's instructions, with a final elution volume of 100 µl. Amplifications were performed with the primers FD8 and RB (Chinain et al. 1999), which amplify the D8-D10 hypervariable region of the large subunit ribosomal RNA gene (LSU rDNA). PCR reactions (25 µl) contained ~5 ng template DNA, 1 x PCR Buffer (500 mM KCL and 100 mM Tris–HCl, pH 8.3), 2 mM $MgCl_2$, 0.8 mM dNTPs, 0.5 µM of each primer, and 0.5 U of AmpliTaq DNA Polymerase (Applied Biosystems Inc., Foster City, CA, USA).

Hot start PCR amplifications were performed in an Eppendorf Mastercycler Nexus thermal cycler (Eppendorf, Hamburg, Germany) as follows: 94° C for 4 min; then 35 cycles of 94 °C for 30 s, 57 °C for 1 min, 72 °C for 2 min, and a final extension of 72 °C for 10 min. PCR amplification products were visualized by electrophoresis on 1% TAE agarose gel adjacent to a 100 bp DNA ladder. Positive PCR products were cloned into the p-GEM-T Easy Vector (Promega, Madison, WI, USA). Clones were screened for inserts by PCR amplification with plasmid primers M13F and M13R, and four positive clones from each PCR amplicon were selected for DNA sequencing (Eurofins MWG Operon, Ebersberg, Germany). Products were sequenced in both the forward and reverse direction.

DNA sequences were edited and assembled using the bioinformatics software Geneious

Pro 6.1.7 (Biomatters, Auckland, NZ), and the consensus sequences were compared with those deposited in GenBank using BLAST sequence similarity searches (National Center for Biotechnology Information). Genetic distances among the strains in this study and closely related taxa were calculated. For these calculations, consensus sequences were aligned with closely related taxa, and positions containing gaps or missing data were eliminated.

CFP Cases

Caribbean Epidemiology Centre (CAREC) Annual Reports were examined (www.carpha.org) to gather data on CFP reports from the Bahamas. Data were compiled on an annual basis for all years from which data were available. The data were then examined to determine if trends were evident over time.

RESULTS AND DISCUSSION

Field Surveys

Gambierdiscus cells were observed at all San Salvador sites examined, including Graham's Harbor (Figure 1). Interestingly, *Gambierdiscus* cells were present at East Beach along the northeastern side of San Salvador, which is on the windward side of the island, a region not typically thought of as conducive for *Gambierdiscus* colonization (Taylor et al. 1985).

While *Gambierdiscus* abundances are usually reported as cells g^{-1} wet wt algae, wet weights were not recorded in this study as algal grab samples were composed of several species to both streamline sampling efforts and diversify host substrates sampled. As a result, *Gambierdiscus* abundances were only recorded in a presence-absence format, with the main result being *Gambierdiscus* was present at all sites sampled in this study. Prior to this study, *Gambierdiscus* has only been sampled for (and encountered in) the western Great Bahamas Bank and the northern region of Little Bahama Bank (Figure 2). Our results demonstrate that *Gambierdiscus* is also present in the eastern Bahamas (San Salvador), which is not on either bank, but rather on the eastern edge of the Bahama Escarpment. It is likely, therefore, that *Gambierdiscus* cells were transported to San Salvador sometime in the past by currents or rafting on drift algae or *Sargassum* (e.g., Bomber et al. 1988).

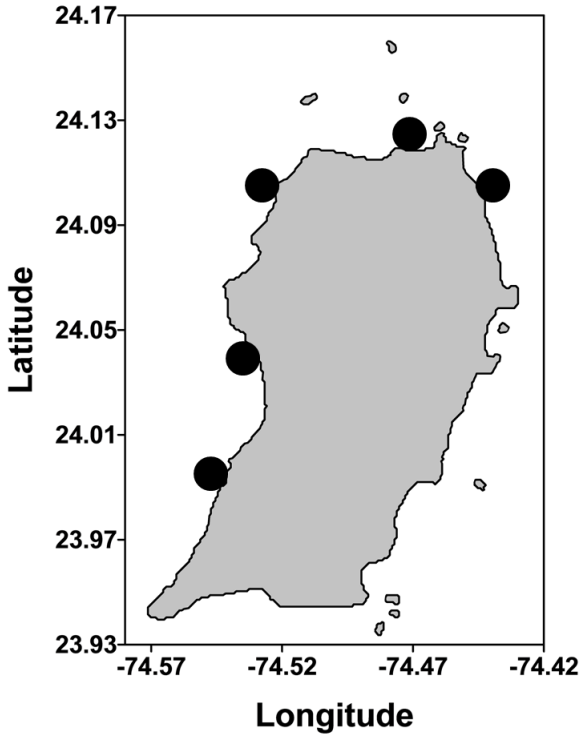

Figure 1. Sampling locations around San Salvador where Gambierdiscus *was found to be present (i.e., all locations sampled).*

Gambierdiscus spp. species diversity

DNA sequences were successfully collected from a subset of ten *Gambierdiscus* isolates from San Salvador, and consensus sequences ranged in length from 889-907 bp. The three clones that were 889 bp in length all had a 17 bp internal deletion located at nucleotide positions 403-420 (compared with *G. carolinianus* strain CM515, GenBank Acc. No. EU770679). All sequences were compared with those deposited in GenBank using BLAST sequence similarity searches (National Center for Biotechnology Information). Nearest matches from BLAST searches for all isolates included sequences of *G. carolinianus* and *G.* sp. *carolinianus*. Genetic

distances values calculated among clones of the same strain ranged from 0 to 0.008, and distances among strains ranged from 0 to 0.012; the latter value was observed between the pair GHCG2-A7 (Acc. No. KJ818266) and several other isolates. Additionally, distance values between these strains and *G. carolinianus* available in GenBank ranged from 0.002 for several pairwise comparisons between the Bahamas isolates and a *G. carolinianus* isolate from Cancun, Mexico (Acc. No. EU770679), to 0.02 for the comparison between GHCG2-A7 (Acc. No. KJ818266) and an isolate from Belize (GU968521).

Figure 2. Locations around the Bahamas where Gambierdiscus *has been confirmed to be present. Closed circles are from Bomber (1987); open circles are from this study.*

Based on these results, we conclude that all *Gambierdiscus* strains established from San Salvador were *G. carolinianus* (Figure 3). DNA sequences obtained in this study were deposited into GenBank (Accession numbers KJ818266 – KJ818274).

CFP Cases

CAREC Annual Reports contained CFP data from the Bahamas for all years between 1995 and 2011. No data could be located outside of this time frame. The highest number of cases reported was in 2001 (263), while the lowest number of cases reported was in 2006 (24). No trends were evident in the data (Figure 4), although more cases of CFP were reported from 2000-2005 versus 2006-2011. On average, 153 cases of CFP were reported each year in the Bahamas. The two low years (1995 and 2006), may reflect how CFP is often under-reported, although their low CFP values are the only data supporting this supposition. No data are available on what type of fish were eaten that led to CFP in the Bahamas, although casual inquiries indicate that barracuda and black grouper have caused CFP in recent years.

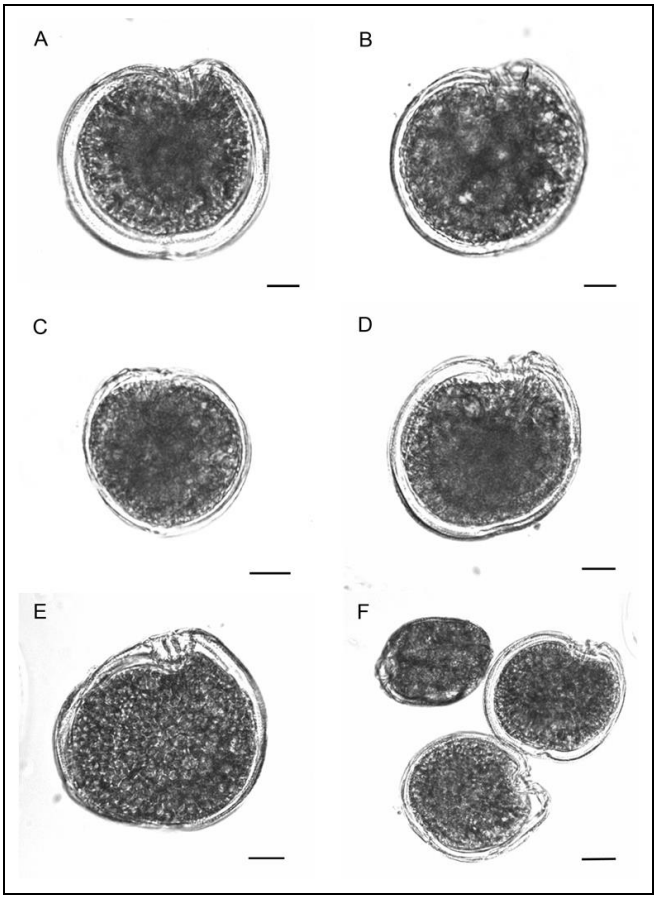

Figure 3. Light micrographs of Gambierdiscus carolinianus *strains isolated from San Salvador, Bahamas, in 2013: (A) apical view of GHCG2-A6; (B) apical and (C) antapical views of GHCG2-B8; (D, E) apical view of GHCG2-A7; (F) GHCG2-A7, multiple cells. Scale bar: 20 µm.*

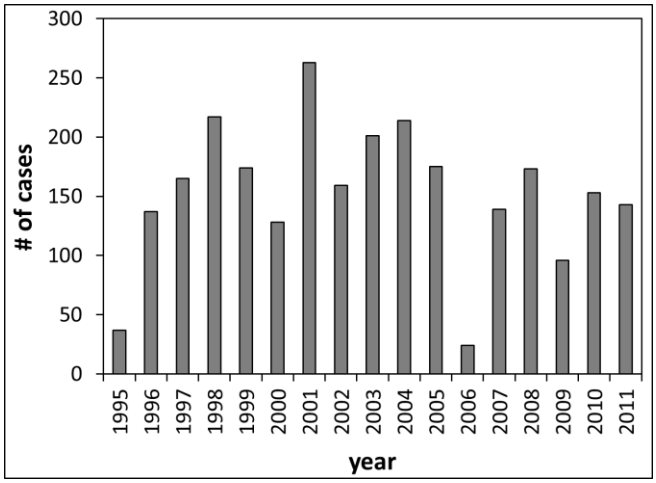

Figure 4. Annual number of cases of CFP in the Bahamas reported to the Caribbean Epidemiology Centre, 1995-2011.

CONCLUSIONS

In conclusion, the results of this study reveal that *Gambierdiscus* is not restricted to the northern and western Bahamas, but is also present in the eastern Bahamas (San Salvador). We know now that *G. carolinianus* is present in the Bahamas, and is the first species recorded here. CFP is common in the Bahamas, with an average of 153 cases reported each year. Considering that only an estimated 10% of cases are actually reported, CFP cases could therefore number well over 1,000 in actuality. The fish most responsible for CFP remains unconfirmed, but barracuda have been historically implicated and are commonly considered to be a source of CFP in the present. Grouper may also be a source of CFP, but additional research needs to be conducted to confirm if grouper should be a vector of concern, or if their apparent involvement in CFP rather reflects their popularity on the dinner table.

Future studies should continue to examine the distribution and diversity of *Gambierdiscus* in the Bahamas, as well as ecological studies to better understand their population dynamics. Other efforts should focus on fish toxicity, to determine which fish represent the biggest threat for CFP, and which regions are most likely to harbor toxic fish. Only by better understanding the dynamics of CFP in the Bahamas can we better safeguard fish resources and protect human health.

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REFERENCES

Ballantine, D.L., A.T. Bardales, T.R. Tosteson, and H. Dupont-Durst. 1985. Seasonal abundance of *Gambierdiscus toxicus* and *Ostreopsis* sp. in coastal waters of southwest Puerto Rico. Pp. 417-422 *in* Delesalle, B. and B. Salvat, eds., *Proceedings of the Fifth International Coral Reef*, vol. 4, Antenne Museum EPHE, Moorea, French Polynesia.

Becker, S.A., and W.E. Sanders, Jr. 1991. Ciguatoxins. FJPH 3:38-41.

Bomber, J.W. 1987. Ecology, genetic variability and physiology of the ciguatera-causing dinoflagellate *Gambierdiscus toxicus* Adachi & Fukuyo. Unpublished Ph.D. Thesis, Florida Institute of Technology, Melbourne, FL, 147 pp.

Bomber, J.W., S.L. Morton, J.A. Babinchak, D.R. Norris, and J.G. Morton. 1988. Epiphytic dinoflagellates of drift algae: another toxigenic community in the ciguatera food chain. Bulletin of Marine Science 43:204-214.

Bomber, J.W., D.R. Tindall, and D.M. Miller. 1989. Genetic variability in toxin potencies among seventeen clones of *Gambierdiscus toxicus* (Dinophyceae). Journal of Phycology 25:617-625.

CAREC. 2010. The Caribbean Epidemiology Center Annual Report 2010. Port of Spain, Trinidad and Tobago: Phoenix Publishing Ltd., 88 pp.

Chateau-Degat, M.L., M. Chinain, N. Cerf, S. Gingras, B. Hubert, and E. Dewailly. 2005. Seawater temperature, *Gambierdiscus* spp. variability and incidence of ciguatera poisoning in French Polynesia. Harmful Algae 4:1053-1062.

Chinain, M., M.A. Faust, and S. Pauillac. 1999. Morphology and molecular analysis of three toxic species of *Gambierdiscus* (Dinophyceae): *G. pacificus*, sp. nov., *G. australes*, sp. nov., and *G. polynesiensis*, sp. nov. Journal of Phycology 35:1282-1296.

Chisholm, C. 1808. On the poison of fish. The Edinburgh Medical and Surgical Journal 4:393-422.

Dickey, R.W., and S.M. Plakas. 2010. Ciguatera: A public health perspective. Toxicon 56:123-136.

Doherty, M.J. 2005. Captain Cook on poison fish. Neurology 65:1788-1791.

Gudger, E.W. 1930. Poisonous fishes and fish poisoning, with special reference to ciguatera in the West Indies. The American Journal of Tropical Medicine 1:43-55.

Hales, S., P. Weinstein, and A. Woodward. 1999. Ciguatera (fish poisoning), El Niño, and Pacific sea surface temperatures. Ecosystem Health 5:20-25.

Halstead, B.W. 1965. *Poisonous and Venomous Marine Animals of the World.* U. S. Government Printing Office, Washington, D.C., 1034 pp.

Helfrich, P., and A.H. Banner. 1963. Experimental induction of toxins of marine ciguatera toxicity in fish through diet. Nature 197:1026–1027.

Helfrich, P., and A.H. Banner. 1968. Ciguatera fish poisoning. 2. General patterns of development in the Pacific. Occasional Papers of the Bernice P. Bishop Museum 23:371-382.

Holmes, M.J., R.J. Lewis, M.A. Poli, and N.C. Gillespie. 1991. Strain dependent production of ciguatoxin precursors (gambiertoxins) by *Gambierdiscus toxicus* (Dinophyceae) in culture. Toxicon 29:761-775.

Hughes, T.P. 1994. Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. Science 265:1547-1551.

Kaly, U.L., and G.P. Jones. 1994. Test of the effect of disturbance on ciguatera in Tuvalu. Memoirs of the Queensland Museum 34:523-532.

Lapointe, B.E. 1989. Caribbean coral reefs: are they becoming algal reefs? Sea Frontiers 35:82-91.

Lapointe, B.E. 1997. Nutrient thresholds for bottom-up control of macroalgal blooms on coral reefs in Jamaica and southeast Florida. Limnology and Oceanography 42:1119-1131.

Legrand, A.M. 1998. Ciguatera toxins: Origin, transfer through the food chain and toxicity to humans. Pp. 39-43 *in* Reguera, B., J. Blanco, M.L. Fernández, and T. Wyatt, eds., *Harmful Algae*, Vigo, Spain, Xunta de Galicia and IOC.

Lehane, L., and R.J. Lewis. 2000. Ciguatera: recent advances but the risk remains. International Journal of Food Microbiology 61:91-125.

Litaker, R.W., M.W. Vandersea, M.A. Faust, S.R. Kibler, M. Chinain, M.J. Holmes, W.C. Holland, and P.A. Tester. 2009. Taxonomy of *Gambierdiscus* including four new species, *Gambierdiscus caribaeus*, *Gambierdiscus carolinianus*, *Gambierdiscus carpenteri* and *Gambierdiscus ruetzleri* (Gonyaulacales, Dinophyceae). Phycologia 48:334-390.

Litaker, R.W., M.W. Vandersea, M.A. Faust, S.R. Kibler, A.W. Nau, W.C. Holland, M. Chinain, M.J. Holmes, and P.A. Tester. 2010. Global distribution of ciguatera causing dinoflagellates in the genus *Gambierdiscus*. Toxicon 56:711-730.

Morton, S.L., and D.R. Norris. 1990. The role of temperature, salinity, and light on the seasonality of *Prorocentrum lima*. Pp. 201–205 *in* Graneli, E., ed. *Toxic marine phytoplankton*, New York, Elsevier.

Mowbray, L.L. 1916. Fish Poisoning (Ichthyotoxismus). Bulletin New York Zoological Society 19:1422-1423.

Murata, M., A.M. Legrand, Y. Ishibashi, and T. Yasumoto. 1989. Structures of ciguatoxin and its congener. Journal of the American Chemical Society 111:8929-8931.

O'Toole, A.C., M.Y.D. Bottein, A.J. Danylchuk, J.S. Ramsdell, and S.J. Cooke. 2012. Linking ciguatera poisoning to spatial ecology of fish: A novel approach to examining the distribution of biotoxin levels in the great barracuda by combining non-lethal blood sampling and biotelemetry. Science of the Total Environment 427:98-105.

Olsen, D.A., D.W. Nellis, and R.S. Wood. 1984. Ciguatera in the eastern Caribbean. Marine Fisheries Review 46:13-18.

Parsons, M.L., K. Aligizaki, M.Y.D. Bottein, S. Fraga, S.L. Morton, A. Penna, and L. Rhodes. 2012. *Gambierdiscus* and *Ostreopsis*: Reassessment of the state of knowledge of their taxonomy, geography, ecophysiology, and toxicology. Harmful Algae 14:107-129.

Pastorok, R.A., and G.R. Bilyard. 1985. Effects of sewage pollution on coral-reef communities. Marine Ecology Progress Series 21:175-189.

Poey, F. 1866. Ciguatera. Pp. 1-39 *in* Poey F, ed. *Reportorio Fisico-Natural de la Isla Cuba*, Havana.

Randall, J.E. 1958. A review of ciguatera, tropical fish poisoning with a tentative explanation of its cause. Bulletin of Marine Science of the Gulf and Carribean 8:236-267.

Richlen, M.L., S.L. Morton, P.H. Barber, and P.S. Lobel. 2008. Phylogeography, morphological variation and taxonomy of the toxic dinoflagellate *Gambierdiscus toxicus* (Dinophyceae). Harmful Algae 7:614-629.

Ruff, T.A. 1989. Ciguatera in the Pacific: A link with military activities. Lancet 333:201-204.

Scheuer, P.J. 1994. Ciguatera: A scientific link between Japan and Hawaii. Pp. 1-8 *in* Hokama, Y., P.J. Scheuer, and T. Yasumoto, eds., *Proceedings of the International Symposium on Ciguatera and Marine Natural Products*, South Kohala, Hawaii.

Scheuer, P.J., W. Takahashi, J. Tsutsumi, and T. Yoshida. 1967. Ciguatoxins: Isolation and chemical nature. Science 156:1267-1268.

Taylor, F.J.R. 1984. Human and domestic animal fatalities, as well as skin reactions, associated with the "rough triggerfish", *Canthidermis maculatus* (Bloch) in Dominica, West Indies, following Hurricane David. Revue internationale d'oceanographie medicale. 73-74:35-40.

Taylor, F.J.R., D.G. Repsilon, and C. Tahiti. 1985. The distribution of the dinoflagellate *Gambierdiscus toxicus* in the eastern Caribbean. Pp. 423-428 *in* Delesalle, B. and B. Salvat, eds., *Proceedings of the Fifth International Coral Reef*, vol. 4, Antenne Museum EPHE, Moorea, French Polynesia.

Tester, P.A., R.L. Feldman, A.W. Nau, S.R. Kibler, and R.W. Litaker. 2010. Ciguatera fish poisoning and sea surface temperatures in the Caribbean Sea and the West Indies. Toxicon 56:698-710.

Yasumoto, T., A. Inoue, T. Ochi, Y. Oshima, Y. Fukuyo, R. Adachi, R. Bagnis, and K. Fujimoto. 1980. Environmental studies on a toxic dinoflagellate (*Gambierdiscus toxicus*) responsible for ciguatera. Bulletin of the Japanese Society of Scientific Fisheries 46:1397-1404.

Yasumoto, T., I. Nakajima, R. Bagnis, and R. Adachi. 1977. Finding of a dinoflagellate as a likely culprit of ciguatera. Bulletin of the Japanese Society of Scientific Fisheries 43:1021-1026.