Universidad de La Laguna

Máster Biología Marina: Biodiversidad y Conservación

# Ciguatera

## A brief ecological perspective

Ciguatera

Una breve perspectiva ecológica

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# AUTORIZACIÓN DEL TUTOR/ES

El Prof. José Javier Fernández Castro, Catedrático de Química Orgánica de la Universidad de La Laguna y la Dra. Ana Raquel Díaz Marrero, Científica Titular adscrita al Instituto de Productos Naturales y Agrobiología del Consejo Superior de Investigaciones Científicas, como Tutor Académico y Tutora Externa, respectivamente,

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Y para que así conste y surta los efectos oportunos, firman el presente informe favorable en San Cristóbal de La Laguna a 5 de julio de 2023.

Fdo. Prof. José J. Fernández Castro　　　　Fdo. Dra. Ana R. Díaz Marrero

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## Abstract

As well known, Ciguatera Fish Poisoning (CP) is a prevalent and non-infectious seafood-borne illness caused by the consumption of reef fish contaminated with ciguatoxins. These potent neurotoxins are produced by *Gambierdiscus* spp., a marine dinoflagellate, and tend to accumulate in the food chain, particularly in carnivorous species. CP affects thousands of individuals worldwide, presenting symptoms such as gastrointestinal issues, cardiovascular problems, and neurological disorders. This illness has significant socioeconomic implications, impacting both the fishery sector and tourism industry in endemic regions. The proliferation of *Gambierdiscus*, attributed to human activities and climate change, has led to an increase in harmful algal blooms. Furthermore, climate change and rising ocean temperatures have profound effects on fish behavior, metabolism, and overall marine ecosystems. Understanding the ecological aspects of CP, including the bioaccumulation, biomagnification, and biotransformation of ciguatoxins in the food chain, is crucial for effectively managing this global health issue. Therefore, this review aims to provide updated information on CTXs, with a specific focus on the Canary Islands.

**Resumen**

La intoxicación por Ciguatera (CP) es una enfermedad prevalente y no infecciosa transmitida por mariscos, causada por el consumo de peces de arrecife contaminados con ciguatoxinas. Estas potentes neurotoxinas son producidas por *Gambierdiscus spp.*, un dinoflagelado marino, y tienden a acumularse en la cadena alimentaria, especialmente en especies carnívoras. CP afecta a miles de personas en todo el mundo, presentando síntomas como problemas gastrointestinales, trastornos cardiovasculares y neurológicos. Esta enfermedad presenta problemas socioeconómicos significativos, impactando tanto en el sector pesquero como en la industria turística en las regiones endémicas. La proliferación de *Gambierdiscus*, atribuida a las actividades humanas y al cambio climático, ha llevado a un aumento de las floraciones de algas nocivas. Además, el cambio climático y el aumento de las temperaturas oceánicas tienen efectos profundos en el comportamiento de los peces, su metabolismo y los ecosistemas marinos en general. Comprender los aspectos ecológicos de CP, incluyendo la bioacumulación, la biomagnificación y la biotransformación de las ciguatoxinas en la cadena alimentaria, es crucial para gestionar eficazmente este problema de salud global. Por lo tanto, esta revisión tiene como objetivo proporcionar información actualizada sobre las CTXs, con un enfoque específico en las Islas Canarias.

***Keywords*:** ciguatoxins, ciguatera, *Gambierdiscus spp.*, bioaccumulation, Canary Islands.

# 1. Marine Biotoxins

Natural toxins are, as its name suggests produced naturally by living organisms from different kingdoms of life, with each having individual biological functions and activities that affect directly or indirectly other organisms, including humans (Nwaji et al., 2023).

Toxins are considered bioactive compounds that are usually produced to provide competitive advantages in the environment the producers live in to thrive or enhancing survival as a whole (Nwaji et al., 2023). These toxins are normally assembled to keep predators away as well as protect colonies and are used as a mechanism to capture prey (Nwaji et al., 2023).

Certain marine biotoxins have a complex chemical structure and toxicity, and can be categorized into two major groups: lipophilic toxins, such as ciguatoxins, yessotoxins and okadaic acid, and hydrophilic toxins, such as domoic acid, paralytic shellfish poisoning toxins, and tetrodotoxins (Alves et al., 2019). Broadly speaking, hydrophilic toxins tend to exhibit greater bioaccessibility than lipophilic toxins (Alves et al., 2019).

Natural toxins can also be divided based on the biological origin, target organ toxicity or mode of action (Nwaji et al., 2023). The mechanisms of action can vary depending on their bioactive compound including mechanisms such as the inhibition of sodium-potassium ATPase, inhibition of angiotensin converting enzyme (ACE), binding to ion channels or binding to and inactivating proteins (Nwaji et al., 2023).

Some marine biotoxins are generated by particular harmful algae that can proliferate and lead to harmful algal blooms (HABs) under specific climatic and environmental circumstances (Alves et al., 2019). These biotoxins have been recognized as a worldwide issue, as they can pose a danger to human health, mainly because of food-borne illnesses. Moreover, these outbreaks of HABs can lead to significant economic losses for the shellfish and fish industries because of the closure of harvesting zones (Alves et al., 2019; Grattan et al., 2016). The reason for this is that many HABs generate toxins that collect in species caught by these industries, leading to the risk of human illness or even death when these contaminated species are consumed in large amounts (Free et al., 2022; Grattan et al., 2016), Figure 1.

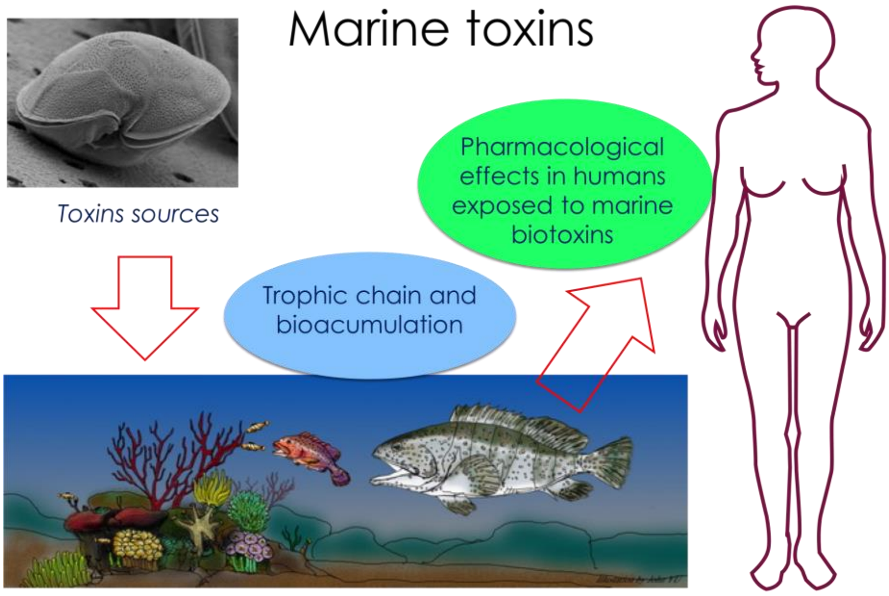


*Figure 1. Aquatic toxins in the environment, animals and humans.*

Humans can experience a variety of disorders known as biointoxications as a result of the accumulation of biotoxins through consuming fisheries products (Morabito et al., 2018). There are five known illnesses that are commonly associated with Harmful Algal Blooms (HABs) (Grattan et al., 2016; Morabito et al., 2018):

- Ciguatera Fish Poisoning (CFP or CP)
- Paralytic Shellfish Poisoning (PSP)
- Neurotoxic Shellfish Poisoning (NSP)
- Amnesic Shellfish Poisoning (ASP)
- Diarrheic Shellfish Poisoning (DSP)

HABs can be classified based on the effects they produce, their chemical structure, or their solubility in solvents (Table 1).

*Table 1. Classification of biotoxins (adapted from Morabito et al., 2018).*

| Biotoxins | Chemical class | Toxin source | Toxic syndrome in humans |
|---|---|---|---|
| **Water soluble marine biotoxins Saxitoxin (STXs)** | Purine-derived | | Paralytic Shellfish Poisoning (PSP) |
| **Domoic acid** | Amino acids | *Pseudo-nitzschia pungens f. multiseries* | Amnesic Shellfish Poisoning (ASP) |
| **DSP toxins: Okadaic acid (OA); Pectenotoxin (PTXs); Yessotoxins (YTXs)** | Linear and macrocyclic polyethers<br>*Trans*-fused polyethers | *Dinophysis spp.* *Prorocentrum spp.* | Diarrhetic Shellfish Poisoning (DSP) |
| **Brevetoxins** | *Trans*-fused polyethers | *Karenia brevis* | Neurotoxins Shellfish Poisoning (NSP) |
| **Ciguatoxins (CTXs)** | Cyclic polyether compounds | *Gambierdiscus spp.* | Ciguatera Fish Poisoning (CP) |

The adverse impacts of HABs on human health are primarily caused by three types of exposure: consumption of contaminated seafood, skin contact with contaminated water, and inhalation of aerosolized biotoxins. The most common and well-documented effect is seafood poisoning resulting from the consumption of contaminated filter feeders, primarily bivalve molluscs, although echinoderms, tunicates, gastropods, and fish that acquire biotoxins through the food chain can also be affected (Morabito et al., 2018).

Seafood species that are vulnerable to toxins are carefully monitored to ensure their safety. When toxin levels exceed safe limits, fisheries and aquaculture operations may be shut down to prevent harm to public health (Free et al., 2022). However, such closures can have negative impacts on the economy, nutrition, and social and cultural aspects of affected communities. Therefore, it is crucial to develop monitoring and management programs for biotoxins that effectively protect public health while minimizing the impact on fishing and aquaculture operations. This is essential for sustaining the viability of coastal communities in the face of a changing ocean (Free et al., 2022).

Recently, there has been observed an increase on the blooms of toxic marine microalgae, both temporal and spatial. The rise in the abundance of  HABs is likely a result of human activities and climate change, which impact the marine planktonic ecosystems (Grattan et al., 2016; Morabito et al., 2018). Anthropogenic activities, such as nutrient contamination, high utilization of coastal areas, changes in water flow dynamics, and species leakage from ships' ballast waters, contribute to the global spread of HABs (Morabito et al., 2018). Climate change, which includes ocean acidification, alterations in temperature, stratification, and increased nutrient input induced by precipitation and light, is also responsible for the proliferation of HABs. Temperature is a major environmental factor that affects the structure and composition of phytoplankton communities. Global warming affects several stages of HAB growth and development, influencing germination, motility, photosynthesis, nutrient uptake, and other physiological activities, which support toxin production in HAB species (Morabito et al., 2018).

One of the most worldwide relevant marine biotoxins are ciguatoxins (CTXs). CTXs are chosen among harmful algal bloom (HAB) toxins due to their significant impact on human health, marine ecosystems, and their ability to bioaccumulate in fish, leading to widespread ciguatera fish poisoning. CTXs are considered as emerging biotoxins in the EU area and a big threat in the Canary Islands. Therefore, the main goal of this review is to provide a brief vision on CP from an ecological point of view. We aim to create an updated review that explains the basic knowledge on this family of toxins, as well as to interpret and detail the processes of bioaccumulation, biomagnification, and biotransformation of these toxins in the food chain. Additionally, the importance of ciguatoxins and their potential impacts on human health and the environment will be provided, with a particular focus on the Canary Islands.

# 2. Ciguatera Fish Poisoning (CP)

Ciguatera Poisoning (CP) is considered as the most prevalent, and non-infectious seafood-borne illness in the world (Chinain et al., 2021, 2021; Grattan et al., 2016; Heimann & Sparrow, 2015; Tanyag et al., 2021). This food-borne illness acts on fish-consumers around the world bringing high consequences in the modern society such as expenses in social security and insurance (Soliño & Costa, 2020).

There is an estimation ranging from around 10.000 to 50.000 cases of CP per year (Chinain et al., 2021; Friedman et al., 2008; Tanyag et al., 2021). The variation in the reported cases of CP can be explained by various factors, such as the isolation of tropical island nations, inadequate medical and technical infrastructure, the preference for traditional remedies, and the tendency to avoid medical help in mild cases (Heimann & Sparrow, 2015). Adding to the issue is the absence of rapid diagnostic kits specifically designed for detecting ciguatera or testing fish for the toxin at markets or on boats before they are distributed to shops, which makes it difficult to diagnose the illness or prevent the distribution of contaminated fish (Heimann & Sparrow, 2015).

CP occurs when humans consume reef fish that have accumulated powerful neurotoxins called ciguatoxins in their muscles and organs. These toxins are produced by a type of marine dinoflagellate called *Gambierdiscus* spp., which grow on various macroalgae or other substrates in coral reef ecosystems (Grattan et al., 2016). Herbivorous fish consume the dinoflagellates, and the toxins are then magnified and accumulated as they move up the food chain through carnivorous species. Ciguatera toxicity can affect over 425 fish species (Lehane & Lewis, 2000; WHO, 2020), with the highest risk for those at the top of the food chain and commercially valuable species, such as predatory carnivores like barracuda (over 70% of which may be toxic). Other high-risk fish include snapper, grouper, wrasse, moray eel, parrotfish and amberjack (Grattan et al., 2016; WHO, 2020).

The disease has been reported in the Pacific, Atlantic, and Indian Ocean (Grattan et al., 2016; Soliño & Costa, 2020) among temperate countries located between 35ºN and 35ºS (Soliño & Costa, 2020). Interestingly, the distribution for the dinoflagellate *Gambierdiscus* has been reported to higher latitudes, as well as into new areas of the East Atlantic, the Mediterranean islands and the Arabian sea and Gulf of Bengal (Soliño & Costa, 2020). This scenario could be caused by the global warming and the increase of sea surface temperatures causing a new issue in these areas, as already mentioned before (Soliño & Costa, 2020), Figure 2.

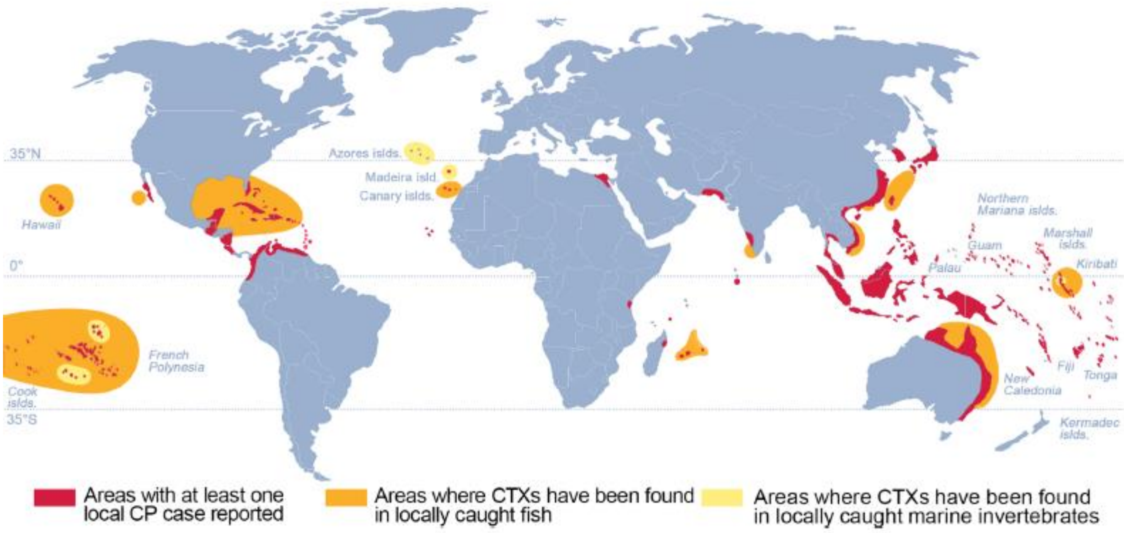


*Figure 2. Areas with at least one local CP case reported (red), CTXs confirmation in locally caught fish (orange), and marine invertebrates (yellow). Picture taken from Chinain et al., 2021).*

The diagnosis of CP is typically based on clinical symptoms in the context of a thorough history of recent consumption of predatory reef fish (Grattan et al., 2016). The symptoms appears to be dependent on the type of toxin ingested as well as the amount of toxin consumed (Litaker et al., 2010). Symptoms of CP usually appear within 12 hours of eating the contaminated fish and start with severe gastrointestinal issues such as nausea, vomiting, diarrhea, and abdominal pain, which typically subside within 24 hours. The acute episode may also involve cardiovascular or neurological symptoms (Heimann & Sparrow, 2015; Lopez et al., 2016). Some patients report persistent symptoms for several years, which are collectively known as the chronic ciguatera syndrome. Overall the most common and distinctive symptoms are: diarrhea, abdominal pain, vomiting, muscular aches, sweating, anxiety, numbness plus tingling in different areas, irregular heartbeat, low blood pressure, reversal of temperature sensation, paralysis and death in some cases (Litaker et al., 2010).

Chronic symptoms may include fatigue, weakness, paresthesias, and depression, which may recur after a presumed recovery or may be triggered by alcohol use or the ingestion of fish with low levels of ciguatoxin. This suggests that those who have had CP before are at a higher risk of repeated illness (Grattan et al., 2016).

As discussed earlier in this chapter, in terms of CP diagnosis, there are two primary barriers to the global attempt to documenting the real incidence rates. The foremost challenge is the absence of an established diagnostic criterion and/or failure on the part of medical professionals to identify CP symptoms, especially in regions where the disease is not endemic. This frequently results in erroneous diagnoses and misrepresentation of CP, both in clinical settings and public health data, which leads to imprecise assessments of the prevalence and incidence of CP (Chinain et al., 2021). The second inadequate representation of CP prevalence can be attributed to a widespread practice of under-reporting CP instances to national health agencies in the majority of the affected regions. There is evidence that suggests ciguatera is underreported by up to 90%, and records indicate that as many as 500,000 individuals are affected annually (Chinain et al., 2021).

CP is still recognised as great major health problem worldwide. Not only has it health consequences but also it impacts on the socioeconomic sector due to the low demand and consumption of fish in that area leading to a negatively effect on the fishery sector (Chinain et al., 2021).

Moreover, the illness has affected the tourism sector in endemic regions. In 2020, 55 countries and islands territories were classified as CP Risk destination by the International Association for Medical Assistance to Travellers (IAMAT, 2017). These circumstances can cause a major problem around these areas causing significant losses in the tourism industry (Chinain et al., 2021). The risk of poisoning increases in populations that have a strong fish consumption habit as well as lack of protein alternatives in coastal areas. With this said, the number of incidents can also vary due to disturbances such as hurricanes and human alterations that bring out blooms of *Gambierdiscus* (Soliño & Costa, 2020).

Loeffler et al., 2021 found that as a consequence of climate change, the world's oceans have absorbed heat and CO2, which can have various impacts. Excess heat can affect the oxygen content of water, leading to increased metabolic costs associated with breathing. This, in turn, can bring about changes in fish behaviour, metabolism, respiration, body size, and life history.

## *2.1. Brief historic evolution of ciguatera*

The first cases of CP were reported in the 17$^{th}$ century, precisely in 1601, in the Southern coast of Mauritius Island (Indian Ocean). In the year 1748, there was a report of 1500 people who died from eating toxic fish in the remote island of Rodrigues. A few years later, Captain Cook and Antonio Parra reported a fish poisoning event that it was interpret as CP by its described symptoms while in an exploration of the Pacific and Caribbean Sea (Chinain et al., 2021; Pasinszki et al., 2020).

While the first CP event ever reported in the Caribbean was in 1862 located around the Mexican coast when a French crew sailing got sick after ingesting parrotfish (Chinain et al., 2021). Eventually, the CP was linked to dinoflagellates production in 1977 (Pasinszki et al., 2020).

Until the year 2000, it was believed that the CP illness was distributed in regions between latitudes 35ºN and 35ºS (the three oceans already mentioned), ant it was not until early 2004 when new ciguatera reports were caught in non-endemic areas. The Macaronesia (Canary Islands, Madeira, Azores and Cape Verde), eastern part of the Mediterranean sea, the coast of Cameroon or the western part of the Gulf of Mexico were the new locations where ciguatera was reported outside the endemic areas range (Chinain et al., 2021). In Europe, in specific the Macaronesia islands including Canary Islands and Madeira, the CTXs are considered an arise threat since the first episode reported in 2004 (Soliño & Costa, 2020).

Data from 2020 shows that 65% of fish sampled in Europe was positive in Ciguatera, indicating that ciguatera is becoming an increasing risk in Europe (Diogène et al., 2021; Raposo-Garcia et al., 2023). Despite this fact, there are not regulatory limits for ciguatoxins in food in Europe yet (Raposo-Garcia et al., 2023).

## *2.2. Ciguatoxins (CTXs)*

Ciguatoxins (CTXs) are marine polyether compounds produced as a result of biotransformation and bioaccumulation of fish metabolism of precursors biosynthesized by benthic dinoflagellates *Gambierdiscus* and *Fukuyoa*.(Clausing et al., 2018; Loeffler et al., 2021; Nicolas et al., 2014; Paredes et al., 2011; Raposo-Garcia et al., 2023; Soliño & Costa, 2018, 2020). These dinoflagellates produce CTX-precursors that are less polar and less potent. However, these toxins undergo biotransformation in the liver of fish through oxidative metabolism and spiroisomerisation, resulting in the formation of more polar ciguatoxins. For instance, an analysis of the chemical structure of ciguatoxins reveals that P-CTX-1, obtained from the moray-eel *Gymnothorax javanicus,* is generated through acid-catalyzed spiroisomerisation and oxidative modification of P-CTX-4A produced by *Gambierdiscus* sp. (Nicholson & Lewis, 2006).

The CTXs are heat stable and extremely oxygenated lipophilic polycyclic ether compounds with molecular weights ranging from 1000 to 1150 Daltons. They are considered neurotoxins having a direct effect on the open voltage gated sodium and potassium channels with different potencies in excitable cells (Heimann & Sparrow, 2015; Raposo-Garcia et al., 2023). CTXs binds to site 5 of the alpha subunits of the sodium channels, keeping the protein channel permanently open producing a normal cell resting membrane potential (Raposo-Garcia et al., 2023).

The way CTXs get in the marine red is via either herbivorous fish or benthic invertebrates that feed on macroalgae where the dinoflagellates *Gambierdiscus* and *Fukuyoa* cells can be settled on (Soliño & Costa, 2020). These dinoflagellates are usually found in a mix of algal grass, however they can also be found in coral superficies, detritus, sand and other types of surfaces (Chinain et al., 2021; Rains & Parsons, 2015; Yong et al., 2018).

As happens in any environment, these organisms are an important diet part of the carnivorous species producing an accumulation and a biotransformation in fish top predators (Paredes et al., 2011; Soliño & Costa, 2020). Following ingestion, CTXs are metabolized in various ways depending on the animal's individual biochemical, biological, and physiological processes. Typically, CTXs are absorbed and initially detected in the gastrointestinal tract and liver. Eventually, they are transferred to the muscle tissue and spread throughout the body and skin of the organisms (Loeffler et al., 2021).

Summing up, CTXs get into the food web through the herbivores and detritivores getting further bio – transformed and bio – magnified as they keep scaling in the food web, ultimately reaching humans that have consumed animals that contained these toxins (Chinain et al., 2021; Loeffler et al., 2021; Paredes et al., 2011).

## *2.3. CTXs classification*

CTXs are bioactive molecules constituted by ether rings (between 13 or 14 rings, generally speaking) with main differences in their number of carbons, structure, and action. They lack odor, color, heteroatoms other than oxygen, and have few conjugated bonds (Dickey & Plakas, 2010)**.** The distinguishing characteristic shared by all CTXs is the elongated and inflexible structure, characterized by the presence of fused ether rings of different sizes. (Nicholson & Lewis, 2006).

Moving on, CTXs slightly differentiate in their structure, toxicity and polarity. The main differences in the structures have allowed to classify them into three geographical groups. Three families of CTXs were described, loosely labeled based on their origin in the Caribbean Sea (C-CTXs), Pacific Ocean (P-CTXs), and Indian Ocean (I-CTXs) (Clausing et al., 2018; Soliño & Costa, 2020). CTXs from the Pacific are more toxic and polar than toxins from the Caribbean and the Indian Ocean (Paredes et al., 2011; Soliño & Costa, 2018). However, it is important not that all the CTXs family have in common the same bioactivity. Theses toxins produce the activation of the voltage gated sodium channels (VGSC) on cellular membranes, giving as a result an increase in sodium ion permeability and cell disruption (Soliño & Costa, 2018).

While these region-based descriptions are still used, the FAO and WHO 'Report of the expert meeting on ciguatera poisoning' recommends a new approach based on known chemical structures and geographical distribution. Under this updated approach, CTXs and their derivatives, are classified into four distinct groups: CTX4A, CTX3C, C-CTX (Caribbean ciguatoxin), and I-CTX (Indian ciguatoxin), as summarized by the FAO and WHO (Loeffler et al., 2021). However, in this review, we will be focused on the original classification based on geographical factors.

### 2.3.1. Ciguatoxins from Pacific Ocean

The ciguatoxins belonging to this group are divided into P-CTX-I (CTX1B type) and II (CTX3C type). Both types have13 rings with differences in the E ring and the lack of the side-chain substituent in analogues from group II (Soliño & Costa, 2018), Figure 3.

The P-CTX-I toxin found in the Pacific Ocean is the most potent one among all this family marine toxins. The primary and most potent ciguatoxin from the Pacific was initially discovered in moray eels in 1967 (Scheuer et al., 1967). While the structural identification and its precursor toxin CTX-4B from *G. toxicus* was accomplished in 1989 (Murata et al., 1989).

The first ciguatoxin (P-CTX-I) is also the most studied hence the one which has the most known information in terms of accumulation, biotransformation and toxicity (Soliño & Costa, 2018). As seen in research, the common structure of all these different groups is characterized by three main features: a structure of 13 rings where the last ring (N) is non-contiguous and can be found as a chain in M-seco analogues, a carbon (C5) side terminal chain and an oxopene ring (E) with six carbons and one oxygen atom. What makes the toxins different is the extremes in both sides of the molecule (R1 and R2). The differences can be either to changes in the oxygen position or the methylene group at C52. For instance, CTX-2 and -3 present a higher number of oxygen atoms in R1 whereas CTX1B has them in both sides (Soliño & Costa, 2018), Figure 3.

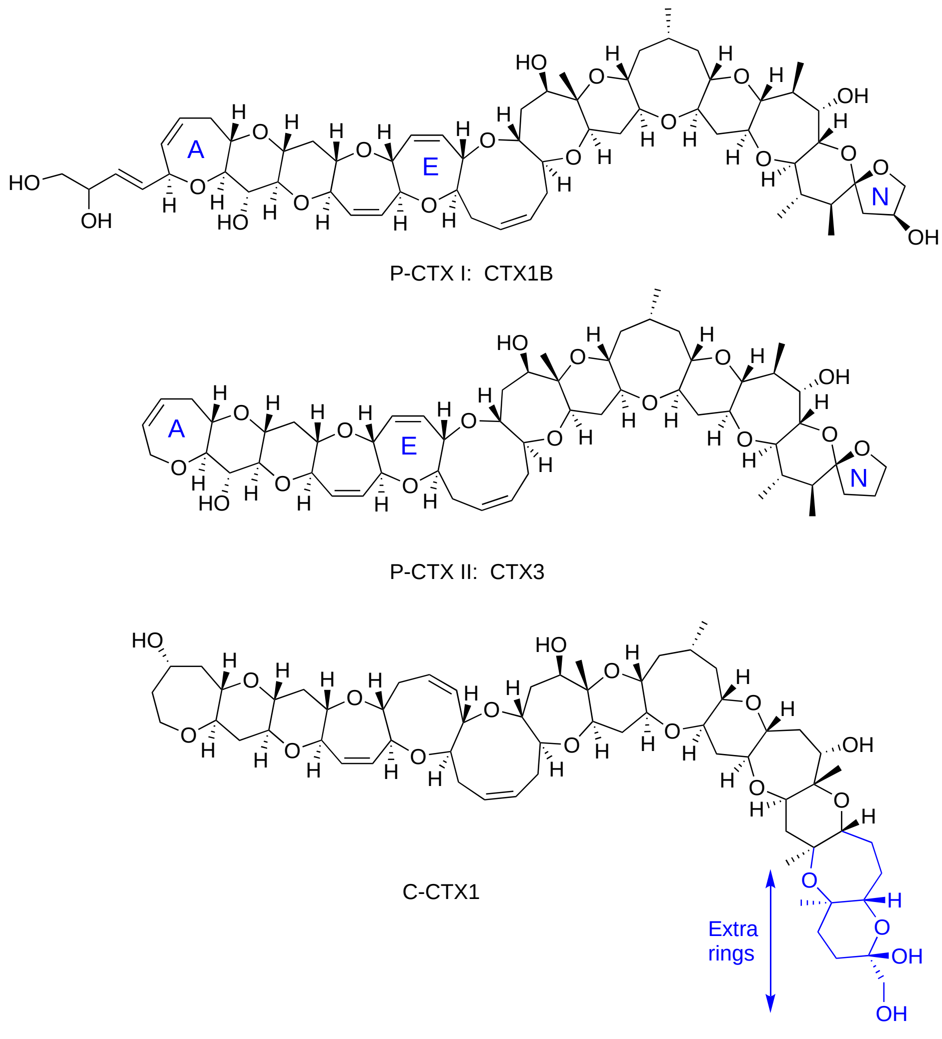


*Figure 3. Most significant features of the chemical structure of ciguatoxins.*

The molecular formula of P-CTX-1, also known as CTX 1B, is $C_{60}H_{86}O_{19}$, and it has a mass of 1110 g/mol. This CTX is highly potent, with an intraperitoneal (i.p.) median lethal dose ($LD_{50}$) of 0.25 µg/kg in mice (Mak et al., 2017; Nicholson & Lewis, 2006).

As it will be discussed in the next chapter, the attachment of P-CTX-I to the voltage-gated sodium channels in excitable tissues like nerves and muscles can cause them to stay open for longer durations, resulting in a greater influx of sodium ions across the membrane (Mak et al., 2017). This can lead to the repetitive firing of action potentials, which can have adverse effects on the undirected locomotion of fishes. These effects include muscle incoordination, tilted or corkscrew swimming, paralysis, and convulsions (Mak et al., 2017).

Furthermore, the exposure of fishes to P-CTX-1 results in a reduction in the heartbeat rate and vasoconstriction, which causes a decrease in the fish' blood flow, leading to a reduced efficiency of oxygen uptake. Additionally, in ciguatoxic fish, a decrease in the levels of ATP synthase and cytochrome c has been observed. These changes in cardiovascular performance and cellular energy metabolism, as well as mitochondrial respiration, may result in a decrease in the activity and predator avoidance ability of the fishes that are exposed to P-CTX-1, and this is ecologically relevant (Mak et al., 2017).

### 2.3.2. Ciguatoxins from Caribbean and Indian Ocean

These two groups have a similar structure to P-CTX-II with extra-fused rings, Figure 3, blue-colored fragment.

Since the 1980s, it has been acknowledged that the toxins responsible for CP in the Caribbean are distinct from those in the Pacific. This distinction was established through epidemiological investigations and the examination of symptoms. It was known that in the Caribbean, gastrointestinal symptoms are more pronounced, while neurological symptoms are less significant compared to CP cases in the Pacific (Soliño & Costa, 2018).

Lewis et al., 1998 first described Caribbean ciguatoxin (C-CTX-1) with a mass of 1140.6 Da and a molecular formula of $C_{62}H_{92}O_{19}$. While in terms of the Indian CTXs, Hamilton, Hurbungs, Jones, et al., 2002 and Hamilton, Hurbungs, Vernoux, et al., 2002 reported the isolation and characterization of these toxins in 2002.

The lack of information regarding CTXs from the Indian Ocean primarily stems from the insufficient research conducted in this specific domain. Therefore, it is imperative that further investigations are undertaken to enhance our understanding of these toxins and elucidate the distinctions within this group of toxins.

## *2.4. Mechanism of action of CTXs*

The classification of marine biotoxin compounds as neurotoxins is based on their ability to interact with ion channels that regulate the flow of sodium, potassium, and calcium ions in nerve and muscle cells. Marine biotoxins primarily affect neuronal function by interacting with ion channels or receptors, which can result in symptoms ranging from paralysis to death. And as mentioned before, these toxins pose a potential threat to consumers as they can accumulate in fish and shellfish (Joseph, 2017).

Ion channels are membrane proteins that facilitate the rapid diffusion of ions across the lipid bilayer. They can be categorized into two major classes: ligand-gated and voltage-gated channels. Voltage-gated channels are regulated by changes in the membrane's voltage potential (Joseph, 2017) whereas ligand-gated channels are regulated by a binding of a specific neurotransmitter (Alexander et al., 2011).

Marine toxins primarily target the sodium channel, a voltage-gated ion channel protein consisting of an alpha subunit and one to three beta subunits. Sodium channels contain six receptor sites, which are the key targets for neurotoxins (Joseph, 2017). Neurotoxins can be broadly classified into three groups based on their receptor site and functional effect:

(1) Pore-blocking toxins

(2) Toxins that affect gating from sites within the membrane

(3) Toxins that affect gating from extracellular sites.

Pore-blocking toxins inhibit ion conductance by binding to the outer mouth of the pore, while toxins that alter voltage gating by binding to intramembrane receptor sites bind to the channel in its active state. This results in persistent activation and prevention of inactivation. Voltage-sensor trapping toxins alter gating by binding extracellularly through electrostatic interactions with specific amino acid residues. Due to their ability to target different receptor sites on the channels, marine neurotoxins exhibit varying molecular pharmacologies (Joseph, 2017).

CTXs are neurotoxins that have the ability to activate voltage-gated sodium channels in nerves, muscles, and the heart tissue, leading to disruption of ion conductance through these channels (Mak et al., 2017). CTXs are one of the marine biotoxins that affect VSGCs directly, but it is important to know that they are not the only ones as there are others like the pyrethroid pesticides and deltamethrin (Raposo-Garcia et al., 2023) (as it is shown in Figure 4).

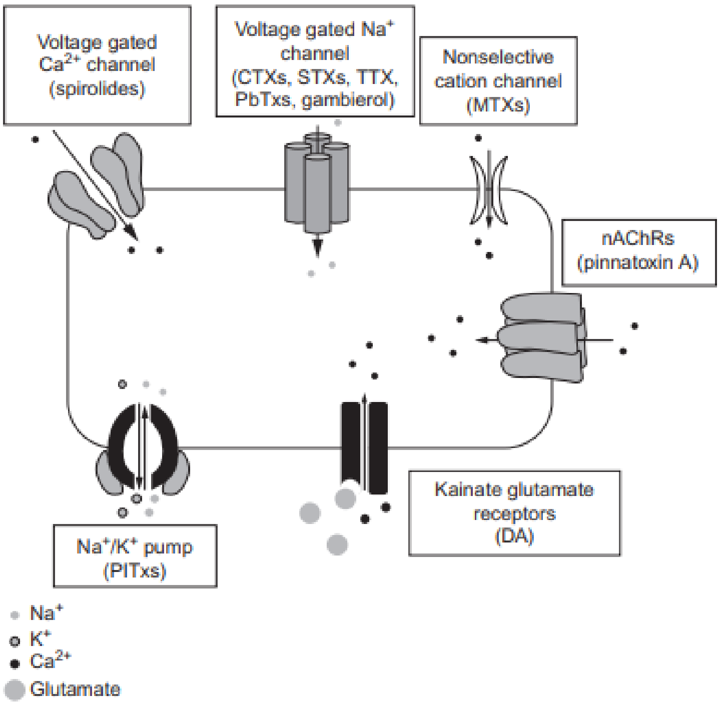


*Figure 4. Schematic representation of the principal targets of marine neurotoxins. The different targets of marine neurotoxins including ion channels and pump as well as receptors are included. Picture taken from Nicolas et al., 2014.*

As showns in Figure 5, voltage-gated sodium channels (VGSCs) are transmembrane proteins that allows the sodium ions cross the membrane causing signals of communication between different tissues. The VGSCs isoforms are found throughout the body and concentrated in different tissues where they are necessary due to their functional properties (Raposo-Garcia et al., 2023).

That is why these channels are considered to be one of the most important, if not the most, of ion channels due to their vital function. Due to its crucial function, they are the target of many natural or synthetic compounds that can have negative effects on human and animal health. These channels oversee functions such as the transmission, generation and propagation of action potentials in excitable cells. (Raposo-Garcia et al., 2023)

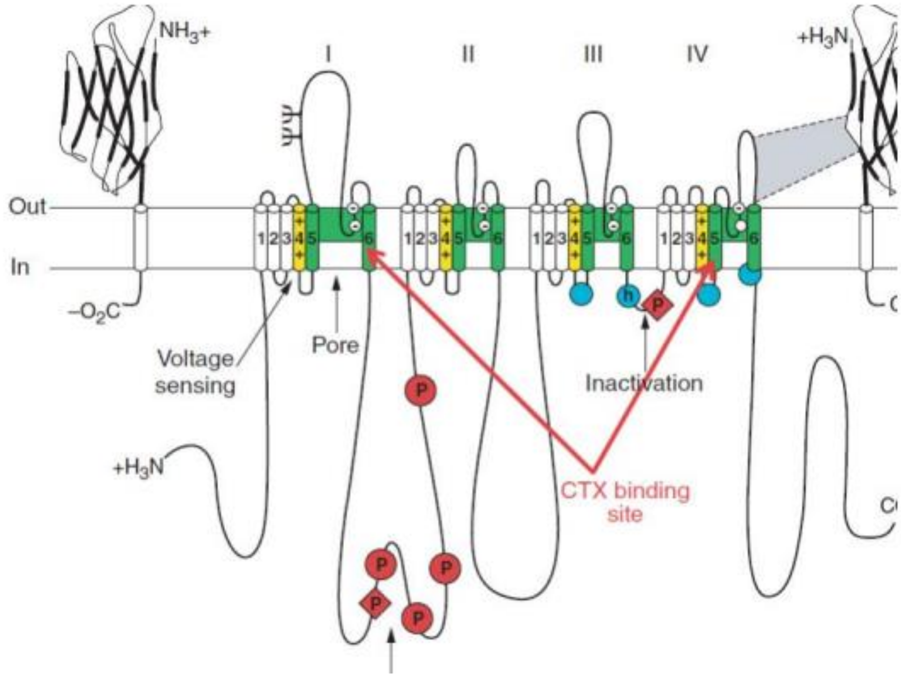


*Figure 5. VGSCs transmembrane structure and binding site of the CTXs in VGSCs. Picture taken from Vale et al., 2015.*

The mechanism of action of CTXs is that the toxins bind to site 5 of the voltage-sensitive sodium channels, resulting in their opening and causing an influx of Na+ ions. Consequently, the depolarization of the involved cells triggers action potentials. As a result of the alteration of the electro-chemical gradient, the plasma membrane cannot control the volume of the cells. The mitochondria then swell, and blebs are formed on the cell's surface (Lehane & Lewis, 2000; Paredes et al., 2011).

The neurological effects of CTXs are attributed to that last mechanism, while CTXs also elicit cardiovascular effects that result from the activation of Na+ voltage-gated channels. Due to the intracellular movement of sodium, the cells extrude calcium. The sarcoplasmic reticulum buffers a significant portion of this increased calcium, which is then available for calcium-induced calcium release. This release is thought to augment the strength of cardiac muscle contraction, which is a common symptom of CTX poisoning. (Lehane & Lewis, 2000; Paredes et al., 2011).

As previously mentioned, CTXs also have gastrointestinal effects, which are also due to the intracellular calcium transport that occurs in the epithelial cells. This results in the alteration of ion-exchange systems, causing fluid secretion and resulting in diarrhoea (Lehane & Lewis, 2000; Paredes et al., 2011).

Nevertheless, the research done by Dickey & Plakas, 2010 and Kumar-Roiné et al., 2008 claim that not all the ciguatera symptoms can be explained by the voltage-gated sodium channels effects. Their proposed explanation, which still needs to be verified, is that the continuous activity of voltage-gated sodium channels may trigger an overproduction of NMDA receptors, leading to the activation of NMDA receptors and subsequent influx of calcium. This, in turn, may activate the constitutive nitric oxide synthase (cNOS) and result in the production of nitric oxide (NO).

## *2.5. Relationship between Gambierdiscus and CTXs*

As already been discussed, the genus *Gambierdiscus* is a dinoflagellate that produces marine toxins such as ciguatoxins among many others that bioaccumulate in tropical and sub-tropical fishes generating ciguatera fish poisoning (CP) (Litaker et al., 2010). The name of both the organism and the toxin were derived from the Gambier Islands, located in French Polynesia, where ciguatera is prevalent and has a high occurrence rate (Heimann & Sparrow, 2015).

*Gambierdiscus* species are armored dinoflagellates that have cellulose plates deposited in thecal vesicles between the inner and outer continuous membranes (the Amphiesma). The tabulation, a complex pattern of plate arrangements resulting from the overlap of thecal plates, is used to differentiate between genera and species of armored dinoflagellates (Heimann & Sparrow, 2015; Nishimura et al., 2014). However, distinguishing between *Gambierdiscus* species based solely on tabulation is challenging, since the differences are subtle, and light- and scanning-electron microscopy are inadequate for clear identification (Heimann & Sparrow, 2015; Murray et al., 2014; Nishimura et al., 2014).

Until 1995, *Gambierdiscus* was thought to be a monotypic taxon recorded as *G. toxicus* and it was not until Chinain et al. (1999) that combined all the morphological data collected throughout the years and with phylogenetic analysis determinated some *Gambierdiscus* species. Later, Litaker et al. (2009) following previous work from that study, provided a complete revision of the genus in 2009.

During the last decade, the fact that there has been a massive extension and diversification of molecular techniques for taxonomic assessments (like PCR, Restriction Fragment Length Polymorphism and Fluorescent *In Situ* Hybridization) has made possible to identify new *Gambierdiscus* species (Chinain et al., 2021). Currently, there are 18 species of *Gambierdiscus* recognized worldwide, of which only 11 have been found to produce CTX compounds. This was measured by liquid chromatography tandem mass spectrometry (LC-MS/ MS) and other functional assays in Chinain et al., 2021 research.

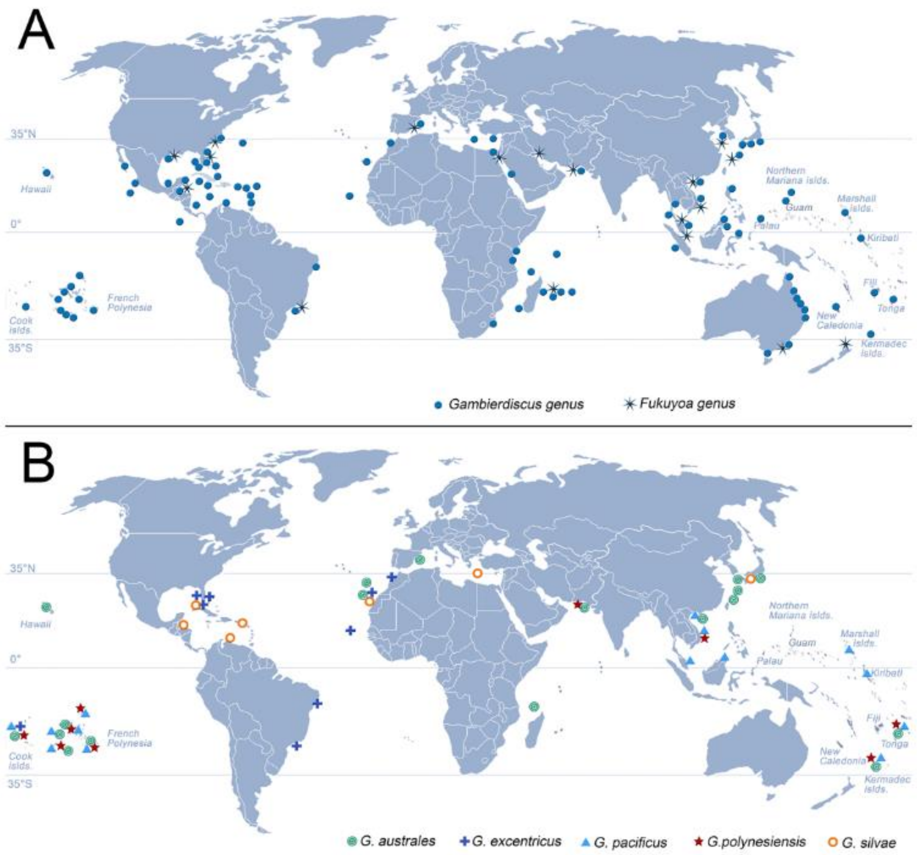


*Figure 6. Global distribution of A) Ciguatera-related Gambierdiscus and Fukuyoa genera, and B) CTX-producing species G. autrales, G.excentricus, G. pacificus, G. polynesiensis and G. silvae. Picture taken from Chinain et al., 2021.*

In other terms, Litaker et al., 2010 was the first research paper presenting the first global distribution of *Gambierdiscus* species, Figure 6. Different phylogenetic analyses showed that there were five species which are endemic to the Atlantic, other five endemic to the tropical Pacific, and two different species that are globally distributed (*G. carpenter* and *G. caribaeus*). The fact that these two species are globally distributed is yet to be determined, but it may be related to an unexpected broad temperature tolerance compared to the rest of the *Gambierdiscus* species. The composition of the *Gambierdiscus* of each region shows a correlation with structural differences in the ciguatoxins reported from Atlantic and Pacific fish, meaning that the species from each region produce different toxins (Litaker et al., 2010).

*Gambierdiscus* has been found in maximum depths of around 60 m in different locations. And this parameter might be an important contemplation since depth is directly correlated with low temperatures and it is known that the more toxic species have lower thermal tolerance (Chinain et al., 2021).

The Pacific region is considered the most diverse in terms of the *Gambierdiscus* species distribution in the CP endemic areas. What it is more, several islands in the South Pacific are also recognized as biodiversity hotspots of *Gambierdiscus*. For instance, there were up to six different *Gambierdiscus* species found co-habitatings within the same part of the Mangareva Island (French Polynesia). Actually, there are only two species not yet reported in the Pacific, and those are *G. carolinianus* and *G. silvae* (Chinain et al., 2021).

The most toxic CTXs are believed to be *G. excentricus* and *G. sylvae*. Apparently, these species are not as frequently come across as other species believing that they have a strong thermal tolerance explaining their distributional pattern (Chinain et al., 2021).

There are some studies that have showed that the CP events can be preceded by an increase in *Gambierdiscus* cell densities, but it is important to note that not all increases in *Gambierdiscus* cell densities lead to CP (Litaker et al., 2010). These results suggest that finding high concentrations of *Gambierdiscus* is a requisite, however is not a sufficient condition for the CP situation (Litaker et al., 2010). The best well-known conditions associated with elevated *Gambierdiscus* abundance and CP occurrence are warmer waters and specific types of environmental disturbances (Litaker et al., 2010). Disturbances such as hurricanes, coral bleaching episodes, anthropogenic or natural nutrient inputs, and coastal development.

The conversion of *Gambierdiscus* metabolites within the food chain results in the production of multiple ciguatoxins, which differ in potency and the symptoms they cause in ciguatera. Furthermore, the levels of toxins present in herbivorous and carnivorous fish vary between different regions of French Polynesia, indicating that toxin storage and dose are significant factors in the severity of ciguatera. The extent to which toxin levels and profiles vary within different fish species is not well understood and may depend on factors such as their diet ratios, digestive properties (such as toxin adsorption, metabolism, and excretion), and the oxidation state of the toxins (Heimann & Sparrow, 2015).

Environmental parameters and physicochemical factors can lead to an increase in ciguatera risk in endemic areas or expansion into new regions. In regions with ciguatera prevalence, coral reefs are ecosystems that can experience temporary or permanent shifts in substrate dominance from coral to macroalgae, due to natural disturbances like storms, cyclones, crown-of-thorns outbreaks, and coral bleaching, as well as anthropogenic activities such as dredging, anchorage, overfishing, and pollution. Identifying the key parameters that influence the expansion of macroalgal substrates is challenging, as reef ecosystems are often subject to multiple stressors in a short period (Heimann & Sparrow, 2015).

The prevailing view is that the proliferation of macroalgal substrates prompts the expansion of *Gambierdiscus* populations, which in turn leads to an increase in ciguatera cases. Increases in the number of ciguateric fish have also been noted in the aftermath of disturbances, especially among herbivorous species. However, there is currently insufficient evidence to establish a link between peaks in *Gambierdiscus* populations and the rise in ciguateric predatory fish (Heimann & Sparrow, 2015).

While the expansion of macroalgal substrates is occurring, it is a known fact that *Gambierdiscus* populations require warm sea surface temperatures (SST) and adequate nutrients. Nevertheless, the influence of other parameters, such as predation by fish, crustaceans, and other marine invertebrates, as well as the dinoflagellate community structure, present a challenge to understanding the growth and toxicity of *Gambierdiscus* populations (Heimann & Sparrow, 2015). The expansion of dinoflagellate populations into new areas is facilitated by the rise in sea surface temperatures resulting from climate change (Heimann & Sparrow, 2015).

In regard to this topic, human activities are unintentionally impacting the creation and dissemination of CTXs by modifying the presence, absence, and abundance of toxin-producing organisms, their carriers, and habitats. Changes in land use and hydrology, including impervious surfaces, breakwaters, deforestation, sediment accumulation, freshwater runoff, and poor water quality due to pollution and nutrient excess, can all alter the benthic habitat coverage, food-web dynamics, and CTXs production, resulting in uncertain and nonlinear impacts. The introduction of invasive species and overfishing can further exacerbate these changes. Ocean warming in the past century has enabled tropical species such as *Gambierdiscus* to expand their range towards the poles (Loeffler et al., 2021).

The loss of habitat, overfishing, competition from invasive species, and long-term changes such as climate change and ocean acidification are major anthropogenic threats to reef fish populations and the overall health of reef ecosystems. However, their short and long-term effects on CP incidences remain unknown. A practical approach currently utilized to mitigate human impacts on marine systems and increase fisheries productivity is to manage the fishery through various measures, such as establishing marine protected areas or 'no-take' zones. Harvest restrictions and marine reserves can also be useful in protecting consumers from consuming CTX-contaminated fish. However, CTX distribution can be geospatially complex, and harvesting at the 'spillover' borders of these protected areas, if not adequately large, may potentially increase the risk of CP (Loeffler et al., 2021).

## *2.6. Impact of Ciguatera*

Ciguatera has a huge impact on the world’s population. The economic impacts of ciguatera can be classified into three categories. Firstly, there are losses in the primary industry due to reduced consumer confidence in the product or restrictions on the sale of certain fish species from specific regions. Secondly, the gastronomy and tourism sectors in areas endemic to ciguatera can also experience significant losses. Finally, there are impacts on the income of those directly affected by ciguatera (Heimann & Sparrow, 2015). However, to accurately assess the economic losses and health costs associated with ciguatera, a more comprehensive understanding of distribution and seasonal patterns, prevalence, and incidence rates is necessary (Heimann & Sparrow, 2015).

As mentioned in the previous chapter, forecasts and data-driven models of sea-level rise and warming waters resulting from climate change and human activity suggest that there will be a surge in Ciguatera cases. This is because there is a rapid proliferation of toxin-producing microalgae, which is causing the increase (Loeffler et al., 2021).

The responsibility for identifying, mitigating, and reducing the incidence of ciguatera primarily lies with resource and human health managers. Countries around the world that are dealing with ciguatera poisoning have implemented domestic policies aimed at regulating the sale or capture of products that are implicated in poisonings, such as fish, by species, size, and region (Loeffler et al., 2021).

However, these solutions are not enough to solve this global seafood-related health problem, which requires a multidisciplinary approach involving scientists, consumers, governments, human health experts, and fishers. To effectively address the remaining challenges of ciguatera, this group of people must work together with a dedicated approach that involves capacity building, a willingness to provide technology and knowledge transfer, and sharing research material (Loeffler et al., 2021).

## *2.7. Ciguatera in the Canary Islands*

The very first CP report for the Archipelago was in 2004 and it was directly linked to an intake of amberjack flesh. Since then, there has been a total of 11 CP outbreaks reported by different species of amberjack and that affected 75 people. (Chinain et al., 2021; Ramos-Sosa et al., 2022).

The Canary Islands are considered a hotspot for CTXs in Europe and it was in 2011 when the Directorate-General for Fisheries of the Canary Government applied a protocol that allows to detect CTXs in some species before the products reach the commercial market (Ramos-Sosa et al., 2022).

One of the reasons why CP is a big threat in the Canary Islands is due to the fact that five species of the genus *Gambierdiscus* have been recorded around the islands out of the 16 species recorded globally, making it a biodiversity hotspot for these species. (Bravo et al., 2019; Costa et al., 2021; Rodríguez et al., 2017). The identified species of the genus *Gambierdiscus* in the Canary Islands are (Bravo et al., 2019), Figure 7:

- *Gambierdiscus australes*
- *Gambierdiscus caribaeus*
- *Gambierdiscus carolinianus*
- *Gambierdiscus excentricus*
- *Gambierdiscus silvae*

The species *G. excentricus* was the first recorded in the Canary Islands and is considered the most abundant and toxic. The study by Bravo et al., 2019 depicted in Figure 7 the first distribution of the five recognized species in the Canary Islands, based on morphological and genetic data. It is observed that the dominant species in the archipelago are *G. excentricus* and *G. australes*. On the other hand, the other three species are more exclusively observed in the westernmost islands, which may be due to the tropical nature of these species (Bravo et al., 2019).

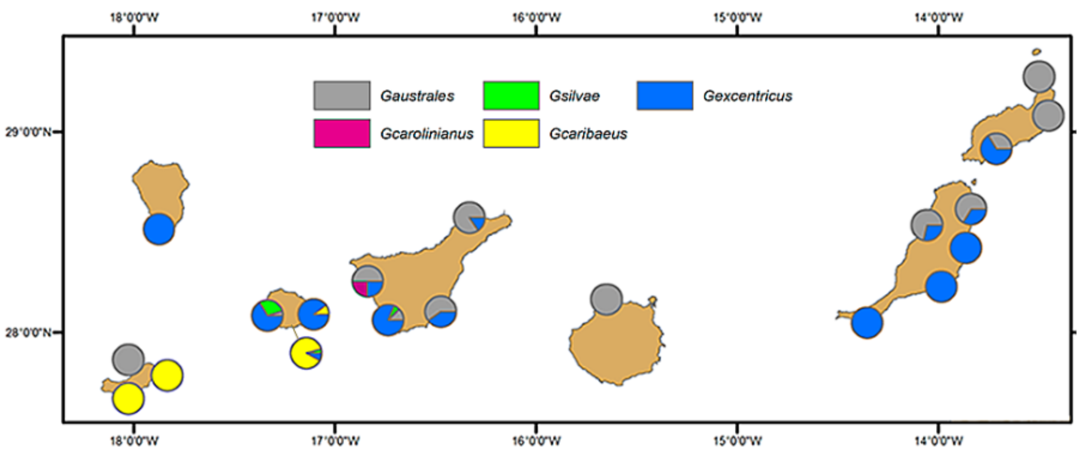


*Figure 7. Geographical distribution of the different species of Gambierdiscus in the Canary Islands. Picture taken from Bravo et al., 2019.*

Furthermore, according to the study by Rodríguez et al., 2017, it was possible to affirm for the first time that the *Gambierdiscus* species found in the Canary Islands are an extended, diverse, and probably indigenous component of the benthic microalgae communities of the islands. The high diversity found, along with its abundance similar to values recorded in endemic areas of the genus, suggests ancient colonization and diversification of this genus in the region. Additionally, there is the possibility that ciguatera outbreaks in this region are due to local populations of *Gambierdiscus*, with the eastern islands (Lanzarote and Fuerteventura) presenting a higher abundance of this genus.

Many regions with a high frequency of CP require regulatory measures to be in place. The control protocol established by the Canary Government is a viable method, which is based on identifying the presence or absence of CTX-like toxicity in the muscle of certain fish species, through a cell-based assay (CBA). This prevents the sale of toxic fish specimens. Detecting CTXs is only possible through various analytical methods as it does not affect the appearance, smell, or taste of the flesh. Furthermore, it has been noted that the concentration of CTXs varies depending on the tissue and species of fish, therefore, individuals with no trace of CTX-like toxicity in muscle could still have CTXs in other tissues (Ramos-Sosa et al., 2022). This topic will be extended in further chapters in this review.

Apart from the analytical methods just mentioned, there is a project called EuroCigua and its main focus is to characterize the risk of CP in Europe. This project also highlighted the importance of the Canary Island for the presence of ciguatoxins in their waters and remarked on some relevant species which accumulate CTXs in their tissues. The relevant species, which are of great fishery value and are distributed worldwide, mentioned are: amberjack (*Seriola spp.*), dusky grouper (*Epinephelus marginatus*), moray eel (family Muraenidae) and *Diplodus vulguaris* (Ramos-Sosa et al., 2022).

# 3. CTXs Bioprocess throughout the natural food chain. Biotransformation, bioaccumulation, and biomagnification

## *3.1 Biotransformation and bioaccumulation*

The process of bioaccumulation is generally described as the rise of concentrations of contaminants in aquatic organisms that have absorbed them from the surrounding environmental medium (Wang, 2016). Basically, the general principle of bioaccumulation is based on the balance between influx and efflux (of any substance either liquid or solid) considering that an organism is a single compartment (or box) without having any consideration at internal transportation (Wang, 2016), Figure 8.

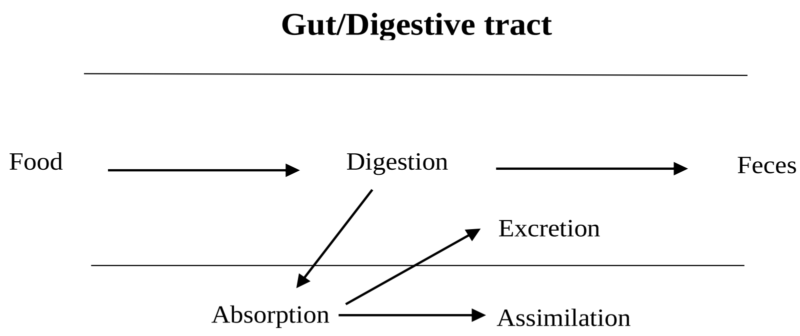


*Figure 8. Processes involved when foods are digested. Picture taken from Wang, 2016.*

Ciguatoxins have been proven to undergo bioaccumulation and biotransformation into more potent forms as they progress up the marine food chain, starting from herbivorous fish that graze on coral or macroalgae, such as parrot fish (*Scaridae spp.)* and mullet (*Mugil cephalus*), and continuing to omnivorous species at higher trophic levels, like wrasse (*Cheilinus spp*.), and carnivorous reef fish that prey upon them, such as Spanish mackerel (*Scomberomorus spp*.) (Hambright et al., 2014).

While CTXs can bioaccumulate in fish at all trophic levels, with reported estimates of ciguatoxin-producing (CP) vectors ranging from 60 to over 400 species

(FAO, 2014), it is the carnivorous fish species that are frequently associated with ciguatera poisoning (CP) cases, as they are commonly targeted by commercial and recreational fishermen. In fact, carnivorous fish species account for 68% of intoxication incidents in French Polynesia and 85% in New Caledonia (J. S. Murray et al., 2020).

Information regarding vectors for the biotransfer of gambiertoxin and ciguatoxin, their distribution, seasonal abundances, as well as their mobility for direct uptake, conversion, and bioaccumulation, is limited. Resolving these processes is essential for the development of biomonitoring processes, comprehensive establishment of risk areas, identification of diets, and understanding the groups in remote island locations (Heimann & Sparrow, 2015).

In the majority of instances, toxic species are present at low concentrations, thus posing minimal impact on the environment or human health. However, in cases where these species occur in high densities within phytoplankton populations, which are then consumed by crustaceans, zooplankton, and herbivorous fish, toxins accumulate in these organisms (bioaccumulation) and subsequently transfer to higher trophic levels in the food chain (bioconcentration), as depicted in Figure 9. This process can lead to various adverse effects (Rutkowska et al., 2019).

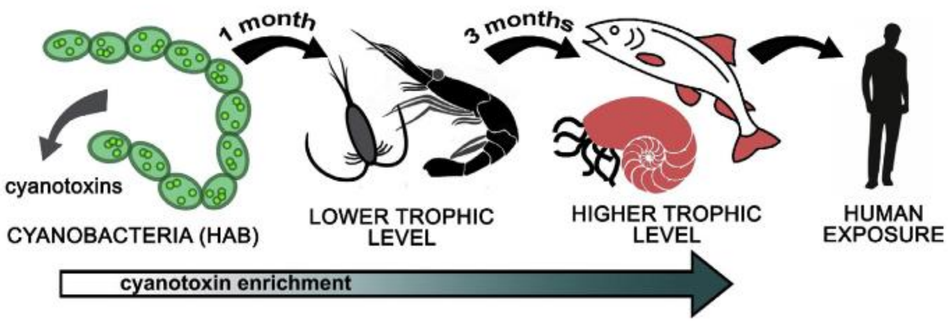


*Figure 9. Bioaccumulation of cyanobacterial neurotoxins in the trophic chain, and potential vector of human exposure. Picture taken from Rutkowska et al., 2019.*

A direct relationship exists between increasing trophic levels and the biomagnification of CTX, resulting in higher concentrations of CTXs in apex predators. Ciguatera poisoning occurrences are often associated with fractional trophic levels (TL) that indicate positions in the food web, with primary producers being TL 1 and higher trophic level species having higher TL values. In Australia, high trophic level fish such as mackerel (*Scombridae or Scomberomorus spp*., TL = 4.5), coral trout (*Plectropomus spp*., average TL = 4.2), barracuda (*Sphyraena jello*, TL = 4.5), grouper (*Epinephelus* *lanciolatus*, TL = 4.0), red bass (*Lutjanus bohar*, TL = 4.1), kingfish (*Seriola spp*., TL = 4.1), chinaman fish (*Symphorus nematophorus*, TL = 4.1), and other tertiary consumers are the species most frequently implicated in ciguatera outbreaks. The biomagnification of CTX in other high-order carnivores, particularly regional apex predators occupying the highest trophic levels, such as sharks with an average TL of 3.65 and numerous large species surpassing a TL of 4, raises doubts (Meyer et al., 2016).

While herbivores, omnivores, and lower-order carnivores have essential roles in the trophic transfer of these toxins, they usually contain relatively low levels of CTXs. The toxin profiles in these species are often dominated by P-CTX-2 and P-CTX-3. Nonetheless, due to the lipophilic nature and high stability of these toxins, particularly P-CTX-1, they persist in the food chain and readily biomagnify to hazardous levels in the higher-order carnivores (Meyer et al., 2016).

However, regional investigations have yielded contradictory findings, as certain herbivorous fish have been found to possess elevated levels of CTXs in their flesh, similar to those observed in fish higher up the food chain. The precise mechanisms by which fish accumulate CTXs in their flesh, reaching concentrations that can cause human intoxication (> 0.1 ng CTX1B $g^{-1}$ of fish; Lewis and Holmes 1993; Hossen et al. 2015), remain unclear and have not been adequately confirmed through experimental validation (Clausing et al., 2018).

Despite the aforementioned context, the significance and potential contribution of invertebrates in the transfer of ciguatoxins through the food chain have not been adequately explored thus far. However, it is possible that herbivorous invertebrates may play a noteworthy role in the transfer of ciguatoxins (Heimann & Sparrow, 2015).

Although, in recent research by Roué et al. (2016) offers evidence of the capacity of giant clams to store CTXs in their tissues, verifying that these mollusks, which are included in the diet of many communities in Pacific Island Countries and Territories (PICTs), may serve as an additional mode of transmission for ciguatera in regions where *Gambierdiscus* populations are prevalent.

Interestingly in recent research, it has been seen that the absorption of P-CTX-1 via yolk sac is rapid and effective, and repeated exposure to low doses can have effects similar to those of a single high-dose exposure. Fish living in ciguatoxic coral ecosystems may be exposed to CTXs via maternal transfer and their diet over their entire lifespan.

The exposure to P-CTX-1 can reduce survivability and the number of fish that reach sexual maturity, leading to a decline in reproductive potential and larval recruitment. So, predators that consume P-CTX-1 contaminated fish are more likely to accumulate P-CTX-1 along the food chain, potentially disrupting aquatic communities, especially in ecologically important coral ecosystems (Mak et al., 2017).

An example of bioaccumulation is shown in Yan et al., 2020 that a dietary exposure to P-CTX-1 affected the reproductive performance and rate of survival of offspring, also causing bioaccumulation, as well as, maternal transfer of P-CTX-1 in marine mendaka.

The extent of CTX metabolization during digestion plays a crucial role in their retention or elimination within fish. Typically, the abundance of oxidized CTX forms rises with metabolization and correspondingly with trophic level, leading to increased potency due to enhanced polarity. The hypothesis proposes that the augmentation of polarity during fish transformation could serve as a mechanism to aid in depuration. In herbivorous fish, the processes of metabolization and depuration are yet to be determined (Clausing et al., 2018).

Biotransformation of the toxins involves several chemical pathways, with oxidative metabolism and carbon-chain backbone spiroisomerizations occurring primarily in the stomach and liver. The resulting compounds are highly stable and can undergo further oxidative transformations depending on the biochemical conditions in various consumers, leading to an increase in toxicity levels. The highly stable P-CTX-1 dominates the toxin profiles of CP causing fish due to subsequent transformations in the case of the Pacific CTXs (Meyer et al., 2016).

The mechanism behind the bioconversion of gambierotoxin to ciguatoxins is not yet fully understood, but it has been suggested that oxidation reactions are catalyzed by cytochromes in the liver of fish (Heimann & Sparrow, 2015; Lehane & Lewis, 2000). CTX 4A is thought to be a precursor toxin for P-CTX 2 and CTX 4, while CTX 4B is the possible precursor for CTX 3 and CTX 1. Predators' oxidative metabolism yields toxins with increasing polarity, which appears to be positively correlated with toxicity. Conversely, reduction of CTX 4A, P-CTX 2, and P-CTX 4 may result in CTX 4B, P-CTX 3, and P-CTX 1, respectively (Heimann & Sparrow, 2015).

Although maitotoxins are not usually associated with ciguatera, recent research has revealed that herbivorous fish and invertebrates can bioconvert them into ciguatoxins (Hambright et al., 2014; Heimann & Sparrow, 2015). This is concerning because maitotoxin precursors are also produced by other toxic dinoflagellates such as *Ostreopsis* spp., which are often found in the same area as *Gambierdiscus* (Hambright et al., 2014; Heimann & Sparrow, 2015). This suggests that maitotoxins could potentially contribute to the development of ciguatera, causing different symptoms due to their ability to interact with voltage-gated calcium channels and cause membrane depolarization and calcium influx (Hambright et al., 2014; Heimann & Sparrow, 2015).

One secondary metabolite thought to be related to CP, which can be detected more readily using LC-MS/MS compared to CTXs, is 44-methylgambierone. This compound has been previously identified as putative maitotoxin-3 (MTX-3) and has recently been structurally analyzed in *G. australes* (J. S. Murray et al., 2019) and *G. belizeanus* (Boente-Juncal et al., 2019; J. S. Murray et al., 2020). Although, based on J. S. Murray et al., 2020 study, it has been established that the intraperitoneal toxicity of 44-methylgambierone is extremely low, indicating that it is highly improbable to contribute to CP.

### *3.2 Importance of CTXs distribution in fish tissues and sizes*

It is essential to understand where the CTXs can be found on the animals for its detection for safe consumption. However, there is not much research done on CTXs distribution in fish tissues, so the main tissue targeted for toxin detection are muscle and the viscera in general. With this said, the toxin concentration is normally higher in viscera, more specific in the liver, than in the muscle. Apparently, CTXs have a tendency to bind to the cytoplasmatic protein found in hepatocytes, explaining why the liver is a significant reservoir for these toxins (Ramos-Sosa et al., 2022).

As stated in studies, CTX is generally uniform throughout the flesh. However, poisoning surveys have claimed that patients that had eaten the fish head or jaw muscle developed more severe illnesses than others that consumed body muscle. However, there have been no reliable studies made on this topic, and more research is needed to confirm these speculations and to understand if there are certain species that can be more prone than others to accumulating toxins throughout the body or in different muscle types (Soliño & Costa, 2020).

It appears that various biological data points can provide valuable information about CTX accumulation. These include age, which can be determined through otolith analysis, as well as dietary shifts resulting from prey availability or ontogeny, which can be determined through stable isotopic analysis to establish trophic feeding level, location, or habitat utilization type. Additional data can be gleaned from the analysis of gut contents to ascertain fish prey information, while standard morphometrics can be used to determine fish size. Fecundity, as indicated by egg production/weight, can reveal the impact of CTXs on fish reproduction, and the liver, viscera, and brain can provide data on bioaccumulation and metabolism (Loeffler et al., 2021).

In terms of correlation with size, the first food chain hypothesis, stated by Randall (1958), claimed that the bigger the fish individual was in the same species, the higher amounts of CTXs would be stored (Soliño & Costa, 2020). This issue has obviously been a real concern among the fish consumers, causing the local governments to act and spread this assumption without any real scientific basis. Yet the relationship between the size and the toxicity has only been shown in a quiet limited number of species, making it non-reliable at this moment (Soliño & Costa, 2020).

There is a nine-year study in French Polynesia that took 856 fishes belonging to 59 species from 12 different families, and it is the most extensive research done to 2020. The results of this survey showed that there is no correlation between CTX concentration and fish total length, except for one species, *Lutjanus bohar*, sampled in Fakarava Island. However, there was a positive correlation when looking at CTX concentration in the muscle and liver of moray eels (*Gymnothorax spp*.) and weight of individuals with samples collected in Marakei and Tarawa, South Pacific. The same results were found between total length or body weight and CTXs concentrations in giants moray (*Gymnothorax javanicus*) in the Kiribatie Islands (Soliño & Costa, 2020).

Additionally, positive correlations between the CTX concentration and fish weight/length were found in different studies and among different species, like in Sanchez-Henao et al. 2019 where higher toxicity levels were observed in heavier individuals of amberjacks (*Seriola spp*.) and dusky grouper (*Epinephelus marginattus*) from the Canary Islands. As a consequence of lack of information on this topic, there are only three species *(Gymnothorax spp., L. bohar, and C. argus*) that have a positive relationship between CTXs content and fish weight/length (Soliño & Costa, 2020).

Apart from the typical forms of ciguatera poisoning, other atypical forms, such as shark poisoning, have been found. These poisonings occur mainly in the Indian Ocean and the Pacific Ocean regions. The same has been found for marine invertebrates, such as clams, sea urchins, and some gastropods species (Chinain et al., 2021). These findings validate that certain types of ocean-dwelling invertebrates, which hold significant value for communities residing in the Pacific and Caribbean islands, may also pose a potential danger for human consumption.

Furthermore, the fact that legal regulations and recommendations use fish length as a threshold to monitor or avoid ciguateric fish is concerning. Despite this, field data indicates that smaller individuals may have higher toxicity levels, and laboratory experiments suggest that CTXs somatic growth dilution may occur. Consequently, slow-growing fish may have higher concentrations of CTXs compared to fast-growing ones. These findings suggest that the quantity of toxins found in certain species may be related to site-fidelity, age, and growth rate, rather than fish weight or length (Sanchez-Henao et al. 2019).

# 4. Analysis and detection

The extraction of CTXs from fish tissue is a crucial step for CTX quantification due to the contamination and poor quantity of the toxins in the tissue. The purification step is also critical because of the removal tissues that can interfere negatively during the analysis (i.e lipids) (Pasinszki et al., 2020).

Lewis et al. suggested the original extraction method but since then multiple different methods have been published. The methods of extraction involve different steps that will be determinant to obtain reliable results. These steps are: 1) extraction of raw, frozen, dried or cooked muscle tissue with a polar organic solvent (acetone or methanol are the commonly used); 2) purification of the extract by liquid-liquid partitioning (with diethyl ether, chloroform, or dichloromethane); 3) the use of hexane or cyclohexane to defeact the extract by liquid-liquid partitioning, and 4) to purificate the crude extract by solid-phase extraction (SPE) (Pasinszki et al., 2020).

## *4.1 Extraction of CTXs*

During several years, there has been research on how to detect CTX presence in fish, from indigenous observations and animal mortality tests to modern analytical techniques (Pasinszki et al., 2020).

At present, the established methods for detecting marine biotoxins in seafood products involve in vivo assays, which are used to quantitatively or qualitatively evaluate substances for impurities, toxicity, or other characteristics. Examples of such methods include the mouse bioassay (MBA), as well as chemical approaches like high-performance liquid chromatography with ultraviolet detection (HPLC-UV), liquid chromatography with fluorescence detection (LC-FLD), and liquid chromatography with mass spectrometric detection (LC-MS/MS) (Nicolas et al., 2014).

These methods are not just particular to CTXs so it might be sometimes inadequate for quantification or expressing the results in “equivalent of a CTX standard”. To monitor the CTXs the most advanced methods are a combination of biological and chemical methods that are divided into two steps: screening fish extract toxicity with a sensitive functional assay and a confirmation of the presence of CTXs via LC-MS/MS (Pasinszki et al., 2020).

## *4.2 Detection of CTX*

The detection of CTX remains a difficult task for laboratories and regulatory bodies due to its colorless, odorless, and tasteless nature, Figure 10. Although traditional mouse bioassays are commonly used for detection, analytical and functional assays have also been developed with technological advancement. However, the lack of reference standards makes these methods impractical. To date there is no standardized method available. Therefore, the MBA is still the primary method for confirming toxicity in fish found in markets and remnants from CFP patients, as there are no reliable, robust, and simple assays for rapid screening of potential ciguateric fish (Tanyag et al., 2021).

The detection of ciguatoxins in the Philippines was historically carried out using Cigua-Check® test kits by the Bureau of Fisheries and Aquatic Resources, but this method posed problems due to the ambiguity of test results (Bienfang et al. 2011).

Therefore, both management and monitoring are necessary to ensure seafood safety for consumers. Different countries have employed various strategies to combat this kind of poisoning, such as implementing checks and controls on fishery products that may contain CTX to prevent them from being placed on the market, and prohibiting the marketing of fish species potentially contaminated with CTX in some regions (Tanyag et al., 2021).

Unfortunately, there is no rapid and reliable test that can detect ciguatoxins on-site or prior to human consumption. As mentioned before, the concentration of toxins in fish is higher in fish viscera than muscle tissue, around 10-50 times higher, therefore the CFP is a big issue in communities that consume fish viscera (Pasinszki et al., 2020).

The species of the genus *Gambierdiscus* that represent a significant threat to humans are G. *polynesiensis* from the Pacific Ocean and *G. excentricus* in the Atlantic Ocean. These two organisms not only produce the CTXs toxins but other marine toxins such as maitotoxins and gambierones (Pasinszki et al., 2020).

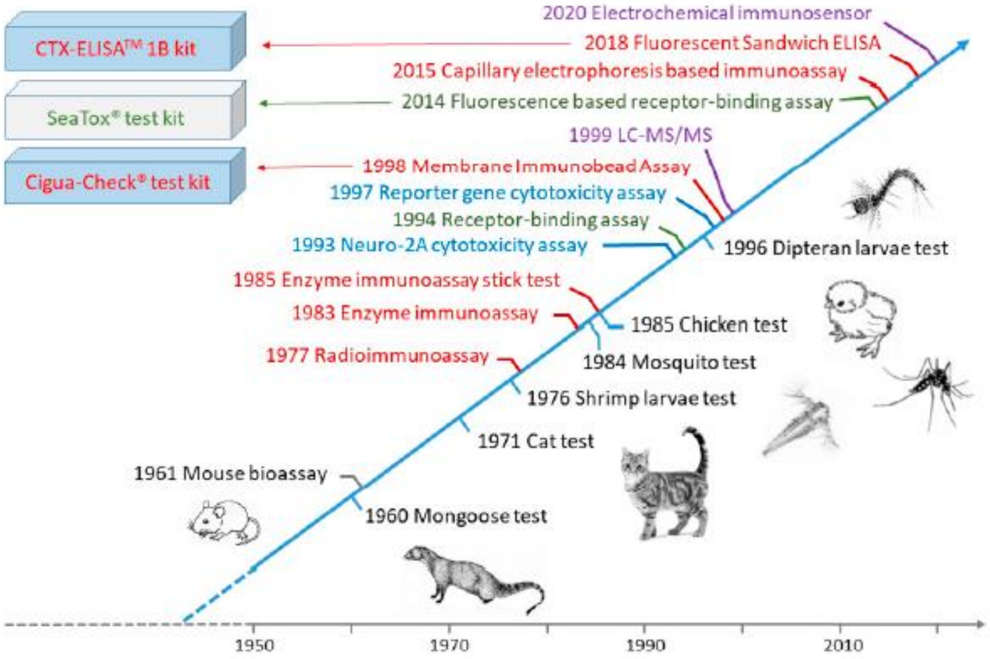


*Figure 10. Timelines of CTX detection methods. Picture taken from Pasinszki et al., 2020.*

Advancements in understanding *Gambierdiscus*, CTXs, and ciguatera poisoning have led to frequent discoveries across all aspects of the problem, from toxin sources to patient care. Methodological and technological improvements have increased the sensitivity of CTX detection, enabling early identification of *Gambierdiscus* and CTXs in regions where their cell abundance and toxin levels are low. These monitoring efforts are continuously refined with the addition of new oceanographic data, input from fisheries scientists, advancements in CTX detection methods, knowledge of food web transmission, and epidemiological surveillance data (Loeffler et al., 2021).

Currently, the primary method for detecting most marine biotoxins in seafood is the MBA, although many laboratories are increasingly turning to chemical testing due to concerns raised by animal welfare activists (Nicolas et al., 2014). The MBA is deemed by many as unethical due to its low reproducibility, low sensitivity, and a high rate of false-positive and false-negative results. Thousands of mice are used as experimental animals each year to detect marine biotoxins in seafood. In 2015, there was a proposal to ban MBA in Europe for the detection of lipophilic toxins. Consequently, efforts have been made to develop alternative methods based on the chemical properties of different marine biotoxins or their specific modes of action (Nicolas et al., 2014).

# 5. Conclusions

Marine biotoxins, naturally occurring bioactive compounds produced by various organisms, pose significant risks to human health and the environment. Harmful algal blooms (HABs) play a crucial role in the proliferation of marine biotoxins, leading to food-borne illnesses and economic losses in the seafood industry. The rise in HABs can be attributed to human activities and climate change, with nutrient contamination, coastal development, and altered environmental conditions contributing to their global spread. It is essential to address these factors and understand the ecological implications of marine biotoxins, such as ciguatoxins, to protect human health, preserve marine ecosystems, and ensure the sustainability of coastal communities.

Ciguatera Poisoning is a significant global health issue caused by consuming fish contaminated with ciguatoxins, resulting in a range of debilitating symptoms and long-term health effects. The prevalence of CP varies due to under-reporting, limited diagnostic tools, and inadequate fish testing. The expansion of CP beyond traditional endemic regions, driven by factors such as climate change and warming seas, highlights the need for increased awareness, monitoring, and prevention strategies. CP not only poses health risks but also has socioeconomic implications, impacting the fishery and tourism industries in affected regions. Efforts to establish standardized diagnostic criteria, enhance surveillance systems, and regulate ciguatoxins in food are crucial for effectively addressing the growing threat of CP and mitigating its impact on public health and global economies.

CTXs enter the marine food web through herbivorous fish and benthic invertebrates that consume macroalgae hosting the dinoflagellates. They undergo biotransformation in fish and accumulate in top predators, ultimately reaching humans through consumption. The primary target of CTXs is the voltage-gated sodium channels in excitable tissues, causing prolonged channel openings and disrupting ion conductance. These interactions lead to a range of adverse effects on fish and potentially humans, including muscle coordination issues, paralysis, convulsions, and cardiovascular disturbances. Further research is needed to better understand CTXs from the Indian Ocean and their distinctions within this toxin group. Understanding the mechanisms and classifications of CTXs is crucial for assessing the risks associated with ciguatera poisoning and developing effective prevention and mitigation strategies.

Ciguatera has a significant impact on the global population, with economic repercussions affecting multiple sectors. Losses in the primary industry, as well as in gastronomy and tourism, can be attributed to reduced consumer confidence and restrictions on fish sales. Additionally, those directly affected by ciguatera experience financial hardships. To accurately assess the economic losses and health costs associated with ciguatera, a comprehensive understanding of its distribution, prevalence, and incidence rates is essential. While countries have implemented domestic policies to regulate the sale and capture of implicated products, a multidisciplinary approach involving scientists, consumers, governments, human health experts, and fishers is necessary to effectively address the challenges of ciguatera. The Canary Islands, known as a hotspot for ciguatoxins in Europe, face particular threats due to the presence of multiple *Gambierdiscus* species. The region has implemented a control protocol to detect CTX-like toxicity in certain fish species, preventing the sale of toxic specimens. However, the concentration of ciguatoxins can vary, requiring additional analytical methods. The EuroCigua project has highlighted the importance of the Canary Islands in assessing the risk of ciguatera in Europe and identified key species that accumulate ciguatoxins. Further exploration of these topics will be discussed in subsequent chapters of this review.

Bioaccumulation and biotransformation of CTXs in marine ecosystems play a significant role in the transfer of these harmful compounds through the food chain. CTXs are known to accumulate in various organisms, with carnivorous fish species frequently associated with ciguatera poisoning cases. The bioaccumulation process, driven by the influx and efflux of contaminants, results in higher concentrations of CTXs in apex predators, posing risks to human health. While the precise mechanisms of CTX accumulation and the role of different species in the transfer of these toxins are not yet fully understood, research suggests that CTXs tend to biomagnify in higher-order carnivores due to their lipophilic nature and stability. The biotransformation of CTXs involves oxidative metabolism and carbon-chain backbone spiroisomerizations, leading to the formation of more potent forms. The bioaccumulation of CTXs in fish tissues, particularly in the liver, is well-documented, but further research is needed to understand the distribution patterns within different muscle types and organs. Size and trophic level do not always exhibit a direct correlation with CTX concentration, emphasizing the complexity of the bioaccumulation process. Overall, a comprehensive understanding of the dynamics of CTX bioaccumulation, distribution, and biotransformation is crucial for assessing the risks associated with ciguatera poisoning and developing effective monitoring and management strategies to protect both ecosystems and human health.

Current detection methods for marine biotoxins, including CTXs, involve a combination of biological and chemical approaches such as in vivo assays, high-performance liquid chromatography, and mass spectrometry. However, these methods may not always be specific to CTXs and can lack standardized protocols and reference standards, making accurate quantification and expressing results in CTX equivalents difficult. The lack of rapid and on-site tests further complicates the detection of CTXs, with the mouse bioassay remaining the primary method for confirming toxicity in fish. To ensure seafood safety, management and monitoring strategies are implemented globally, including checks and controls on fishery products and the prohibition of marketing potentially contaminated fish species. Advancements in understanding *Gambierdiscus*, CTXs, and ciguatera poisoning have led to improved detection methods, enhanced monitoring efforts, and increased sensitivity in identifying low levels of toxins. However, the ethical concerns associated with the mouse bioassay have prompted the development of alternative chemical-based methods. Further research and collaboration are necessary to establish standardized and reliable methods for the extraction and detection of CTXs, ultimately safeguarding human health and preventing ciguatera poisoning incidents.

In conclusion, prioritizing research in various areas is crucial to effectively tackle the challenges posed by marine biotoxins and ciguatera poisoning. Firstly, understanding the ecological implications of marine biotoxins and their association with harmful algal blooms is essential to mitigate their impact on human health and marine ecosystems. Secondly, efforts should be focused on enhancing diagnostic tools, surveillance systems, and fish testing methods to accurately assess the prevalence and incidence rates of ciguatera poisoning. Thirdly, further research is necessary to unravel the mechanisms, classifications, and distinctions of ciguatoxins, enabling enhanced risk assessment and the development of prevention and mitigation strategies. Additionally, conducting comprehensive studies on the economic losses and health costs linked to ciguatera will provide valuable insights for policy-making and resource allocation. Furthermore, investigating the bioaccumulation, biotransformation, and distribution patterns of ciguatoxins in marine ecosystems will aid in understanding the risks posed by contaminated fish and guide monitoring and management endeavors. Lastly, establishing standardized and reliable methods for the extraction and detection of ciguatoxins is imperative to ensure seafood safety and prevent ciguatera poisoning incidents. By addressing these research areas, we can safeguard human health, preserve marine ecosystems, and promote the sustainability of coastal communities.

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