



Ciguatoxin – an Emerging Biological Hazard among Reef Fishes of India

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Abstract

Ciguatera Fish Poisoning (CFP) is an emerging food safety hazard which has been reported in southern peninsular India in recent times. Ciguateric fishes mostly associated with coral reef ecosystem are implicated in food poisoning outbreaks. USFDA guidance for fish and fishery products classifies CFP as “reasonably likely to occur” in fishes harvested from coral reef regions. Ciguatera Fish Poisoning has been reported from tropical or subtropical areas around the world between latitudes 35°N and 35°S, particularly in the Caribbean, Pacific and Indian Ocean and in the Flower Garden Banks area in the northern Gulf of Mexico. Action levels for CFP limits are now listed as 0.01 ppb for Pacific and 0.1 ppb for Caribbean ciguatoxin. CFP is considered as a natural toxin and USFDA has listed out 12 group of fishes under ciguatera hazard category. With recent EU import rejections of some of the seafood consignments originated from India, CFP has emerged as an important food safety concern. Although, no fatality has been reported so far, morbidity symptoms observed from cases of hospitalization is a definite concern to the export trade. This review deliberates on the significance of CFP, its distribution and hazard control measures.

Keywords: Ciguatoxin, CFP, mouse bioassay, reef fish, mass spectrometry, food safety

Harmful Algal Blooms and Biotoxins

Phytoplankton is the most important constituent of the marine food web and comprises 40% of the total fixed global primary productivity (Falkowski, 1984; D'Silva et al., 2012). In a typical algal bloom

scenario, algal cells multiply upto 10^5 to 10^6 cells l^{-1} of seawater (Smith et al., 1993). Around 60-80 phytoplankton species are known to be harmful or toxic, in which 75% are contributed by dinoflagellates (Van Dolah, 2000; Wells et al., 2015; Roberts et al., 2004) and mostly responsible for the production of Harmful Algal Bloom (HAB). Reports of HABs are increasing in frequency, intensity and geographic distribution due to climate change and increased rates of coastal eutrophication (Paerl, 1988; Smayda, 1992; Hallegraeff, 1993; Nixon, 1995; Richardson & Jorgensen, 1996; Daranas et al., 2001). Seafoodborne intoxications, caused by marine biotoxins like Ciguatoxin (CTX), Saxitoxin (STX), Okadaic Acid (OA), Brevetoxin (PbTx), Domoic Acid (DA), Palytoxin (PLTX), Pectenotoxin (PTX), Tetrodotoxin (TTX) and Yessotoxin (YTX) result from the ingestion of contaminated fish and shellfish with the marine algal toxins (MATs) (Garthwaite, 2000; Botana, 2008; Lawrence et al., 2011; Shi, 2012). Marine biotoxins or the seafood toxin comes under the category of naturally occurring chemical hazards (FDA, 2011).

Incidents of biotoxin related hazards are reported globally from Europe, Africa, North America, Central and South America, Asia, Oceania etc. (Yasumoto et al., 1978; Underdal et al., 1985; Perl et al., 1990; Rodrigue et al., 1990; Morris et al., 1990; McMahon & Silke, 1998; DeSchrijver et al., 2002; FAO, 2004; Aune et al., 2007). Hence, producers of shell fishes and fin fishes have to ensure that the product must not contain the marine biotoxins in quantities that exceed $80 \mu g kg^{-1}$ for PSP, $20 mg kg^{-1}$ of DA for ASP, $160 \mu g kg^{-1}$ of OA equivalents (Dinophysis toxins and Pectenotoxins in combination), one milligram of Yessotoxin equivalents per kilogram for YTX, 160 micrograms of Azaspiracid equivalents per kilogram for AZP and any detectable level 100 g of fish for Ciguatoxin (FDA, 2011). Table 1 describes the major seafood poisoning syndromes, sources, mechanism of action, clinical

Received 28 June 2016; Accepted 20 July 2018

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symptoms and treatments. The extent of threat on human health from HABs in Indian waters remains unreported and unregulated. Until 1980s, the phenomenon of Paralytic Shellfish Poisoning (PSP) was virtually unknown in Indian waters. Indian waters are regularly seen with algal bloom occurrences and a report stated that a total of 101 bloom incidents and 39 causative species responsible for blooms during the period from 1908 to 2009, of which *Noctiluca scintillans* and *Trichodesmium erythraeum* were the most common species contributing to HAB events (D'Silva et al., 2012).

Ciguatera toxin

Existence of ciguatoxicity cannot be indicated by any highly visible surface phenomenon such as red tide as seen in the case of Paralytic Shellfish Poisoning (DeFouw et al., 2001). Hence an early warning to alert incidence of CFP is not possible. Until 2016, the occurrence of Ciguatera Fish poisoning was virtually unknown to fishes from Indian Coast. This region specific biotoxin has been reported very recently from Mangalore and Kerala coast (Rajeish et al., 2016; Rajisha et al., 2017a;b). In reports of CFP cases *Lutjanus bohar* commonly known *Chempalli* fish species was detected as ciguatoxic fish from south west coast of India and caused intoxication in local population. Incidence of ciguatoxin from Indian Coast led to the fact that prevalence of ciguatera toxin in fishes from our reef ecosystem is mainly due to bioaccumulation or biotransformation of toxic dinoflagellate *Gambierdiscus toxicus* through the food web.

As per the observation of UNESCO (2016), there has been a shift in the distribution and occurrence of biotoxins around the world because of climate change. This phenomenon can be attributed to the consecutive import rejections from the European Union for Indian seafood consignments for presence of Ciguatoxin. Intoxication due to CTX was reported from fish samples collected from South West Coast of India (Rajeish et al., 2016; Rajisha et al., 2017a;b). CFP is emerging as an important food safety concern which has to be addressed. There is no fatality reported so far, but the symptoms of ciguatera exist as a primary concern to the fisheries sector and export trade.

History of Ciguatoxin

Ciguatera fish poisoning (CFP) is a seafood-borne illness associated with a wide variety of gastrointes-

tinal, neurological and cardiovascular symptoms in humans. The term "cigua" refers to intoxication caused by the ingestion of coral reef fishes (Juranovic & Park, 1991; Scheuer, 1994; DeFouw et al., 2001), which was first used by Don Antonio Para in Cuba in 1787 as a trivial name in Spanish to represent a univalve mollusk *Turbo livona pica* (Dickey, 2008). The incidence of ciguatera has been depicted from centuries back, since the time of Alexander the Great (356-323 B.C) (Scheuer, 1994; Pearn, 2001; Wong et al., 2014) and Homer's Odyssey (800 B.C) (Ragelis, 1984).

Bioaccumulation of ciguatoxin in fish

Ciguatoxin (CTX) is an important biotoxin resulting from the consumption of coral associated fishes. Ciguateric fish orally accumulates complex, more polar and sodium channel activating ciguatoxin (CTX) through the food web (Lewis, 2001). A benthic dinoflagellate known as *Gambierdiscus toxicus*, is responsible for the production of Gambiertoxin. *G. toxicus* is responsible for the production of less polar toxin precursors known as Gambiertoxins. It is transferred and metabolized into the more polar Ciguatoxin (CTX) by the fish itself through the food web (Holmes et al., 1991; DeFouw et al., 2001; Friedman et al., 2017). CTXs are bio accumulated and concentrated in the food chain and both herbivorous and carnivorous fish can become toxic. Small fish ingest the toxin and then are being devoured by larger fish, so that the fish in the higher trophic level of the food web contains high CTX concentrations which are in turn consumed by humans (Banner et al., 1960; Gillespie et al., 1986; Crump et al., 1999; Lehane & Lewis 2000; Dickey & Plakas 2010). Yasumoto et al. (1977) was first to consider *G. toxicus* as the responsible species for CTX accumulation based on the hypothesis of Randall (1958).

Worldwide distribution of ciguatera

Estimated number of people affected from this intoxication has been reported to be ranging from 10,000 to 50,000 on annual basis as per earlier reports (Baden et al., 1995, Lewis, 2001). Recent reports indicate the range has increased to 50,000 to 5,00,000 individuals annually (Lehane & Lewis, 2000; Caillaud et al., 2010), which signifies the intensity of occurrence even though it is difficult to ascertain the under reporting of cases (Tester et al., 2010, Skinner et al., 2011, Friedman et al., 2017). Fig. 1 shows the current global distribution of CFP according to FAO (2017).

Table 1. Marine Algal Toxins, source, clinical symptoms and treatments of poisoning syndromes (Caillaud et al., 2010; Shi, 2012; Friedman et al., 2017)

Toxin Group	Seafood Poisoning Syndromes					
	Fishes			Shell fishes		
	Ciguatera fish Poisoning (CFP)	Paralytic Shellfish Poisoning (PSP)	Diarrhetic Shellfish Poisoning (DSP)	Amnesic Shellfish Poisoning (ASP)	Neurotoxic Shellfish Poisoning (NSP)	Azaspiracid Shellfish Poisoning (AZP)
	Ciguatoxin (CTX)	Saxitoxin (STX)	Okadaic Acid (OA)	Domoic Acid (DA)	Brevetoxin (BTX)	Azaspiracid (AZA)
Source	<i>Gambierdiscus toxicus</i>	<i>Alexandrium catenella</i> <i>A. minutum</i> <i>A. tamarense</i> ; <i>Gymnodinium catenatum</i> ; <i>Pyrodinium bahamense</i> <i>Alteromonas tetraodonis</i> ; <i>Moraxella sp.</i>	<i>Dinophysis acuminata</i> ; <i>D. acuta</i> ; <i>D. fortii</i> ; <i>D. norvegica</i> ; <i>Prorocentrum lima</i>	<i>Pseudo-nitzschia multiseriata</i> and <i>P. australis</i>	<i>Karenia brevis</i>	<i>Protoperdinium crassipes</i> ; <i>Azadinium spinosum</i>
Action on	Nerve, Muscle, Heart, Brain	Nerve, Brain	Enzymes	Brain	Nerve, Muscle, Lungs, Brain	
	VGSC* openers	VGSC* blockers	Protein phosphatases inhibitors	Glutamate receptors stimulators	VGSC* openers	
Symptoms	<i>Mild case</i> After 3-5 h - Diarrhea, nausea, vomiting, and abdominal pain. After 12-18 h - hot-cold inversion, muscular aches, tingling and numbness of lips, tongue, and perioral region Metallic taste, dryness of mouth, anxiety, prostration, dizziness, chills, sweating, dilated eyes, blurred vision, and temporary blindness. <i>Extreme Case</i> Paralysis and death may occur in a few extreme cases.	<i>Mild case</i> Within 30 min - Tingling sensation or numbness around lips, gradually spreading to face and neck, prickly sensation infingertips and toes, headache, Dizziness, nausea, vomiting, diarrhea. <i>Extreme Case</i> Muscular paralysis, pronounced respiratory difficulty, choking sensation, death through respiratory paralysis may occur within 2-24 h after ingestion.	<i>Mild case</i> After 30 min to a few hours (seldom more than 12 h): diarrhea, nausea, vomiting, abdominal pain. <i>Extreme Case</i> Chronic exposure may promote tumor formation in the digestive system.	<i>Mild case</i> After 3-5 hours - nausea, vomiting, diarrhea, abdominal cramps. <i>Extreme Case</i> Decreased reaction to deep pain, dizziness, hallucinations, confusion, short-term memory loss, seizures.	<i>Mild case</i> After 3-6 h - chills, headache, diarrhea, muscle weakness, muscle and joint pain, nausea, vomiting <i>Extreme Case</i> Paresthesia, altered perception of hot and cold, difficulty in breathing, double vision, trouble in talking and swallowing	<i>Mild case</i> Nausea, vomiting, severe diarrhea, and stomach cramps, similar to DSP and NSP <i>Extreme Case</i> Suspected carcinogen
Treatment	Symptomatic, Gut emptying and decontamination with charcoal is recommended.	Gastric lavage, artificial respiration. No lasting effects.	Recovery after 3 days, irrespective of medical treatment.	Supportive care	Supportive care	Supportive care

* Voltage Gated Sodium Channel

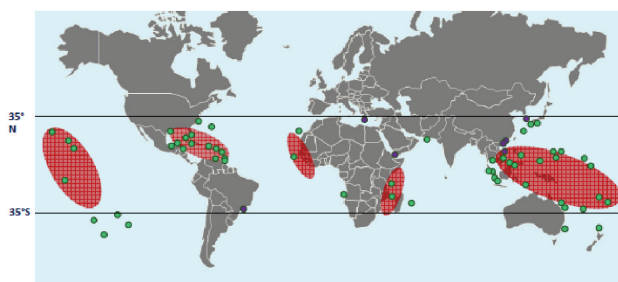


Fig. 1. Current distribution of toxins (Source FAO, 2017)

CFP recognized worldwide as CTX group toxins, is classified into three major categories according to their occurrence as Caribbean (C-CTXs), Pacific (P-CTXs) and Indian Ocean (I-CTXs) origin. It was identified for the first time in fish caught from Europe (EFSA, 2010) and hence the geographical distribution of CFP affects globally in the tropical and subtropical regions. Around 400 reef associated fin fish species (Halstead, 1978; Caillaud et al., 2010) are found to be ciguateric due to the process of biotransformation in the food web, but comparatively small number of species are regularly implicated in ciguatera poisoning (Lehane & Lewis, 2000). Dinoflagellates responsible for the production of ciguatoxin exist within coastal waters between 35°N and 35°S of the equator (Lewis, 2001; FDA, 2011). Spread of ciguatera has been ascribed to many factors that include increased number of fishing community, consumption of contaminated fishes associated with oceanic oil rings habitats, increased number of travel and trade, increase in ocean temperature, importation of contaminated fish in to new areas where it is not reported earlier (Frenette et al., 1988; Morton et al., 1992; Glaziou & LeGrand, 1994; Moulignier et al., 1995; Bruneau et al., 1997; Lewis, 2001; Pottier et al., 2001; DeHaro et al., 2003; Sheppard & Rioja, 2005; Villareal et al., 2007).

Ciguateric fish

Most of these ciguateric fishes comes under the category of top selling, good tasting and highly demanded food fishes in the world market (Friedman et al., 2017). FDA (2011) has listed common reef associated fin fishes implicated in CFP (Table 2).

Other different ciguateric fish species are also reported around the world from various researchers (Blythe et al., 1992; Vernoux & Lejeune, 1994; Hokama et al., 1998; Lewis et al., 1999; Hsieh et al., 2009; Azziz et al., 2012; Chan, 2013). Gillespie et al.

Table 2. Common reef associated fin fishes with Ciguatera toxicity (FDA, 2011; Friedman et al., 2017)

Species	Family
Barracuda	Sphyraenidae
Amberjack	Seriola
Grouper	Serranidae
Snapper	Lutjanidae
Po'ou (<i>Cheilinus</i> spp.)	Labridae
Jack	Carangidae
Trevally (<i>Caranx</i> spp.)	Carangidae
Wrasse	Labridae
Surgeon fish	Acanthuridae
Moray eel	Muraenidae
Roi (<i>Cephalopholis</i> spp.)	Serranidae
Parrot fish	Scaridae

(1986) reported narrow-barred Spanish mackerel, *Scomberomorus commersoni* ciguateric from Australian coastline. Chinain et al. (2010a) reported Scarids (Parrotfish) and Acanthurids (Unicorn fish) as high-risk ciguateric fish species from French Polynesia.

Clinical diagnosis of ciguatera symptoms

Clinical criteria include a wide array of symptoms characterized into three major groups: neurological, gastrointestinal and cardiac. Preliminary symptoms start with gastrointestinal (e.g. nausea, diarrhea and vomiting, abdominal pain) problems which begin within 6-12 h of fish consumption and resolve spontaneously within 1-4 days. Secondly, the neurological symptoms which affects the central and peripheral nervous systems (e.g. paresthesia in the extremity and circumoral regions, pruritis, dysuria, myalgia, hallucinations, depression, cold allodynia, giddiness, vertigo, visual, balance and behavioral disturbance, loss of consciousness) set in, but in some cases they may starts simultaneously with the initial symptoms (DeMotta & Noceda 1985; Pearn, 2001; Arena et al., 2004; Friedman et al., 2007; Stewart et al., 2010). Third category includes the cardiac symptoms (e.g. hypotension, bradycardia) at the early stage of toxicity and proceed in combination with the initial two categories of symptoms (Chateau-Degat et al., 2007a; Katz et al., 1993). All these symptoms start within 2-30 h after toxic fish consumption (Caillaud et al., 2010) and may persist from weeks to months and years. According to Chan (2016), CFP is rarely fatal (<0.1% fatality) and Lewis

(2000) reported that it may be higher in the Indian Ocean. Certain foods and behaviours potentiate the ciguatoxic symptoms like nuts, caffeine, pork, chicken, alcohol consumption, tobacco smoking, fish consumption etc. (Gillespie et al., 1986; Glaziou & Martin, 1993; Lewis, 2000; Lewis, 2001; Chateau-Degat et al., 2007b). Clinical diagnosis of ciguatera fish poisoning is considered as a challenge to emergency physicians because of the patients exhibit or present with a mixture of gastrointestinal, neuro-cutaneous and constitutional symptoms (Cheng & Chung, 2004). There is no effective treatment for this poisoning syndrome and the available remedy is based on acute symptomatic and supportive care for the patients (Friedman et al., 2017). Intravenous mannitol (one gram/kilogram body weight over a 30 to 45 min period) and atropine (0.5 mg every 3-5 min) was administered as dosage (Pearn et al., 1989; Baden et al., 1995; Lewis, 2001). CFP diagnosis is done in suffered individuals based upon the visible symptoms, time of onset and previous history of fish consumption (whether reef associated or its toxic history). There are no reliable biomarkers or documentation

symptoms yet discovered to confirm the exposure of this toxicity. Distinct differences are present in case of CFP symptoms according to various geographical distributions. In the Pacific CTX, neurological symptoms are dominated, whereas in the Caribbean, gastrointestinal problems are highly dominated (Lewis, 2001). Indian Ocean CTX exhibits a group of symptoms like hallucinations, mental depression, lack of coordination, etc. along with typical ciguatera symptoms (Lewis, 2001). Table 3 describes common CFP associated symptoms in humans.

Detection methods for Ciguatoxin

Ciguatoxin emerging as a new toxin from our coast and the absence of purified standards and complex nature of CTX in fish tissue will be a major concern for the development of a laboratory analytical method. USFDA and NOAA laboratories in Japan and Australia have been developed *in-vitro* assay protocol for determination of ciguatoxin in fish (Dickey, 2008). Traditional methods are practiced among local population, which include animal

Table 3. Clinical diagnosis of ciguatera symptoms (Morris et al., 1982; Coleman, 1990; Lewis, 2001; Arena et al., 2004; Chateau-Degat et al., 2007a; Baumann et al., 2010; Friedman et al., 2017)

Category	Symptoms
Gastrointestinal	Abdominal pain, Vomiting, Diarrhoea, Nausea
Neurological	Generalized weakness, Vertigo Lingual paraesthesia, Extremities Paraesthesia, Circumoral Paraesthesia Arthralgia and Myalgia Dental pain, Ataxia Paradoxical Temperature sensation Respiratory paralysis Coma, Weakness in the extremities, Headache Myalgia and Arthralgia
Cardiovascular	Dizziness Hypotension (systolic BP <100 mmHg) Bradycardia (pulse rate <60 beats/min) Chest pain
Others	Chills Sweating Shortness of breath Itching (two to three days) Nightmares, mental depression, hallucinations Lack of coordination and loss of equilibrium

testing, observing the bleeding at the tail of the fish fillet, observing silver coins turning black on a hypothetical cooked fish, feeling a sensation on tingling when rubbing the liver on gums etc. (Banner et al., 1963; Chinain et al., 2010b). These methods are practically not suitable for the determination of CTX toxicity. An ideal or recognized official method for CTX detection in fish is not yet established (Caillaud et al., 2010; Friedman et al., 2017). Ciguatoxin is a lipid soluble compound and most of the sample preparation methods are based on acetone or methanol extraction (Caillaud et al., 2010).

Mouse Bioassay and other *in vivo* and *in vitro* methods

Mouse bioassay (MBA) has been widely used for the selective determination of ciguatoxicity in fishes introduced by Banner et al. (1960) and further refined by Yasumoto et al. (1984). In this method the lethality is estimated in terms of Mouse Units (MU). After intra peritoneal injection of crude fish ether extract into mice, signs of toxicity is observed up to 24 h. The toxicity and relationship between dose and time to death is used to quantify toxicity (Lewis, 1995). Other *in vivo* assays for the detection of CTX include chicken assay (Kosaki et al., 1968), Brine Shrimp assay (Granade et al., 1976; Bienfang et al., 2008), mosquito larvae assay (Bagnis et al., 1985), Diptera Larvae assay (Labrousse & Matile, 1996), etc. These assays are not recommended for CTX quantification, hence not widely used in laboratories for screening of ciguatoxin (Caillaud et al., 2010). MBA is followed as an official testing method for Paralytic and Diarrheic shellfish toxins as per European Union and FDA guidelines (FDA, 2011; AOAC, 2012). In case of CFP, MBA is used for the screening of ciguatera implicated reef fish samples. Yasumoto et al. (1984) and Caillaud et al. (2010) suggested that any fish containing above 2.5 Mouse Unit (MU) 100 g^{-1} should be avoided as food, since it has long term neurological effects. Sub lethal doses were in the range between 0.18 and 0.45 MU 20 mg^{-1} of ether extract (Wong et al., 2005). The utility of MBA method is limited by the requirement of dose response curve because of the lapse of purified CTXs for accurate quantification; hence the curve is not linear (Hoffman et al., 1983; Lehane & Lewis, 2000; Lewis, 2003). Lewis (1995; 2003) revised the MBA extraction protocol and Wong et al. (2005; 2009) developed a solid phase extraction (SPE) clean up method for CTX fish extract for MBA. Routine

analysis of samples by mouse bioassay cannot be recommended since it is non-specific and ethically objectionable (Abraham et al., 2012). Sodium channel specific cytotoxicity (Manger et al., 1993; 1995) and sodium channel receptor binding in rat brain synaptosomal preparations (Lombet et al., 1987; Lewis et al., 1991; Poli et al., 1997) were developed as an alternative to *in vivo* assay. *In vitro* mouse neuroblastoma assay was used as a screening procedure, using an ouabain-veratridine dependent method by Dickey et al. (1999) and Manger et al. (1995). EFSA (2009) recommended *in vitro* assay as an alternative to *in vivo* animal assays for monitoring and investigation of Marine Algal Toxins (MATs).

Physico-chemical detection of CTX in fish

USFDA applied a two- tiered protocol for monitoring of CFP which includes *in vitro* assay and Mass Spectrometry analysis (FDA, 2011; Friedman et al., 2017).

Mass Spectrometry

The main physico-chemical methods used in toxin analysis are chromatographic methods with optical (UV and fluorescent detectors) or mass spectrometric detectors (Quilliam, 2003). A typical LC-MS system comprises HPLC for analyte separation, an atmospheric pressure ionization interface to produce ionized molecules and mass spectrometer (MS) in which ions are separated and detected in a high vacuum environment. Various ciguatoxin congeners were quantified using mass spectrometry method for Pacific, Caribbean and Indian Ocean forms. For Pacific ciguatoxin, P-CTX-1, P-CTX-2 and P-CTX-3 congeners with molecular masses identified as 1111.6 & 1095.5 Da were isolated from carnivorous fishes (Lewis et al., 1991; 1993; Lewis & Jones, 1997). Another two congeners for P-CTX are CTX-3B (49-epi-CTX-3C) and CTX-3C with molecular ions 1023.6 Da and M-seco-CTX-3C with m/z 1041.6 Da were isolated from *Gambierdiscus toxicus* (Satake et al., 1993; Chinain et al., 2010b; Roeder et al., 2010). Molecular mass of m/z 1061.6 Da identified for CTX-4B (GT-4B) isolated from *Gambierdiscus* sp. and herbivorous fish as source organisms and also for 52-epi-ciguatoxin-4B (CTX-4A; GT-4A) isolated from *G. toxicus* (Murata et al., 1990; Satake et al., 1996; Yasumoto et al., 2000; Roeder et al., 2010). $[M+H]^+$ ions 1057.6 Da for CTX-2A1 congener were isolated from both *G. discus* and carnivorous fish and 1039.5 Da for CTX-2C1 was determined from *G. toxicus* as

source organism (Satake et al., 1998; Roeder et al., 2010). Almost 10 congeners are identified for Pacific ciguatoxin as fish and algae as causative species and six congeners for Caribbean ciguatoxin (C-CTX) were isolated from carnivorous fishes as source organisms. C-CTX having a m/z 1141.6 Da for C-CTX-1 and C-CTX-2, 1127.6 Da for C-CTX-1127, 1143.6 Da for C-CTX-1143, 1157.6 Da for C-CTX-1157 and 1159.6 for C-CTX-1159 have been identified as congeners by various researchers (Vernoux & Lewis, 1997; Lewis et al., 1998; Lewis et al., 1999; Pottier et al., 2002a,b; Pottier et al., 2003). Indian Ocean ciguatoxin (I-CTX) was isolated from carnivorous fishes with molecular masses m/z 1141.6 Da for I-CTX-1 & I-CTX-2 and 1157.6 Da for I-CTX-3 & I-CTX-4 congeners (Hamilton et al., 2002a,b). Mass Spectrometry (MS) is an excellent tool for the identification and characterization of ciguatoxin congeners, since it is provided enhanced sensitivity and selectivity by measuring accurate masses or a series of fragment ions. Lewis et al. (1994) introduced Ion Spray (IS) as ion source for MS analysis. Yogi et al. (2014) carried out an LC-MS/MS analysis using Triple Quadrupole Mass Spectrometry, in which 14 reference toxins were used and pure CTX-1B and CTX3C were prepared from fish samples collected from Japan.

Nuclear Magnetic Resonance (NMR)

Modern NMR has proved as a vital technique for the full elucidation of the chemical structures of novel biotoxins (Shi et al., 2012). Proton (^1H) NMR and Carbon-13 (^{13}C) enables determination of the proton environment (number and configuration of neighboring protons) and identification of the number and type of carbon atoms in an organic molecule respectively. The combination of ^{13}C and ^1H NMR in 2D experiments along with FT IR and UV Visible NIR allows the elucidation of the carbon connectivity and 3-dimensional chemical structure of complex organic molecules (Shi et al., 2012). So far only Pacific and Caribbean CTXs are structurally elucidated and NMR is the key technique used for this purpose. CTX is a group of highly oxygenated and cyclic polyether molecules and structurally related with the Brevetoxin (PbTx) group (Lewis, 2001). Murata et al. (1989) started the pioneer work in the structural confirmation of Pacific CTX and its precursor from *G. toxicus* using NMR methods. From the Pacific P-CTX-1(m/z 1111 Da), P-CTX-2 and P-CTX-3 (both has m/z 1095 Da) were structurally isolated from carnivorous fish and P-CTX-3C

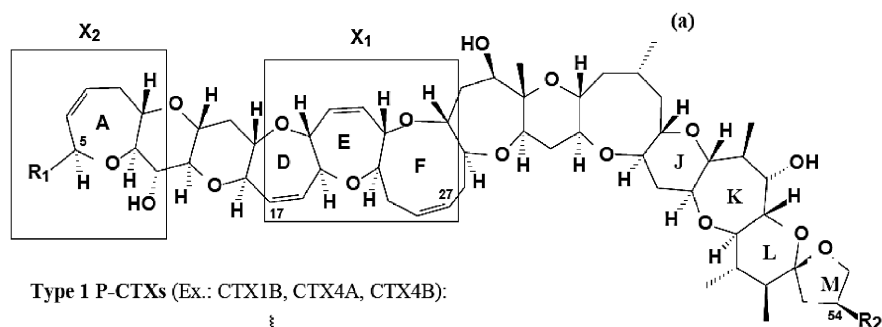
(m/z 1045) was isolated from *G. toxicus* (Lewis et al., 1991; 1993; Satake et al., 1993; 1996; 1998). Caribbean C-CTX-1 and C-CTX-2 with molecular mass 1141 Da were structurally elucidated from carnivorous fish (Vernoux & Lewis, 1997; Lewis et al., 1998). P-CTX-4A and P-CTX-4B with molecular mass 1061 Da has been structurally elucidated from *G. toxicus* and herbivorous fishes (Murata et al., 1990). CTX are structurally distinct from other biotoxins (Yasumoto & Murata, 1993) and using Mass Spectrometry and NMR techniques, several minor toxins are also detected (Lewis & Jones, 1997; Vernoux & Lewis, 1997). Around 20 ciguatoxin congeners are structurally elucidated by Yasumoto et al. (2000) using high energy Mass Spectrometry and NMR techniques.

Chemical and Structural Properties of CTX based on NMR analysis

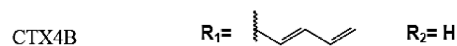
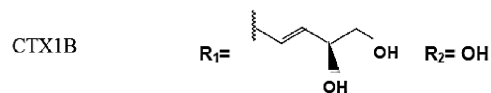
Pacific CTX was divided into Type I and Type II based on number of carbon atoms (60 and 57) respectively and the structure of the ether ring (Murata et al., 1990; Legrand et al., 1998). Caribbean CTXs contains 62 number of carbon and 14 numbers of E rings. Vernoux & Lewis (1997) first isolated and structurally identified two C-CTXs and later Pottier et al. (2002a; 2002b) identified additional congeners. Hamilton et al. (2002b) isolated four I-CTXs, but their structural characteristics were unidentified. Structure of P-CTX (Type I and Type II) and C-CTX elucidated by different researchers were given in Fig. 2. (Murata et al., 1990; Lewis et al., 1991; Satake et al., 1996; Lewis & Jones, 1997; Vernoux & Lewis, 1997; Lewis et al., 1998; Yasumoto et al., 2000; Lewis, 2001; FAO, 2004; Caillaud et al., 2010; FDA, 2011)

Risk assessment of CFP for food safety

EFSA, 2010 panel on contaminants in the food chain assessed, Ciguatoxin as an emerging biotoxin for which widely screened toxicity assay MBA has found limitations due to insufficient detection and ethical concerns. *In vitro* assay and receptor binding assay have been developed as alternatives; but they need further development and only few laboratories have the needed facility for cell based assays (Caillaud et al., 2010). Hence, LC-MS/MS Tandem Mass Spectrometry is only considered as valued method, in which reference standards need to be developed taking into account the distinctive nature of CTX sourced from various geographical regions (Friedman et al., 2017). The major preventive measures for ciguatera include, avoiding ciguateric



Type 1 P-CTXs (Ex.: CTX1B, CTX4A, CTX4B):



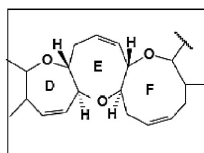
CTX4A: epimer of CTX4B at C52

Type 2 P-CTXs (Ex.: CTX3C, CTX2A1):

- CTX3C

$R_1 = R_2 = H$

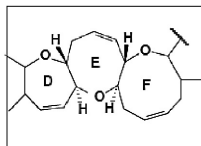
$X_1 =$



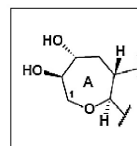
- CTX2A1 (2,3-dihydroxyCTX3C)

$R_2 = H$

$X_1 =$

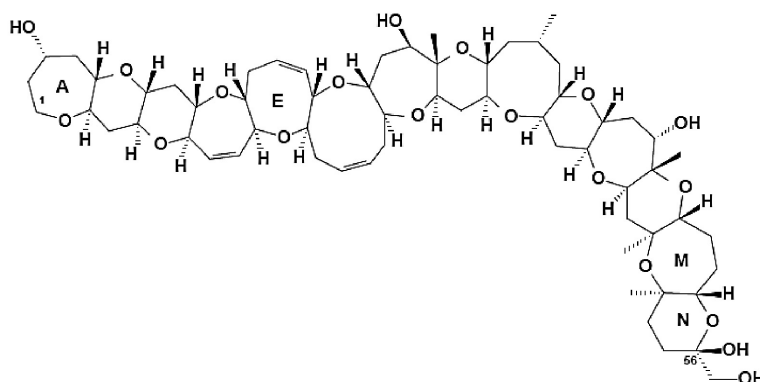


$X_2 =$



a) P-CTX Type I with 13 number of E rings and 60 number of carbons & Type II with 57 number of carbons

(b) C-CTX-1 (C-CTX2 is an epimer of C-CTX-1 at C-56).



b) Caribbean CTX 1 & CTX 2 with 14 number of E rings and 62 number of carbons

Fig. 2 a) Structure of Pacific Ciguatoxin (Type I & II) and b) structure of Caribbean Ciguatoxin (Lewis et al., 1998; Lewis, 2001; FAO, 2004; Caillaud et al., 2010)

fish, proper surveillance and reporting of incidence based on clinical data, community outreach and education to avoid misdiagnosis and under reporting of cases (Friedman et al., 2017). The ciguatera transmission from person to person which include effects on the embryo/ fetus via placenta and breast feed infant via mothers milk showed the risk of ciguatera toxicity in humans (Bagnis & Legrand, 1987; Blythe & De Silva, 1990; Ruff & Lewis, 1994; Karalis et al., 2000). The amount of toxins is directly correlated to the size of the fish and results indicated that large sized fishes had more ciguatoxin in comparison to small fishes (Pottier et al., 2001). Hence, it is advisable for the consumers to take only fishes of small size. Ban or size restrictions on certain reef fish species can be taken as an initial safety measure to protect the consumers from the lethal effects of this toxicity. European Union regulation states that “Fishery products containing biotoxins such as ciguatoxin or muscle-paralyzing toxins must not be placed on the market” but there is no reference analytical method suggested for CFP samples, which restricts the implementation of regulatory safety limits (Caillaud et al., 2010).

Indian seafood trade is heavily dependent upon the export of the highly prized coral reef fishes. In one such case the remnant head waste of *Lutjanus* species deemed for export was implicated in CFP toxicity in Mangalore coast during September 2016 (MTNN, 2016; Times of India, 2016). The workers of nearby exporting fish firm were also hospitalized due to consumption of fish heads, which was considered as a waste in the fish export factory. This incident showed the failure of hazard identification from the industry and most of the industries handling the export of reef fishes have the responsibility for the proper checking and diagnosis of their consignments, so as to ensure the export safety of our products. Coral reef fisheries mainly contributed by major species such as snappers, reef cods, croakers, trevally and barracuda are highly demanded species in the foreign markets. Pre-export testing is an important hazard control measure for coral reef fishes. Although reports on the existence of ciguatera from our coast is rare, ICAR-CIFT has initiated monitoring of coral reef fishes for presence of ciguatoxin (Rajeish et al., 2016; Rajisha et al., 2017a; b). Climate change and globalization of trade has led to an increase in the spread of ciguatera, hence guidance is needed for those countries where CFP risk management programmes has not yet been implemented (FAO, 2017).

Future Directions

Ciguatoxin has not been reported from Indian coastal regions before 2016. Now there is a prevalence of ciguatoxin from Indian Coast. That indicates that there may be climate change induced shift in the habitat of *G. toxicus*, which is the causative organisms responsible for producing CTX. Hence, risk management approach is required for protection of our aquatic habitats against demographic expansion of *G. toxicus* and further bioaccumulation in coral reef fishes. Now, there is an urgent need to periodically monitor all coral reef species that hugely contributes to the export basket as well as for domestic consumption. There is a distinct possibility of bioaccumulation of ciguatoxin in our ecosystem and efforts should be initiated for complete characterization of Indian Ocean Ciguatoxin and development of validated analytical methods.

Acknowledgement

The authors are grateful to the Director, ICAR-Central Institute of Fisheries Technology, Cochin, for the constant support and encouragement.

References

- Abraham, A., Jester, E. L. E., Granade, H. R., Plakas, S. M. and Dickey, R.W. (2012) Caribbean ciguatoxin profile in raw and cooked fish implicated in ciguatera. *Food Chem.* 131: 192-198
- AOAC (2012) Association of Analytical Communities. Official methods of analysis, Natural Toxins, Ch. 49: 86-88
- Arena, P., Levin, B., Fleming, L. E., Friedman, M. A. and Blythe, D. (2004) A pilot study of the cognitive and psychological correlates of chronic ciguatera poisoning. *Harmful Algae.* 3: 51-60
- Aune, T., Larsen, S., Aasen, J. A., Rehmann, N., Satake, M. and Hess, P. (2007) Relative toxicity of dinophysistoxin-2 (DTX-2) compared with okadaic acid, based on acute intraperitoneal toxicity in mice. *Toxicon.* 49(1): 1-7
- Azziz, B. E., Luber, G., Conklin, L., Tosteson, T. R., Granade, H. R., Dickey, R. W. and Backer, L. C. (2012) Assessing the incidence of ciguatera fish poisoning with two surveys conducted in Culebra, Puerto Rico, during 2005 and 2006. *Environ. Health Perspect.* 120: 526-529
- Baden, D., Fleming, L. E. and Bean, J. (1995) Marine Toxins in handbook of clinical Neurology: Intoxications of the Nervous System Part-II, National Toxins and Drugs. (deWolff, F.A., Ed) Elsevier Press: Amsterdam, The Netherlands: 21: 141-175

- Bagnis, R. and Legrand, A. M. (1987) Clinical features on 12,890 cases of ciguatera (fish poisoning) in French Polynesia. In: Progress in Venom and Toxin Research (Gopalakrishnakone, P. and Tan, C. K., Eds). National University of Singapore and International Society of Toxicology, Asia-Pacific Section, Singapore. pp 372-377
- Bagnis, R., Chanteau, S., Chungue, E., Drollet, J., Lechat, I., Legrand, A., Pompon, A., Prieur, C., Roux, J. and Tetaria, C. (1985) Comparison of the cat bioassay, The mouse bioassay and the mosquito bioassay to detect ciguatoxicity in fish. In: Proceedings of the fifth international coral reef congress, Tahiti, French Polynesia. pp 491-496
- Banner, A. H., Helfrich, P., Scheuer, P. J. and Yoshida, T. (1963) Research on ciguatera in the tropical Pacific. In: Proceedings of the 16th ann. Session Gulf, Caribbean Fisheries Institute. pp 84-98
- Banner, A., Scheuer, P., Sasaki, S., Helfrich, P. and Alender, C. (1960) Observations on ciguatera type toxin in fish. Ann. N.Y. Acad. Sci., 90: 770-787
- Baumann, F., Bourrat, M. B. and Pauillac, S. (2010) Prevalence, symptoms and chronicity of ciguatera in New Caledonia: Results from an adult population survey conducted in Noumea during 2005. *Toxicon*. 56(5): 662-667
- Bienfang, P., Oben, B., DeFelice, S., Moeller, P., Huncik, K., Oben, P., Toonen, R., Daly-Engel, T. and Bowen, B. (2008) Ciguatera: the detection of neurotoxins in carnivorous reef fish from the coast of Cameroon, West Africa. *Afr. J. Mar. Sci.* 30: 533-540
- Blythe, D. G. and de Sylva, D. P. (1990) Mother's milk turns toxic following fish feast. *J. Am. Med. Assoc.* 264 (16): 2074
- Blythe, D. G., De Sylva, D. P., Fleming, L. E., Ayyar, R. A., Baden, D. G. and Shrank, K. (1992) Clinical experience with I.V. Mannitol in the treatment of ciguatera. *Bull. Soc. Pathol. Exot.* 85: 425-426
- Botana, L. M. (2008) Seafood and Freshwater Toxins. Pharmacology, Physiology, and Detection; Ed.; CRC Press: Boca Raton, FL, USA. 1215
- Bruneau, A., Mahanty, S., al-Azraqi, T., MacLean, J., Bourque, M. and Desroches, F. (1997) Ciguatera fish poisoning linked to the ingestion of barracuda in a Montreal restaurant-Quebec. *Can. Commun. Dis. Rep.* 23(20): 153-156
- Caillaud, A., de la Iglesia, P., Darius, H. T., Pauillac, S., Aligizaki, K., Fraga, S., Chinain M. and Diogène, J. (2010). Update on Methodologies Available for Ciguatoxin Determination: Perspectives to Confront the Onset of Ciguatera Fish Poisoning in Europe, *Marine Drugs*. 8 (6): 1838-1907
- Chan, T. Y. K. (2013) Ciguatera caused by consumption of humphead wrasse. *Toxicon*. 76: 255-259
- Chan, T. Y. K. (2016) Characteristic features and contributory factors in fatal ciguatera fish poisoning-implications for prevention and public education. *Am. J. Trop. Med. Hyg.* 94: 704-709
- Chateau-Degat, M. L., Huin-Blondey, M. O., Chinain, M., Darius, T., Legrand, A. M., Ngoc, L. N., Laudon, F., Chansin, R. and Dewailly, E. (2007a) Prevalence of chronic symptoms of ciguatera disease in French Polynesian adults. *Am. J. Trop. Med. Hyg.* 77: 842-846
- Chateau-Degat, M., Beuter, A., Vauterin, G., Nguyen, N. L., Chinain, M., Darius, T., Legrand A., Chansin, R. and Dewailly, E. (2007b) Neurologic signs of ciguatera disease: Evidence of their persistence. *Am. J. Trop. Med. Hyg.* 77: 1170-1175
- Cheng, C. C. and Chung, C. H. (2004) Ciguatera fish poisoning: a challenge to emergency physicians Hong Kong *J. Emerg. Med.* 11(3): 173-177
- Chinain, M., Darius, H. T., Ung, A., Fouc, M. T., Revel, T., Cruchet, P., Pauillac, S. and Laurent, D. (2010a) Ciguatera risk management in French Polynesia: The case study of Raivavae Island (*Australes archipelago*). *Toxicon*. 56(5):674-690
- Chinain, M., Darius, H., Ung, A., Cruchet, P., Wang, Z., Ponton, D., Laurent, D. and Pauillac, S. (2010b) Growth and toxin production in the ciguatera-causing dinoflagellate *Gambierdiscus polynesiensis* (Dinophyceae) in culture. *Toxicon*. 56(5): 739-750
- Coleman, A. M. E. (1990) Ciguatoxin-induced food poisoning in a community - implications for disease surveillance and medical-practice in Jamaica. *West Indian Med. J.* 39: 233-238
- Crump, J. A., McLay, C. L. and Chambers, S. T. (1999) Ciguatera fish poisoning. *Postgrad. Med. J.* Nov. 75: 678-679
- D'Silva, M., Anil, A., Naik, R. and D'Costa, P. (2012) Algal blooms: a perspective from the coasts of India, *Natural Hazards. ISPMNH.* 63(2): 1225-1253
- Daranas, A. H., Norte, M. and Fernández, J. J. (2001) Toxic marine microalgae. *Toxicon*. 39(8): 1101-1132
- DeFouw, J., Egmond, H. and Speijers, G. (2001) Ciguatera fish poisoning: a review. National Institute of Public Health and Environment. RIVM, P.O. Box 1, 3720 BA, Bilthoven, RIVM report 388802021. 66p
- DeHaro, L., Pommier, P. and Valli, M. (2003) Emergence of imported ciguatera in Europe: report of 18 cases at the Poison Control Centre of Marseille. *J. Toxicol. Clin. Toxicol.* 41(7): 927-930s
- DeMotta, E. G. and Noceda, D. G. (1985) Epidemiological, clinical and experimental aspects of ciguatera in Puerto Rico. In Proceedings of the 5th International Coral Reef Congress, Tahiti, French Polynesia. pp 415-416

- DeSchrijver, K., Maes, I., De Man, L. and Michelet, J. (2002) An outbreak of diarrhoeic shellfish poisoning in Antwerp, Belgium. *Eurosurveillance Monthly Archives*. 7(10): 138-141
- Dickey, R. W. (2008) Ciguatera Toxins: Chemistry, Toxicology and Detection. In *Seafood and Freshwater Toxins: Pharmacology, Physiology, and Detection* (Botana, L. M., Ed), 2nd edn., CRC Press-Taylor & Francis: Boca Raton, FL, USA. pp 479-500
- Dickey, R. W. and Plakas, S. M. (2010) Ciguatera: A public health perspective. *Toxicon*. 56 (2): 123-136
- Dickey, R., Jester, E., Granade, R., Mowdy, D., Moncrief, C., Rebarchik, D., Robl, M., Musser, S. and Poli, M. (1999) Monitoring brevetoxins during a *Gymnodinium breve* red tide: comparison of sodium channel specific cytotoxicity assay and mouse bioassay for determination of neurotoxic shellfish toxins in shellfish extracts. *Nat. Toxins*. 7: 157-165
- EFSA (2009) European Food Safety Authority. Marine biotoxins in shellfish - Yessotoxin group - Scientific Opinion of the Panel on Contaminants in the Food chain. *EF SAJ*(907): 1-62
- EFSA (2010) European Food Safety Authority. Panel on Contaminants in the Food Chain. Scientific opinion on marine biotoxins in shellfish - emerging toxins: brevetoxin group. *EFSAJ*. 8(7): 1677
- Falkowski, P. G. (1984) Physiological response of phytoplankton to natural light regimes. *J. Plankton Res.* 6(2): 295-307
- FAO (2004) Marine biotoxins. FAO food and nutrition paper, Food and Agriculture Organisation of the United Nations, Rome. 80(7): 192p
- FAO (2017) The State of World Fisheries and Aquaculture, Food and Agriculture Organisation of the United Nations, Rome, Italy, 2014: 223p
- FDA (2011) Food and Drug Administration, Fish and Fishery Products Hazards and Controls Guidance Fourth Edition, CHAPTER 6: Natural Toxins, 468p. <https://www.fda.gov/downloads/Food/GuidanceRegulation/UCM251970.pdf>
- Frenette, C., MacLean, J. D. and Gyorkos, T. W. (1988) A Large Common-Source Outbreak of Ciguatera Fish Poisoning. *J. Infect. Dis.* 158(5): 1128-1131
- Friedman, M. A., Arena, P., Levin, B., Fleming, L., Fernandez, M., Weisman, R., Bernstein, J., Schrank, K., Blythe, D., Backer, L. and Reich, A. (2007) Neuropsychological study of ciguatera fish poisoning: A longitudinal case-control study. *Arch. Clin. Neuropsychol.* 22: 545-553
- Friedman, M. A., Fernandez, M., Backer, L. C., Dickey, R. W., Bernstein, J., Schrank, K., Kibler, S., Stephan, W., Gribble, M. O., Bienfang, P., Bowen, R. E., Degrasse, S., Flores Quintana, H. A., Loeffler, C. R., Weisman, R., Blythe, D., Berdalet, E., Ayyar, R., Clarkson-Townsend, D., Swajian, K., Benner, R., Brewer, T. and Fleming, L. E. (2017) An Updated Review of Ciguatera Fish Poisoning: Clinical, Epidemiological, Environmental and Public Health Management. *Mar. Drugs*. 15(3): 72
- Garthwaite, I. (2000) Keeping shellfish safe to eat: a brief review of shellfish toxins, and methods for their detection. *Trends in Food Sci. Technol.* 11(7): 235-244
- Gillespie, N. C., Lewis, R. J. and Pearn, J. H. (1986) Ciguatera in Australia. Occurrence, clinical features, pathophysiology and management. *Med. J. Aust.* 145 (11-12): 584-590
- Glaziou, P. and Martin, P. M. (1993) Study of factors that influence the clinical response to ciguatera fish poisoning. *Toxicon*. 31(9): 1151-1154
- Glaziou, P. and Legrand, A. M. (1994) The Epidemiology of Ciguatera fish Poisoning. *Toxicon*. 32(8): 863-73
- Granade, H. R., Cheng, P. C. and Doorenbos, N. J. (1976) Ciguatera I: brine shrimp (*Artemia salina* L.) larval assay for ciguatera toxins. *J. Pharm. Sci.* 65(9): 1414-1415
- Hallegraeff, G. M. (1993) A review of harmful algal blooms and their apparent global rise. *Phycologia*. 32: 79-99
- Halstead, B. (1978) *Poisonous and Venomous Marine Animals of the World*; Darwin Press: Princeton, NJ, USA. 1043p
- Hamilton, B., Hurbungs, M., Jones, A. and Lewis, R. J. (2002a) Multiple ciguatoxins present in Indian Ocean reef fish. *Toxicon*. 40(9): 1347-1353
- Hamilton, B., Hurbungs, M., Vernoux, J. P., Jones, A. and Lewis, R. J. (2002b) Isolation and characterization of Indian Ocean ciguatoxin. *Toxicon*. 40(6): 685-693
- Hoffman, P., Granade, H. and McMillan, J. (1983) The mouse ciguatoxin bioassay: a dose-response curve and symptomatology analysis. *Toxicon*. 21(3): 363-369
- Hokama, Y., Takenaka, W. E., Nishimura, K. L., Ebesu, J. S. M., Bourke, R., Sullivan, P. K. (1998) A simple membrane immunobead assay for detecting ciguatoxin and related polyethers from human ciguatera intoxication and natural reef fishes. *J. AOAC Int.* 81: 727-735s
- Holmes, M. J., Lewis, R. J., Poli, M. A. and Gillespie, N. C. (1991) Strain dependent production of ciguatoxin precursors (gambiertoxins) by *Gambierdiscus toxicus* (Dinophyceae) in culture. *Toxicon*. 29(6): 761-775
- Hsieh, C. H., Hwang, K. L., Lee, M. M., Lan, C. H., Lin, W. F. and Hwang, D. F. (2009) Species identification of ciguatoxin-carrying grouper implicated in food poisoning. *J. Food Prot.* 72(11): 2375-2379

- Juranovic, L. R. and Park, D. L. (1991) Food borne toxins of marine origin: ciguatera. *Rev. Environ. Contam. Toxicol.* 117: 51-94
- Karalis, T., Gupta, L., Chu, M., Campbell, B. A., Capra, M. A. and Maywood, P. A. (2000) Three clusters of ciguatera poisoning: clinical manifestations and public health implications. *Med. J. Aust.* 172(4): 160-162
- Katz, A. R., Terrell-Perica, S. and Sasaki, D. M. (1993) Ciguatera on Kauai: Investigation of factors associated with severity of illness. *Am. J. Trop. Med. Hyg.* 49: 448-454
- Kosaki, T. I., Stephens, B. J., Anderson, H. H. (1968) Marine toxins from the pacific: III. Comparative bioassay of ciguatoxin (s) in the mouse and chicken. In: *Proceeding of the West Pharmacol Soc.* 11: 126-128
- Labrousse, H. and Matile, L. (1996) Toxicological biotest on Diptera larvae to detect ciguatoxins and various other toxic substances. *Toxicon.* 34 (8): 881-891
- Lawrence, J., Loreal, H., Toyofuku, H., Hess, P., Iddya, K. and Ababouch, L. (2011) FAO Fisheries and Aquaculture Technical Paper-551, Assessment and Management of Biotxin Risks in Bivalve Molluscs; Food and Agriculture Organisation of the United Nations: Rome, Italy. 337p
- Legrand, A. M., Teai, T., Cruchet, P., Satake, M., Murata, K. and Yasumoto, T. (1998) Two structural types of ciguatoxin involved in ciguatera fish poisoning in French Polynesia. In: *VIII International Conference on Harmful Algae* (Reguera, B., Blanco, J., Fernandez, M. L., Wyatt, T., Eds), Xunta de Galicia and International Oceanographic Commission of UNESCO: Paris, France, pp 473-475
- Lehane, L. and Lewis, R. J. (2000) Review Ciguatera: recent advances but the risk remains. *Int. J. Food Microbiol.* 61: 91-125
- Lewis, R. J. (1995) Detection of Ciguatoxins and related Benthic Dinoflagellate Toxins: in vivo and in vitro Methods. In *Manual on Harmful Marine Microalgae* (Hallegraeff, G., Anderson, D. and Cembella, A., Eds), IOC Manuals and Guides No.33, UNESCO: Paris, France. 33: 135-161
- Lewis, R. J. (2000) Ciguatera management. *SPC Live Reef Fish Inf. Bull.* (7): 11-13
- Lewis, R. J. (2001) The changing face of ciguatera. *Toxicon.* 39 (1): 97-106
- Lewis, R. J. (2003) Detection of toxins associated with ciguatera fish poisoning. In *Manual on Harmful Marine Microalgae*. (Hallegraeff, G. M., Anderson, D. M. and Cembella, A. D., Eds), UNESCO: Paris France. pp 267-277
- Lewis, R. J. and Jones, A. (1997) Characterization of ciguatoxins and ciguatoxin congeners present in ciguateric fish by gradient reverse-phase high-performance liquid chromatography/mass spectrometry. *Toxicon.* 35 (2): 159-168
- Lewis, R. J., Holmes, M. J., Alewood, P. F. and Jones, A. (1994) Ionspray mass spectrometry of ciguatoxin-1, maitotoxin-2 and-3 and related marine polyether toxins. *Nat. Toxins.* 2(2): 56-63
- Lewis, R. J., Norton, R. S., Brereton, I. M. and Eccles, C. D. (1993) Ciguatoxin-2 is a diastereomer of ciguatoxin-3. *Toxicon.* 31(5): 637-643
- Lewis, R. J., Sellin, M., Poli, M. A., Norton, R. S., MacLeod, J. K. and Sheil, M. M. (1991) Purification and characterization of ciguatoxins from moray eel (*Lycodontis javanicus*, Muraenidae). *Toxicon.* 29(9): 1115-1127
- Lewis, R. J., Vernoux, J. P. and Brereton, A. M. (1998) Structure of Caribbean ciguatoxin isolated from *Caranx latus*. *J. Am. Chem. Soc.* 120 (24): 5914-5920
- Lewis, R. Jones, A. and Vernoux, J. (1999) HPLC/tandem electrospray mass spectrometry for the determination of Sub-ppb levels of Pacific and Caribbean ciguatoxins in crude extracts of fish. *Anal. Chem.* 71: 247-250
- Lombet, A., Bidard, J. N. and Lazdunski, M. (1987) Ciguatoxin and brevetoxins share a common receipt or site on the neuron a voltage-dependent Na⁺ channel. *FEBS Lett.* 219. pp 355-359
- Manger, R. L., Leja, L. S., Lee, S. Y., Hungerford, J. M. and Wekell, M. M. (1993) Tetrazolium-based cell bioassay for neurotoxins active on voltage-sensitive sodium channels: semiautomated assay for saxitoxin, brevetoxin, and ciguatoxin. *Anal. Biochem.* 214: 190-194
- Manger, R. L., Leja, L. S., Lee, S. Y., Hungerford, J. M., Hokama, Y., Dickey, R. W., Granade, H. R., Lewis, R. J., Yasumoto, T. and Wekell, M. M. (1995) Detection of sodium channel effectors: directed cytotoxicity assays of purified ciguatoxins, brevetoxins, saxitoxin and seafood extracts. *J. AOAC Int.* 78: 521-527
- McMahon, T. and Silke, J. (1998) Re-occurrence of winter toxicity. *Harmful Algae News.* 17: 12-16
- Morris, J. G., Lewin, P., Hargrett, N. T., Smith, C. W., Blake, P. A. and Schneider, R. (1982) Clinical-features of ciguatera fish poisoning - a study of the disease in the United-States Virgin Islands. *Arch. Intern. Med.* 142: 1090-1092
- Morris, P. D., Campbell, D. S. and Freeman, J. I. (1990) Ciguatera fish poisoning: an outbreak associated with fish caught from North Carolina coastal waters South. *Med. J.* 83(4): 379-382
- Morton, S. L., Norris, D. R. and Bomber, J. W. (1992) Effect of temperature, salinity and light intensity on the growth and seasonality of toxic dinoflagellates associated with ciguatera. *J. Exp. Mar. Biol. Ecol.* 157(1): 79-90

- Moulignier, A., Binet, D. and Frottier, J. (1995) Ciguatera fish poisoning: also in Europe. *J Neurol. Neurosurg. Psychiatry*. 59(2): 192
- MTNN (2016) Many hospitalized, consuming fish head curry in Ullal. [online newspaper] Mangalore Today News Network, Oct - 01, 2016. <http://www.mangaloretoday.com/main/Many-hospitalized-consuming-fish-head-curry-in-Ullal.html?fromNewsdog=1>
- Murata, M., Legrand, A. M., Ishibashi, Y. and Yasumoto, T. (1990) Structures and configurations of ciguatera toxin from the moray eel *Gymnothorax javanicus* and its likely precursor from the dinoflagellate *Gambierdiscus toxicus*. *J. Am. Chem. Soc.* 112(11): 4380-4386
- Murata, M., Legrand, A. M., Ishibashi, Y. and Yasumoto, T. (1989) Structures of ciguatera toxin and its congener. *J. Am. Chem. Soc.* 111(24): 8929-8931
- Nixon, S.W. (1995) Coastal marine eutrophication: a definition, social consequences and future concerns. *Ophelia*. 41: 199-220
- Paerl, H. W. (1988) Nuisance phytoplankton blooms in coastal, estuarine and inland waters. *Limnol. Oceanogr.* 33: 823-847
- Pearn, J. (2001) Neurology of ciguatera. *Br. Med. J.* 70(1): 4-8
- Pearn, J. H., Lewis, R. J., Ruff, T., Tait, M., Quinn, J., Murtha, W., King, G., Mallett, A. and Gillespie, N. C. (1989) Ciguatera and mannitol: Experience with a new treatment regimen. *Med. J. Aust.* 151: 77-80
- Perl, T. M., Bedard, L., Kosatsky, T., Hockin, C. J., Todd, E. C. D. and Remis, R. S. (1990) An outbreak of toxic encephalopathy caused by eating mussels contaminated with domoic acid. *N. Engl. J. Med.* 322: 1775-1780
- Poli, M. A., Lewis, R. J., Dickey, R. W., Musser, S. M., Buckner, C. A. and Carpenter, L. G. (1997) Identification of Caribbean ciguateras as the cause of an outbreak of fish poisoning among U.S. soldiers in Haiti. *Toxicon*. 35(5): 733-741
- Pottier, I., Hamilton, B., Jones, A., Lewis, R. J. and Vernoux, J. P. (2003) Identification of slow and fast-acting toxins in a highly ciguateric barracuda (*Sphyraena barracuda*) by HPLC/MS and radiolabeled ligand binding. *Toxicon*. 42(6): 663-672
- Pottier, I., Vernoux, J. P., Jones, A. and Lewis, R. J. (2001) Ciguatera fish poisoning in the Caribbean islands and Western Atlantic. *Rev. Environ. Contam. Toxicol.* 168: 99-141
- Pottier, I., Vernoux, J. P., Jones, A. and Lewis, R. J. (2002b) Analysis of toxin profiles in three different fish species causing ciguatera fish poisoning in Guadeloupe, French West Indies. *Food Addit. Contam.* 19(11): 1034-1042
- Pottier, I., Vernoux, J. P., Jones, A. and Lewis, R. J. (2002a) Characterization of multiple Caribbean ciguateras and congeners in individual specimens of horse-eye jack (*Caranx latus*) by high-performance liquid chromatography/mass spectrometry. *Toxicon*. 40(7): 929-939
- Quilliam, M. A. (2003) The role of chromatography in the hunt for red tide toxins. *J. Chromatogr. A*. 1000(1-2): 527-548
- Ragelis, E. P. (1984) Ciguatera seafood poisoning: overview. In: *Seafood Toxins* (Ragelis, E. P., Ed), American Chemical Society, Washington, DC. pp 22-36
- Rajeish, M., Shekar, M., Madhushree, H. N. and Venugopal, M. N. (2016) Presumptive case of ciguatera fish poisoning in Mangalore, India. *Research Communications, Curr. Sci.* 111(9): 1543-1547
- Rajisha, R., Kishore P., Panda, S. K., Ravishankar, C. N. and Kumar, A. K. (2017a) Confirmation of Ciguatera Fish Poisoning in Red Snapper, *Lutjanus bohar* (Forsskal, 1775) by Mouse Bioassay. *Fish. Technol.* 54: 1-4
- Rajisha, R., Kishore, P., Panda, S. K., Harikrishnan, G., Ajitha, K.C., Suresh, M. K., Chowdhury, L. M., Ravishankar, C. N. and Kumar, A. K. (2017b) Incidence of Ciguatera Fish Poisoning in Trivandrum, India. *Indian J. Fish.* 64(4): 129-133
- Randall, J. E. (1958) A Review of Ciguatera, Tropical Fish Poisoning, with a Tentative Explanation of its Cause. *Bull. Mar. Sci.* 8(3): 236-267
- Richardson, K. and Jorgensen, B. B. (1996) Eutrophication, definition, history and effects. In: *Eutrophication in Coastal Marine Ecosystems* (Jorgensen, B. B. and Richardson, K., Eds) American Geophysical Union, Washington, DC. 1-20
- Roberts, D., Davies, C., Mitchell, A., Moore, H., Picton, B. and Portig, A. (2004) Strangford Lough Ecological Change Investigation (SLECI) Report to Environment and Heritage Service by the Queen's University, Belfast. Available online at <http://www.ni-environment.gov.uk/print/0execsum.pdf>.
- Rodrigue, D. C., Etzel, R. A., Hall, S., de Porras, E., Velasquez, O. H. and Tauxe, R.V., Kilbourne, E. M. and Blake, P. A. (1990) Lethal paralytic shellfish poisoning in Guatemala. *Am. J. Trop. Med. Hyg.* 42: 267-271
- Roeder, K., Erler, K., Kibler, S., Tester, P., Van The, H., Nguyen-Ngoc, L., Gerdt, G. and Luckas, B. (2010) Characteristic profiles of Ciguatera toxins in different strains of *Gambierdiscus* spp. *Toxicon*. 56(5): 731-738
- Ruff, T. A. and Lewis, R. J. (1994) Clinical aspects of ciguatera: an overview. *Mem. Queensl. Mus.* 34: 609-619

- Satake, M., Fukui, M., Legrand, A., Cruchet, P. and Yasumoto, T. (1998) Isolation and structures of new ciguatoxin analogs, 2, 3-dihydroxyCTX3C and 51-hydroxyCTX3C, accumulated in tropical reef fish. *Tetrahedron Lett.* 39(10): 1197-1198
- Satake, M., Ishibashi, Y., Legrand, A. and Yasumoto, T. (1996) Isolation and structure of ciguatoxin-4 A, a new ciguatoxin precursor, from cultures of dinoflagellate *Gambierdiscus toxicus* and parrot fish *Scarus gibbus*. *Biosci. Biotechnol. Biochem.* 60(12): 2103-2105
- Satake, M., Murata, M. and Yasumoto, T. (1993) The structure of CTX3C, a ciguatoxin congener isolated from cultured *Gambierdiscus toxicus*. *Tetrahedron Lett.* 34(12): 1975-1978
- Scheuer, P. J. (1994) Tetrahedron perspective number 2: Ciguatera and its off-shoots-chance encounters en route to a molecular structures. *Tetrahedron.* 50(1): 3-18
- Sheppard, C. and Rioja-Nieto, R. (2005) Sea surface temperature 1871-2009 in 38 cells in the Caribbean region. *Mar. Environ. Res.* 60(3): 389-396
- Shi, F. (2012) Chemical and toxicological investigation of the toxic dinoflagellate, *Karenia brevisulcata*. Ph.D. Thesis, Lincoln University, New Zealand. 216p
- Shi, F., McNabb, P., Rhodes, L., Holland, P., Webb, S., Adamson, J., Immers, A., Gooneratne, R. and Holland, J. (2012) The toxic effects of three dinoflagellate species from the genus *Karenia* on invertebrate larvae and finfish. *New Zeal. J. Mar. Fresh.* 46(2): 149-165
- Skinner, M. P., Brewer, T. D., Johnstone, R., Fleming, L. E. and Lewis, R. J. (2011) Ciguatera fish poisoning in the Pacific islands (1998 to 2008). *PLoS Negl. Trop. Dis.* 5(12): 1416
- Smayda, T. J. (1992) Global epidemics of noxious phytoplankton blooms and food chain consequences in large ecosystems. In: *Food Chains, Models and Management of Large Marine Ecosystems* (Sherman, K., Alexander, L. M. and Gold, B. D. Eds). Westview Press, San Francisco. pp 275-307
- Smith, P., Chang, F. H. and Mackenzie, L. (1993) Toxic phytoplankton and algal blooms, summer 1992/93. Paper presented at the Marine toxins and New Zealand shellfish, proceedings of a workshop on research issues, 10-11 June 1993
- Stewart, I., Eaglesham, G. K., Poole, S., Graham, G., Paulo, C., Wickramasinghe, W., Sadler, R. and Shaw, G. R. (2010) Establishing a public health analytical service based upon chemical methods for detecting and quantifying Pacific ciguatoxin in fish samples. *Toxicon.* 56(5): 804-812
- Tester, P. A., Feldman, R. L., Nau, A.W., Kibler, S. R. and Litaker, R. W. (2010) Ciguatera fish poisoning and sea surface temperatures in the Caribbean Sea and the West Indies. *Toxicon.* 56(5): 698-710
- Times of India (2016) Hundreds taken ill after consuming fish heads in Ullal, [online news] Times of India, Oct 1, 2016. <https://timesofindia.indiatimes.com/city/mangaluru/Hundreds-taken-ill-after-consuming-fish-heads-in-Ullal/articleshow/54630403.cms>
- Underdal, B., Yndestad, M. and Aune, T. (1985) DSP intoxication in Norway and Sweden, Autumn 1984 - Spring 1984. In: *Toxic Dinoflagellates* (Anderson, D. M., White, A. W. and Baden, D. G., Eds). Amsterdam, The Netherlands: Elsevier. <http://agris.fao.org/agris-search/search.do?recordID=US8644422>
- UNESCO (2016) United Nations Educational, Scientific and Cultural Organization, Climate Change Education and COP22, Tackling climate change through Education for Sustainable Development, <http://unesdoc.unesco.org/images/0024/002464/246479E.pdf>.
- Van Dolah, F. M. (2000) Marine algal toxins: origins, health effects, and their increased occurrence. *Environ. Health Perspect.* v 108 (Suppl. 1): 133-141
- Vernoux, J. P. and Lewis, R. J. (1997) Isolation and characterization of Caribbean ciguatoxins from the horseeye jack (*Caranx latus*). *Toxicon.* 35(6): 889-900
- Vernoux, J. P. and Lejeune, J. (1994) Ciguatera in the French West Indies. *Mem. Qld. Mus.* 34: 631-638
- Villareal, T. A., Hanson, S., Qualia, S., Jester, E. L. E., Granade H. R. and Dickey, R. W. (2007) Petroleum production platforms as sites for the expansion of ciguatera in the northwestern Gulf of Mexico. *Harmful Algae.* 6(2): 253-259
- Wells, M. L., Trainer, V. L., Smayda, T. J., Karlson B. S. O., Trick, C. G., Kudela, R. M., Ishikawa, A., Bernard, S., Wulff, A., Anderson, D. M. and Cochlan, W. P. (2015) Harmful algal blooms and climate change: Learning from the past and present to forecast the future. *Harmful Algae.* 49: 68-93
- Wong, C. K., Hung, P., Lee, K. L. H. and Kam, K. M. (2005) Study of an outbreak of ciguatera fish poisoning in Hong Kong. *Toxicon.* 46(5): 563-571
- Wong, C. K., Hung, P. and Lo, J. Y. (2014) Ciguatera fish poisoning in Hong Kong—a 10-year perspective on the class of ciguatoxins. *Toxicon.* 86: 96-106
- Wong, C., Hung, P., Lee, K. and Kam, K. (2009) Solid-phase extraction clean-up of ciguatoxin contaminated coral fish extracts for use in the mouse bioassay. *Food Addit. Contam.* 26: 236-247
- Yasumoto, T. and Murata, M. (1993) Marine toxins. *Chem. Rev.* 93: 1897-1909
- Yasumoto, T., Igarashi, T., Legrand, A., Cruchet, P., Chinain, M., Fujita, T. and Naokis, H. (2000) Structural elucidation of ciguatoxin congeners by fast-atom bombardment tandem mass spectroscopy. *J. Am. Chem. Soc.* 122: 4988-4989

- Yasumoto, T., Nakajima, I., Bagnis, R. and Adachi, R. (1977) Finding of a dinoflagellate as a likely culprit of ciguatera. *Bull. Jpn. Soc. Sci. Fish.* 43(8): 1021-1026
- Yasumoto, T., Oshima, Y. and Yamaguchi, M. (1978) Occurrence of a New Type of Shellfish Poisoning in the Tokohu District. *Bull. Jap. Soc. Sci. Fish.* 44(11): 1249-1255
- Yasumoto, T., Raj, U. and Bagnis, R. (1984) Seafood Poisonings in Tropical Regions, Laboratory of Food Hygiene, Faculty of Agriculture: Tohoku University, Japan. 74p
- Yogi, K., Sakugawa, S., Oshiro, N., Ikehara, T., Sugiyama, K. and Yasumoto, T. (2014) Determination of Toxins Involved in Ciguatera Fish Poisoning in the Pacific by LC/MS. *J AOAC Int.* 97(2): 398-402