Harmful Algae 139 (2024) 102735

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Ciguatera poisoning: A review of the ecology and detection methods for *Gambierdiscus* and *Fukuyoa* species

Joseph C. Perkins [a,*], Kyall R. Zenger [a], Yang Liu [b], Jan M. Strugnell [a]

[a] Centre for Sustainable Tropical Fisheries and Aquaculture, James Cook University, Townsville 4811, Qld, Australia
[b] College of Science and Engineering, James Cook University, Townsville 4811, Qld, Australia

ARTICLE INFO

Keywords:
Ciguatoxins
Climate change
Dinoflagellates
Harmful algae
Molecular methods

ABSTRACT

Ciguatera poisoning is the most prevalent non-bacterial seafood illness globally, with an estimated 10,000 to 50,000 human cases reported annually. While most symptoms are generally mild, some cases can result in severe and long-lasting neurological and psychological damage, and in some instances, even death. The known causative agents of ciguatera poisoning are benthic toxic dinoflagellate species belonging to the genera *Gambierdiscus* and *Fukuyoa*. These species produce highly potent ciguatoxins that bioaccumulate through the marine food chain, eventually reaching humans through seafood consumption. Although *Gambierdiscus* and *Fukuyoa* species are widespread in tropical waters worldwide, the full extent of their distribution remains uncertain. This review provides a detailed examination of the ecological dynamics of these dinoflagellates and explores the diverse range of detection methods used to monitor them. These include a focus on molecular techniques for detection, alongside morphological methods, emerging technologies, and a toxin detection overview. Additionally, we offer recommendations on how the field can advance, highlighting novel solutions and next steps for improving detection and monitoring practices. By assessing the strengths and limitations of current approaches and proposing directions for future research, this review aims to support efforts in better understanding and mitigating the risk of ciguatera poisoning.

1. Introduction

1.1. Ciguatera poisoning

Ciguatera poisoning (CP) is the most common seafood toxin-related illness globally (Chinain et al., 2021; Dickey and Plakas, 2010; Friedman, 2008). The documented number of human CP cases each year ranges from 10,000–50,000 (Chinain et al., 2021; Friedman, 2008). However, some reports predict that this number could be higher, with 2–10 million people affected every year (World Health Organisation, 2020). Although most symptoms are mild, some can be more severe, causing long term neurological and psychological damage or even death (Quiroz et al., 2020). CP is caused by two genera from a diverse group of eukaryotic dinoflagellates, *Gambierdiscus* and *Fukuyoa* (Kohli et al., 2015; Laza-Martínez et al., 2016; Wang et al., 2022).

Gambierdiscus and *Fukuyoa* spp. are recognised as harmful algal bloom species (HABs) and benthic toxic dinoflagellates (BTD). These benthic dinoflagellates commonly inhabit marine macroalgae, dead corals, rocky substrates, and artificial reefs (e.g., shipwrecks and oil structures) and are predominately found in shallow tropical and subtropical waters (Chinain et al., 2020; Han and Liu, 2014; Schulze et al., 2020). They are known to produce ciguatoxins (CTXs) that are highly potent neurotoxins, with ladder-shaped polyether compounds and bioactive properties (Loeffler et al., 2021). These toxins are characterised by their ability to dissolve in lipids and resist high temperatures (Dickey and Plakas, 2010; Ramos-Sosa et al., 2022). This in turn affects human sodium channels as they interact with polyether molecules, causing various physiological processes, including nerve conduction and muscle contractions (Resiere et al., 2022). Herbivores, omnivores, or detritivores consume algae that serve as hosts for CTX producing dinoflagellates. CTXs then bioaccumulate further in carnivores that prey on these organisms and ultimately in humans who consume seafood (Caillaud et al., 2010; Chinain et al., 2021; Loeffler et al., 2021). CTXs are some of the most complex marine toxins known; the diversity of the toxins, the dinoflagellates that produce them, and the mechanisms that contribute to the bioaccumulation process, are still poorly understood (Pulido, 2014; Quiroz et al., 2020). While it is understood that CP cases are significantly underreported, there has nonetheless been an increase

* Corresponding author.
E-mail address: Joe.perkins@my.jcu.edu.au (J.C. Perkins).

https://doi.org/10.1016/j.hal.2024.102735
Received 8 February 2024; Received in revised form 2 October 2024; Accepted 2 October 2024
Available online 10 October 2024
1568-9883/

in occurrences reported globally (Chinain et al., 2021; Friedman et al., 2017; World Health Organisation, 2020).

1.2. Human health

Diagnosis of CTXs is not possible by sight, or smell, and toxin potency is not reduced by cooking or freezing. In addition, there is currently not a commercially available CTX test, therefore guidance regarding CP avoidance is vague and inconsistent (Pasinszki et al., 2020; Yang et al., 2016). For example, New South Wales, Australia, government guidelines recommend following several rules to minimise the risk of exposure to ciguatoxins, including; "vary the type of warm water fish eaten" (NSW NSW Government Food Authority, 2022), which refers to marine fish species commonly found in tropical and subtropical regions, where ciguatera is more prevalent. Similarly, the Hong Kong government guidelines state, "buy fish from reliable seafood retailers" (Centre for Food Safety: The Government of the Hong Kong Special Administrative Region, 2021). These vague recommendations highlight the need for a deeper understanding on marine species that accumulate harmful CTXs and underlines the need for such research to be conversed with authorities and governing bodies to guarantee seafood safety.

CP can cause a diverse range of symptoms (Sup. Table 1) including neurological (i.e., headache, loss of feeling, memory loss and double vision) and gastrointestinal (i.e., diarrhea and abdominal pain). Depending on the dose of CTX consumed, symptoms can develop anywhere from a few minutes after consumption to 48 h later (Chinain et al., 2019). In most cases symptoms are mild and patients recover quickly, but some cases can be severe or chronic, and some severe cases have even caused fatalities (Friedman, 2008; Zha and Silvestri, 2022). Treatment is lacking for CP as there is an absence of effective and specific antidotes. Some cases have shown effective treatment from injecting mannitol (a type of sugar alcohol), if it is applied within a few hours of toxin consumption (Mullins and Hoffman, 2017). In the south Pacific islands, traditional remedies such as orally consumption of octopus bush *Heliotropium foetherianum* have been shown to be effective against CP symptoms (Kumar-Roiné et al., 2011).

1.3. History of ciguatera poisoning

Historical records show CP to be an age-old affliction, with incidences recorded centuries ago by Homer and Alexandra the Great (Soliño and Costa, 2020). Some suggest CP forced the famous Polynesian voyagers (AD 1000 to 1450) to explore new and safe fishing grounds, resulting in the discovery of new lands (Rongo et al., 2009). Some of the first accounts came from the West Indies as early as 1511, followed by reports of CP being responsible for the deaths of several captains in the Spanish army off the Gulf of Guinea in 1521 (Rongo et al., 2009; Soliño and Costa, 2020). The word "ciguatera" was devised in the 18th century by European settlers on the island of Cuba, who ingested a gastropod, locally named "cigua" (*Livonia* spp.) and subsequently encountered neurological symptoms (Pearn, 2001). It was initially thought all cases resulted from the consumption of these snails, but later reports came from cases around the globe of similar symptoms from the consumption of fish. In 1771, Captain James Cook recorded a fish poisoning incident sailing around the nation of Vanuatu in the Pacific Ocean, and the symptoms described are now recognised as being caused by CP (Doherty, 2005; Halstead, 1965). After centuries of speculation about the cause of the neurological symptoms that came after consuming fish from tropical waters, Randall (1958) hypothesised a benthic organism, most likely to be micro-algae from tropical regions, to be the source of the toxin. He proposed that algae grazers (herbivores and detritivores) would then feed on this alga, before in turn being consumed by larger carnivorous fish which would accumulate these toxins. This bioaccumulation process is now widely accepted and will be discussed further throughout this review.

1.4. Global ciguatera poisoning occurrences

CP is highly endemic throughout the tropics, in particular Pacific Island nations and the Caribbean (Abraham et al., 2012; Chinain et al., 2019; Litaker et al., 2017). In the last few decades there has been a 60 % increase in CP cases across Polynesian islands, with no Polynesian location completely safe from the disease (Chinain et al., 2021; Kohli et al., 2014b). CP has been reported on every continent except Antarctica, whether that be endemic or imported cases (Fig.1).

Due to globalisation, seafood has become the most widely traded food globally. As a result, CP is not limited to endemic regions alone but also affects other regions through seafood importation. For example, France, Germany, and the UK have experienced CP cases linked to the importation of tropical fish from Southeast Asia and the Caribbean (Caillaud et al., 2010). Similarly, the USA and Canada have reported CP cases from fish imported from Pacific Island nations and the Caribbean (Dickey and Plakas, 2010). In a remarkable case, a CP outbreak occurred close to Antarctica, on the island of South Georgia, resulting from the suspected consumption of a singular imported Grouper fish from the Caribbean (Agrawal and Grab, 2022). Chinain et al. (2021) provide a detailed review of CP occurrences by region along with global trends, further highlighting the widespread impact of CP.

1.5. Cultural, economic and food safety impacts

Apart from posing significant health risks, CP also has adverse economic implications. In Moorea Island, French Polynesia, CP cases are common. It is estimated because of these incidences there is an estimated annual economic loss of $80,000 USD. This loss can be attributed to expenses incurred on hospital bills, medication, and the loss of productivity due to sick days taken for recovery (Morin et al., 2016; Soliño and Costa, 2020). For an island with a small population of ~16,000 this is a substantial loss. Costs of CP are not solely health related, there are potential losses of economic profit, costs of monitoring and management, and loss of tourism (CP from hotels, tours, and restaurants). The occurrence of CP in affected areas not only has a direct impact on public health but also leads to adverse cultural consequences. Inhabitants of these areas are often compelled to modify their dietary patterns, which can subsequently result in decreased fishing activities. Fishing is a fundamental aspect of island culture across the globe (Morin et al., 2016; Trick et al., 2020).

Research has indicated that if fish contaminated with CTXs are found in a particular location, that area may continue to be at risk of CP incidences for an extended period (Chinain et al., 2019; Loeffler et al., 2021; Yang et al., 2016). This is because the toxic dinoflagellates responsible for causing CP can thrive and proliferate in the ideal conditions found in that area. Also, once CTXs have entered the food web, large, long-living carnivorous fish can accumulate the toxins over many years; meaning some nations must constantly be cautious of this threat (Soliño and Costa, 2020; Chinain et al., 2019). The people of Polynesian Islands heavily rely on seafood as a primary source of sustenance, with an average fish consumption of around 100 kg per person per year in French Polynesia. However, this reliance on seafood exposes them to the risk of CP. Incidences of CP are a concern for these communities as no Polynesian Island is entirely immune to this condition (Chinain et al., 2019). A thorough review by Chinain et al. (2023) has extensively examined the distribution and toxicity of the causative organisms of CP (*Gambierdiscus* spp.) and the related impacts on human health in French Polynesia. This area has been the site of rich and active CP research since the 1960s, making it a crucial region for understanding CP dynamics and informing effective management and mitigation strategies.

Additionally, it is important to consider the impact of CP in fish with very large home ranges, such as Spanish Mackerel (*Scomberomorus commerson*). Farrell et al. (2017) reported that between 2014 and 2016, five cases of CP, involving twenty-four individuals, were linked to Spanish Mackerel caught in the coastal waters of New South Wales

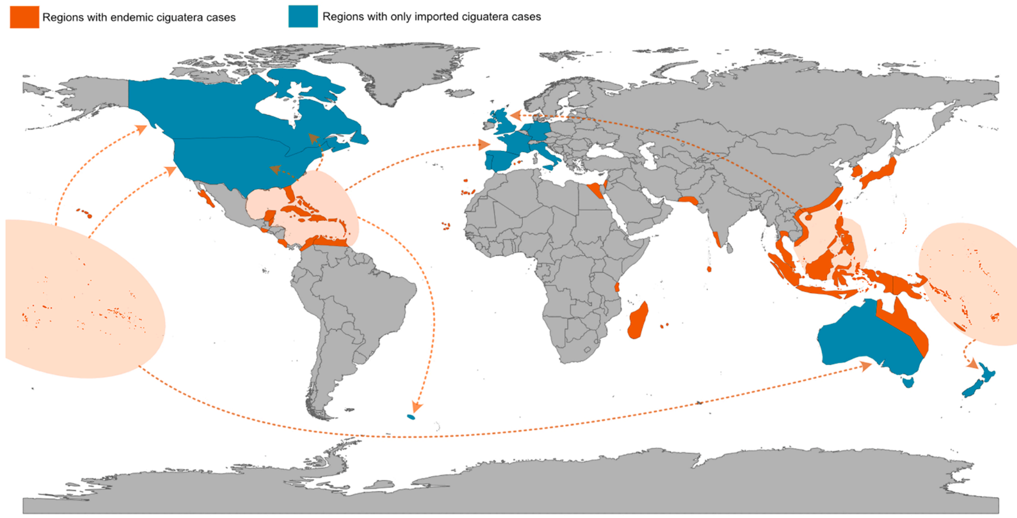

Fig. 1. Global map of endemic ciguatera areas (orange) and imported ciguatera areas (blue). The orange arrows indicate examples of areas in which imported fish have originated and caused CP cases in another location (Identified regions adapted from references cited from sup. Table 5).

(NSW) on the east coast of Australia. This highlights that Spanish Mackerel migrating down the east coast of Australia can accumulate CTXs along their migration, leading to increased incidences of CP in areas previously considered low risk.

The persistence and increase of fish with CTXs in a region also limit the ability to export fish. There have been cases of mislabelling seafood products from such areas, resulting in CP incidences in non-endemic nations (Loeffler et al., 2022). One example, detailed in Kusche & Hanel (2021), reported a shipment of tropical fish to Germany that was labeled as "snapper" to be several different species. Out of the 103 individually labeled "snapper", 84 were identified as CP-associated species such as the two-spotted red snapper *Lutjanus bohar*, yellow-edged lyretails Variola louti and the common coral trout Plectropomus leopardus from tropical waters (i.e., French Polynesia, Vietnam, and Indonesia) where CP is prominent. This highlights the vulnerability of global seafood safety and the need for thorough traceability and provenance checks.

1.6. Toxins produced by Gambierdiscus and Fukuyoa species

Ciguatoxins are a group of neurotoxins that are lipid-soluble and heat stable-compounds. They bind to open voltage-gated sodium channels in nerve cells when produced by certain *Gambierdiscus* and *Fukuyoa* spp. as they enter the marine food chain (Pasinszki et al., 2020). While previously emphasised, it is worth reiterating that CTXs stand out as some of the most toxic and intricate molecules known. Their inherent complexity, diverse structures, and transformations as they bioaccumulate through the marine food web underscore the challenges and significance of understanding these compounds (Soliño and Costa, 2018).

Currently, over 30 CTX congeners have been identified and are categorised into four groups based on three geographical groups: Pacific CTXs (CTX4A and CTX3C), Caribbean CTXs (C—CTX), and Indian Ocean CTXs (I-CTX) (Loeffler et al., 2022; World Health Organisation, 2020). Within these groups, Pacific CTXs are the most diverse and extensively studied globally (Soliño and Costa, 2018; Wang et al., 2022). There is increasing evidence of overlap in these regional distinctions and advances in structural elucidation now allow for the classification of CTXs based on their chemical structures. Recent research by Mudge et al. (2024) identified new Caribbean CTX congeners (C—CTXs) in *Gambierdiscus* spp. from the Caribbean, including a precursor, C—CTX5, reduced into C—CTX1. This study proposes a naming convention aligning with Pacific CTXs, aiding identification of structure and stereochemistry among C—CTX analogues.

As the number of oxygen atoms in the chemical structure of CTXs increases, the toxins become more polar and toxic. This phenomenon has led to the theory that oxidations occur within fish, which allows for the excretion of these toxins, and results in the toxins becoming more potent along this pathway. Several studies have suggested that organisms at lower trophic levels accumulate less oxidized CTXs, whereas carnivorous fish at the top of the food chain tend to contain higher levels of oxidized, polar, and toxic CTXs (Lewis and Holmes, 1993; Satake et al., 1993; Soliño and Costa, 2018). Although research is limited on toxin production and the levels of CTXs considered safe for human consumption (Sup. Tables 2,3 and 4), there have been reviews that provide more detailed information For more comprehensive insights into *Gambierdiscus* and *Fukuyoa* toxin production see reviews by Wang et al. (2022) and Chinain et al. (2023).

Genetic mechanisms that regulate CTX production from *Gambierdiscus* and *Fukuyoa* spp. are not yet clear, although it is thought biosynthesis is facilitated by polyketide synthesis. This assumption is made due to evidence of this pathway from the structurally similar brevetoxins (Chinain et al., 2010; Van Dolah et al., 2020). Research into genetic signatures that dictate CTX production is sparse and an area that should also be investigated further.

1.7. Other toxins produced by Gambierdiscus and Fukuyoa

Other marine polyether compounds are also produced by *Gambierdiscus* and *Fukuyoa* spp.; maitotoxins (MTXs) and gambieraric acids, gambierol and gambierone. MTXs are among the most potent marine toxins, but they are water-soluble compounds, rarely accumulating in tissues, thus making them a minor risk to seafood safety. So far, there has been no evidence to show that MTXs are involved in CP (Holmes et al., 2021; Pasinszki et al., 2020). Gambierone and gambierol have been isolated from *Gambierdiscus* and *Fukuyoa* spp. and shown to have extremely low potency, deemed not to be harmful to humans. They have never been detected in other marine organisms, but they have been shown to act on the same sodium channel as CTXs, leading to questions on their role in CTX production and bioaccumulation processes and something that should be explored further (Raposo-Garcia et al., 2022; Satake et al., 1993).

1.8. Ciguatoxin vectors

According to the World Health Organization (2020), over 425 species of fish have been associated with CP, with most of them being carnivorous fish found in coral reefs. Although CP is primarily considered a fish-borne syndrome, research has revealed that marine invertebrates such as urchins, gastropods, bivalves, crab, lobster, and octopus can also contain high levels of CTXs (Mak et al., 2013; Chinain et al., 2019; Darius et al., 2018). As a result, the traditional name of ciguatera fish poisoning (CFP) has been changed throughout recent literature to ciguatera poisoning (CP) to reflect the broader range of sources for this toxin.

From published data spanning from 1994 to 2022, 181 marine specimens were identified using established detection methods to positively detect CTXs (Sup. Table 5). They covered seven classes: Actinopterygii (174), Chondrichthyes (2), Bivalvia (1), Cephalopoda (1), Chondrichthyes (1), Echinoidea (1) and Gastropoda (1). The ten families that were most common across all marine organisms globally were: Serranidae (grouper), Lutjanidae (snapper), Carangidae (jacks), Scaridae (parrotfishes), Acanthuridae (surgeonfishes), Labridae (wrasses), Lethrinidae (emperors), Muraenidae (Moray eels), Scombridae (mackerel) and Sparidae (breams) (Fig. 2).

Although more than half of the species where CTXs have been detected belong to carnivorous families such as Serranidae, Lutjanidae, and Carangidae, it is interesting to highlight that herbivorous families like Scaridae and Acanthuridae also have a significant number of species where detectable and harmful CTXs are found. These herbivorous species are not typically considered a threat for CP in most locations globally as they are not highly sought after seafood species. However, most detections of CTXs in herbivorous species comes from studies conducted in French Polynesia and the Caribbean, where a greater variety of seafood is consumed, and there is a greater reliance on seafood compared to other regions (Chinain et al., 2023; Gaboriau et al., 2014; Mak et al., 2013; Morin et al., 2016; Soliño and Costa, 2020). Studies have demonstrated that higher levels of harmful CTXs are present in carnivorous species (Mak et al., 2013; Meyer et al., 2016; Oshiro et al., 2024). Nevertheless, it is important to note that dietary and cultural preferences may also play a role in CP cases globally and contribute to sampling bias. Many studies tend to focus on large carnivorous species when investigating CTXs, potentially overlooking the presence of toxins in other species from lower trophic levels.

1.9. Bioaccumulation and biomagnification

Ciguatoxins are recognised for their ability to accumulate gradually through the food chain (Loeffler et al., 2021). This process, known as bioaccumulation, is widely acknowledged as the primary mechanism by which CTXs travel through the food web. However, the rate of bioaccumulation for CTXs is a complex and poorly understood phenomenon. In addition, CTXs are believed to undergo biomagnification, a process in which an organism takes in CTXs at a faster rate than the toxin can be eliminated. This phenomenon can lead to elevated levels of toxins in certain tissues or organs within an organism, as demonstrated by studies that have shown the concentration of CTXs in specific tissues (Loeffler et al., 2021; Meyer et al., 2016; Yang et al., 2016).

It is widely believed that large carnivorous fish found in coral reefs are likely to have higher concentrations of CTXs due to the processes of bioaccumulation and biomagnification (Chinain et al., 2021; Loeffler et al., 2021). However, the evidence supporting this assumption is inconclusive, with conflicting studies that have observed no correlation between CTX concentrations and factors such as fish age, weight, or length (Darius et al., 2018; Gaboriau et al., 2014). Furthermore, research has shown that herbivorous fish such as parrot fish (Scaridae spp.) that graze on algae can also contain extremely high levels of CTXs, contradicting the notion that only large carnivorous fish accumulate high levels of toxins (Mak et al., 2013).

A study conducted by Ramos-Sosa et al. (2022) in the Canary Islands, Spain was the first to compare levels of CTXs between the liver and flesh of fish, as well as across four different species - amberjack *Seriola* spp., dusky grouper *Epinephelus marginatus*, black moray eels *Muraena helena*, and two-banded seabream *Diplodus vulgaris*. The results showed that all liver samples from the different species had higher levels of CTXs than the flesh samples, suggesting that biomagnification occurs mostly within the liver of organisms. This study also detected the presence of CTXs in the common two-branded seabream, an omnivorous species that is highly valued commercially in the Mediterranean.

Establishing the knowledge on the distribution and drivers of *Gambierdiscus* and *Fukuyoa* spp. is important to help prevent current and future CP incidences. As different geographical locations have different taxa, environmental conditions, and food web dynamics, this could mean CP risks differ depending on location (Holmes et al., 2021; Ting and Brown, 2001). Previous studies have found the same species that have high CTX levels in one area, don't necessarily possess similar CTX

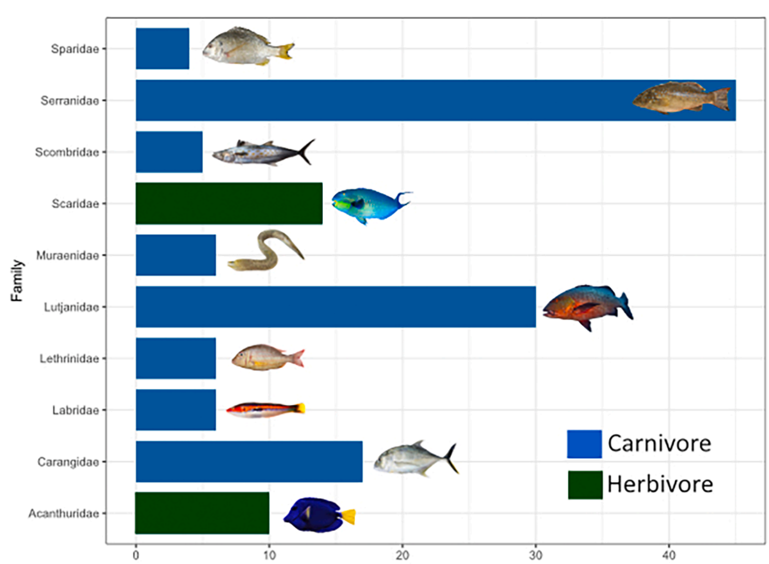

Fig. 2. A bar chart showing the ten most common fish families associated with CP occurrences in alphabetical order along the y-axis. The x-axis represents the number of different species that have been implicated in CP occurrences within each family (Identified fish families adapted from references cited from sup. Table 5).

levels in other areas, even though both locations are considered CP 'hotspots' (Holmes et al., 2021). This could be due to the occurrence of different *Gambierdiscus* or *Fukuyoa* species that are less toxic, in lower abundance, or because herbivorous/omnivorous marine species diets differ or vary between different locations. Therefore, this review will focus on two main areas: (1) the ecology of *Gambierdiscus* and *Fukuyoa* species, and (2) the detection methods to identify and monitor these benthic toxic dinoflagellates.

2. *Gambierdiscus* and *Fukuyoa* dinoflagellates

2.1. Global distribution of Gambierdiscus and Fukuyoa

Gambierdiscus toxicus, was initially described and named by Adachi & Fukuyo (1979). Bagins et al. (1980) then definitively linked *G. toxicus* to CP on the reefs off the Gambier Islands in French Polynesia. Prior to these findings, Yasumoto et al. (1977) were among the first to suggest the involvement of a dinoflagellate in ciguatera, conducting experiments that highlighted the relationship between dinoflagellate cells and toxicity in samples from ciguatera-endemic areas. Their work indicated that *Diplopsalis* sp. *nov* could be a likely cause of CP, based on the extraction and identification of toxins similar to ciguatoxins.

It remained the only known species in the genus until 1995, when another species, *Gambierdiscus belizeanus*, was discovered and described in Belize (Faust, 1995). Currently, there are 22 known species across two genera: nineteen *Gambierdiscus* species and three *Fukuyoa* species (Fig. 3). In addition, there are several unnamed genetic clades (i.e., *Gambierdiscus* sp. ribotypes which are variations in ribosomal RNA sequences and *Gambierdiscus* sp. types) which could represent new species that have yet to be described (Chinain et al., 2020). It is expected more species of *Gambierdiscus* and *Fukuyoa* will be discovered in coming years as more research is conducted in areas which are relatively unstudied, such as the Indian Ocean, Great Barrier Reef, (Australia), and temperate locations globally.

In 2015, Gómez et al. (2015) distinguished the genus *Fukuyoa* as a separate entity from *Gambierdiscus* based on molecular and morphological characteristics of two species, now identified as *F. yasumotoi* and *F. ruetzleri* (previously *Gambierdiscus* sp.) In addition, a third *Fukuyoa* species was subsequently described; *F. paulensis* (Gómez et al., 2015). Although subsequent studies detected no CTX-like activity (Gómez et al., 2015; Larsson et al., 2019), one cultured strain of *F. paulensis* from the western Mediterranean Sea was shown to produce CTX-like activity (Laza-Martínez et al., 2016), providing evidence of the potential danger of the genus *Fukuyoa*.

Gambierdiscus and *Fukuyoa* spp. are mainly restricted to tropical and subtropical latitudes, with the main global hotspots identified from tropical Polynesian and the Caribbean Islands (Chateau-Degat et al., 2005; Kibler et al., 2015; Litaker et al., 2017; Morin et al., 2016). Although, in recent years the detection of *Gambierdiscus* and *Fukuyoa* spp. have been found in temperate latitudes such as the Balearic Islands, Greek Islands, Japan, New Zealand and New South Wales (NSW) in Australia (Funaki et al., 2022; Gaiani et al., 2022; Kohli et al., 2014b; Rhodes et al., 2017; Rhodes et al, 2020).

To date, only two species of *Gambierdiscus* have been identified to produce ciguatoxins: *G. silvae* (Mudge et al., 2023) and *G. polynesiensis* (Rhodes et al., 2014). Other species, such as *G. australes, G. excentricus*, and *G. pacificus*, have shown CTX-like activity in cell-based assays (N2A), but their production of ciguatoxins has not yet been confirmed by mass spectrometry (Caillaud et al., 2011; Tudó et al., 2020). Records and studies prior to 1995 attributed all evidence of CTX production to originate from a single species '*Gambierdiscus toxicus*' and so species identification prior to this time is uncertain. It is also important to consider that there is a lack of evidence to confirm other *Gambierdiscus* and *Fukuyoa* spp. do not produce potent CTXs. Additionally, it is unknown if certain species that are considered non or less toxic, could become more toxic in higher abundance, and if highly toxic species do not need to be highly abundant to contribute to CTX accumulation (Soliño and Costa, 2018; Xu et al., 2014, 2016).

2.2. Habitat and life cycle

Gambierdiscus and *Fukuyoa* spp. are autotropic (primary producers) BTDs that are thought to live in tropical coral reef areas and rocky substrates. They are particularly abundant on dead corals which provide ideal substrates for colonisation by macroalgae (Rains and Parsons, 2015). Most research has investigated the environmental conditions that are suitable for *Gambierdiscus* and *Fukuyoa* spp. growth, but little work has observed their habitat and the role of the macroalgal host.

Some *Gambierdiscus* and *Fukuyoa* species have been observed exploiting the three-dimensional structure of their host macroalgae by actively moving to different depths on their host throughout the day. Studies, such as those conducted by Villareal & Morton (2002), have demonstrated that light intensity significantly influences the cell densities and behaviours of these dinoflagellates on their epiphytic hosts. The dynamic movement of these dinoflagellates throughout the day can have profound implications for trophic transfer pathways, particularly

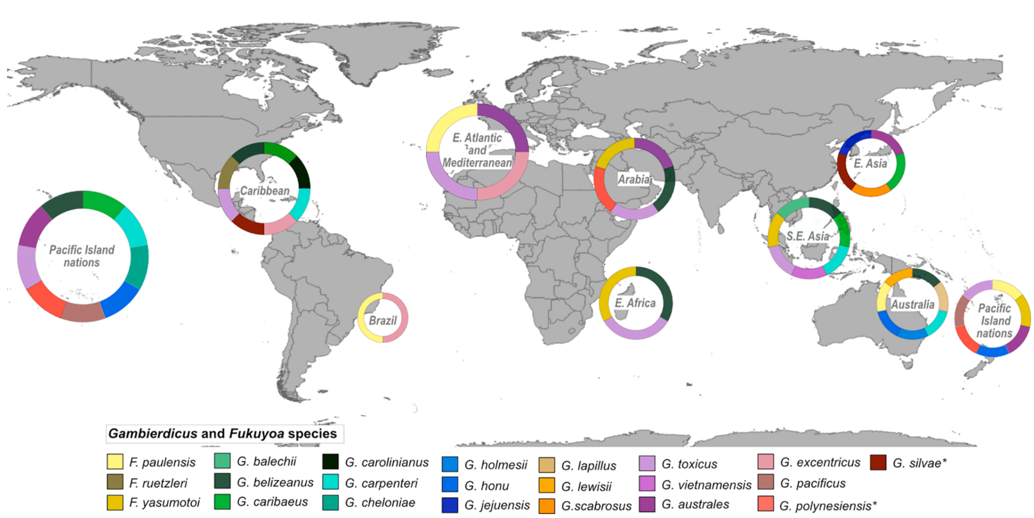

Fig. 3. Global distribution of the 22 known *Gambierdiscus* and *Fukuyoa* dinoflagellate species from multiple global studies (Identified species distribution adapted from references cited from sup. Table 6).
*Species with confirmed ciguatoxin production.

due to the feeding habits of algal-grazing marine organisms (Rains and Parsons, 2015). Increased understanding of *Gambierdiscus* and *Fukuyoa* spp. and their algal host interactions will improve knowledge of basic pathways of CTXs into the food web.

Gambierdiscus and *Fukuyoa* spp. are mainly associated with shallow habitats (0–8 m) including shallow intertidal zones or reefs (Funaki et al., 2022; Litaker et al., 2010). However, investigations into the detection of *Gambierdiscus* spp. were prompted by a series of CP incidences from fish caught in deeper waters In Flower Bank National Marine Sanctuary, located off the coast of Texas, USA. These investigations revealed that *Gambierdiscus* spp. were present in depths of 40–60 m, challenging previous assumptions about their suitable habitat (Tester et al., 2014). In accordance, a study from Japan detected *Gambierdiscus* spp. at depths of approximately 30 metres (Funaki et al., 2022). In controlled settings Yoshimatsu et al. (2016) found some *Gambierdiscus* species could continue to grow at 87 m depth. Clarifying the distribution of *Gambierdiscus* and *Fukuyoa* spp. both horizontally and vertically in the marine ecosystem will help assess CP pathways into the food web.

Despite advancements in understanding the habitat of *Gambierdiscus* and *Fukuyoa* spp., significant gaps remain in our knowledge of their life cycles. There is currently no evidence to support that these species produce cysts, a life stage commonly found in other benthic dinoflagellates. Studies such as Ramilo et al. (2021) indicate that rigorous and complete investigations into the possible existence of resting cysts in *Gambierdiscus* are lacking. Understanding cell division, morphological variability, and the triggers for vegetative growth and blooms is crucial for a comprehensive understanding of these species and their impacts on marine ecosystems. As noted by the Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB, 2012) more detailed studies on the life cycles of *Gambierdiscus* and *Fukuyoa* spp. are necessary to better understand and manage their ecological and toxicological impacts.

2.3. Environmental influences

2.2.1. Salinity

Gambierdiscus and *Fukuyoa* spp. typically occur in the ocean at salinities of 34–38 ppt but can grow in a broader range of salinities. Some strains/species can grow between 15 and 41 ppt in laboratory conditions (Xu et al., 2016). Kibler et al. (2012) observed that eight *Gambierdiscus* species: *G. australes, G. belizeanus, G. caribaeus, G. carolinianus, G. carpenteri, G. pacificus, G. ruetzleri* (now *F. ruetzleri*) and *Gambierdiscus* ribotype 2, demonstrated reduced growth in salinities below 20 parts per thousand and this may explain their reduced abundance in river mouths and estuaries. *Gambierdiscus* and *Fukuyoa* spp. attain maximum growth in salinity ranges between 25 and 35 ppt (Kibler et al., 2012; Xu et al., 2016).

2.2.2. Temperature

Gambierdiscus and *Fukuyoa* species typically inhabit warm tropical waters (24–29 °C) (Kohli et al., 2014b). There is an established relationship between temperature tolerance, distribution, and life traits of certain *Gambierdiscus* and *Fukuyoa* spp. supported throughout the literature. Ramilo et al. (2021) conducted temperature experiments to assess the optimal growth and thermal limits of five *Gambierdiscus* species (*G. australes, G. caribaeus, G. carolinianus, G. excentricus*, and *G. silvae*) isolated from the Canary Islands. They found that *G. australes* exhibited a higher tolerance across a wider range of temperatures, which may explain their circumtropical distribution. Additionally, the study observed a higher abundance of zygotes (fertilised cells) at warmer temperatures, suggesting that sexual reproduction is promoted under these conditions. *G. carpenteri* has also demonstrated tolerance to a wide range of temperatures in laboratory conditions (Kohli et al., 2014b; Sparrow et al., 2017), potentially explaining its widespread distribution, as it has been identified in colder waters (16.5–17 °C) like New South Wales, Australia, where *Gambierdiscus* and *Fukuyoa* spp. are not expected to survive (Kohli et al., 2014a; Sparrow and Heimann, 2016). Further research is needed to investigate the relationship between temperature tolerance and the distribution of *Gambierdiscus* and *Fukuyoa* spp. This knowledge is crucial in predicting and comprehending the current and future habitats of these organisms, particularly in the face of the well-documented increase in ocean temperatures, and in preventing future CP incidences.

2.2.3. Irradiance

Dinoflagellates require light to photosynthesise, survive and grow, thus research on tolerance of light intensities is important for helping resolve distribution and depth limits (Kibler et al., 2012; Tawong et al., 2016). A study by Kibler et al. (2012) investigating eight *Gambierdiscus* species response to the effects of irradiance found they were all able to maintain growth between 6 and 17 umol photons W/m^2.

Using such measures, it was possible to estimate maximum depth at which *Gambierdiscus* cells can maintain growth, which was between 125 and 150 metres. To date, in the wild, *Gambierdiscus* spp. have been detected at depths of up to 60 metres (Litaker et al., 2009).

2.2.4. pH

The pH levels in the ocean can vary, but typically fall within a range of 7.5 to 8.4 (Jiang et al., 2019). Fluctuating levels of pH is known to alter acid-base balances, rates of photosynthesis, direct growth of algae and contribute to harmful algae blooms (Raven et al., 2020). A study investigating the effects of pH on *G. polynesiensis* in cultured conditions found significant increases in CTX analogs when pH was lowered from pH=8.4 to pH=7.0 (Longo et al., 2020). It is well understood that oceanic pH levels are declining globally through ocean acidification (Garcia-Soto et al., 2021), potentially indicating an increase in CTX production around the globe.

2.2.5. Nitrogen (N): phosphorus (P)

High levels of nitrogen with conditions of abundant phosphorus (N:P ratio) have previously been shown to be constant throughout the world's oceans at 16:1; named as the Redfield ratio (Redfield, 1933). Although, in recent times high inputs of nitrogen and phosphorus along coastal waters have become more prevalent and the ratio balance altered due to increases in anthropogenic eutrophication (Accoroni et al., 2015; Cooper et al., 2014; HAIS-UNESCO, 2022). There is evidence showing that alerting N:P ratios is correlating with increased HABs. For example, the genus *Ostreopsis* is another BTD that is commonly found with *Gambierdiscus* and *Fukuyoa* spp. throughout tropical regions. *Ostreopsis* blooms have been associated with faunal damage and human respiratory issues (Berdalet et al., 2018) and annual blooms in the Adriatic Sea have been attributed to warming temperatures, which at >25.8 °C is optimal for cyst germination, along with injections of P-rich water, allowing for both cyst germination and cell proliferation. Research focusing on *Gambierdiscus* and *Fukuyoa* spp. should also aim to investigate the effects on changes in N:P ratios.

2.4. Climate change and other anthropogenic pressures

While there is no direct evidence linking CP events to climate change, there has been an increased incidence of CP reported globally. Furthermore, there has been an increase in endemic fish caught in cooler waters, potentially contributing to CP outbreaks. Evidence suggests that *Gambierdiscus* and *Fukuyoa* spp. have been detected in areas not traditionally associated with the disease (Farrell et al., 2017; Funaki et al., 2022; Kohli et al., 2014b; Ramos-Sosa et al., 2022; Rhodes et al., 2020; Soliño and Costa, 2020) possibly influenced by changes in oceanic currents due to climate change. For instance, the strengthening of the East Australian Current along the East coast of Australia has extended warmer waters further south. Globally, temperate waters are warming at a significant rate, such as the observed increase of 0.75 °C per century in Australia since the early 20th century. This warming trend has facilitated the detection of migratory pelagic fish species and expanded the range of *Gambierdiscus* and *Fukuyoa* spp. beyond their typical distributions in recent decades (Farrell et al., 2017; Kohli et al., 2014b).

Analysis of the time-series data available for a limited number of countries indicates the highest incidence rates are consistently reported from two historical CP-endemic areas, the Pacific and Caribbean regions. This is partly due to the strong reliance of local communities on marine resources (Chinain et al., 2021, 2023; Litaker et al., 2010; Tester et al., 2010). Since 2000, an expansion of the geographical range of CP has been observed in several areas such as Macaronesia and East and Southeast Asia. In some of these locales, random surveys confirmed the presence of CTXs in locally sourced fish, consistent with the concurrent report of novel CP incidents (e.g., Canary Islands, Madeira, Selvagens Islands, New South Wales) (Chan, 2015; Estevez et al., 2020; Kohli et al., 2015; Otero et al., 2010).

However, it is important to interpret these observations with caution. The increased detection of CP and associated species in new areas might also be due to improved detection methods and heightened research efforts rather than solely a consequence of climate change. Further research is needed to disentangle the effects of climate change from other contributing factors to CP incidence and distribution.

The El Niño–Southern Oscillation (ENSO) is an oceanic event that occurs irregularly in the tropical Pacific, characterized by higher-than-average water temperatures. Such oceanic events are becoming more frequent and enduring for longer periods due to climate change. This phenomenon has various adverse environmental effects, such as coral bleaching due to prolonged elevated sea temperatures (Hoegh-Guldberg et al., 2019; Hughes et al., 2018). El Niño is predicted to intensify, persist for more extended periods, and have more severe negative consequences on both the environment and economies (Cai et al., 2014, 2020) Researchers have reported correlations between El Niño events and increases in CP in Pacific waters, as elevated sea surface temperatures associated with El Niño lead to the proliferation of *Gambierdiscus* spp. (Gingold et al., 2014; Hales et al., 1999; Llewellyn, 2010). A study by Rongo and van Woesik (2013), from the island of Rarotonga, southern Cook Islands, found there had been a decrease in CP incidences and between 2006 and 2011 cases were the lowest in 20 years. Relationships between CP cases from 1992 to 2011, along with coral cover, reef disturbances and densities of herbivorous fish, were examined. They found reef disturbance and high densities of herbivores were strong predictors of CP, as after major cyclones, reefs became disturbed and densities in herbivorous fish increased. Since 2006 the number of cyclones has markedly decreased in Rarotonga due to a negative phase of the Pacific Decadal Oscillation (a long-lived El Niño-like pattern), therefore there has been less reef disturbance, lower densities of herbivore fish, and lower CP incidences.

Artificial reefs can create suitable habitats for *Gambierdiscus* and *Fukuyoa* spp. in areas in which habitats would not be suited to their epiphytic nature (Sparrow and Heimann, 2016; Villareal et al., 2007). An example of this is in the north-western Gulf of Mexico, which is characterised by soft, muddy benthic substrates which are not the typical habitat for these species. Interestingly, the gulf hosts approximately 4000 petroleum production platforms. Research by Villareal et al. (2007) found barracuda that predated on fish around these petroleum platforms to have harmful levels of CTXs. Further investigation found these artificial substrates harboured the growth of *Gambierdiscus* spp. responsible for CP. This research highlights that anthropogenic disturbances such as the creation of artificial reefs can create suitable habitat for *Gambierdiscus* and *Fukuyoa* spp. in habitats that would not usually be suitable.

3. Methods to detect and monitor *Gambierdiscus* and *Fukuyoa* species

The detection and monitoring of *Gambierdiscus* and *Fukuyoa* spp. are increasingly vital, given their expanding geographical range and the challenges associated with diagnosing ciguatera poisoning in both patients and seafood. This section of the review aims to explore methods employed for the direct identification and detection of these dinoflagellates, as well as techniques utilised for their ongoing monitoring.

Effective management and mitigation strategies necessitate a comprehensive understanding of the distribution and abundance of these BTDs. Current methodologies encompass a spectrum from traditional or conventional approaches like microscopy and cell counting to advanced techniques such as genetic sequencing. Moreover, some studies utilise a combination of these methods to enhance accuracy and reliability. This section will provide an overview of each detection and monitoring method, discussing their respective advantages and disadvantages (Table 1), offering insights into the diverse tools available for advancing our understanding of *Gambierdiscus* and *Fukuyoa* dynamics. It is noteworthy that, although the detection of toxins is crucial in CP research, this review will not cover methods specific to CTX in detail but provide an overview of some CTX detection methods.

3.1. Field sampling strategies

Effective field sampling strategies are crucial for studying benthic toxic dinoflagellates (BTDs) such as *Gambierdiscus* and *Fukuyoa*. Sampling methods must consider the habitats, distribution, seasonal dynamics, and downstream analysis of these dinoflagellates to ensure accurate and representative data collection.

Substrate sampling involves collecting macroalgae, coral rubble, and other materials from various depths and locations within a reef (Funaki et al., 2022; Rains and Parsons, 2015; Richlen et al., 2024; Villareal and Morton, 2002). Techniques like scraping and brushing directly collect benthic dinoflagellates attached to surfaces, providing a direct assessment of populations on natural substrates (Monserrat et al., 2024; Moshynets et al., 2022). Quadrat sampling standardises the area sampled, making data comparable across sites and times (Monserrat et al., 2024), though both methods can be labour-intensive and potentially disturb the habitat.

Artificial substrates, such as glass slides, ceramic tiles, or rope, offer a non-destructive alternative. These substrates are deployed in the field to attract and collect benthic dinoflagellates over a specified period. Artificial substrates allow for controlled exposure periods and minimise habitat disturbance, but they may not fully represent natural colonisation patterns (Parsons et al., 2024; Tester et al., 2022). Tester et al. (2022) compared the advantages of macrophyte and artificial substrate methods, noting that macrophytes with higher surface area per unit biomass harbour higher concentrations of *Gambierdiscus* cells. Their research suggests there is no definitive evidence that cells actively select specific macrophytes, which supports using artificial substrates for assessing cell abundances in complex habitats.

Sampling strategies must account for temporal and spatial variability in *Gambierdiscus* and *Fukuyoa* populations. Seasonal sampling during different periods can identify peak abundance and environmental triggers, and spatial coverage across various reef zones ensures a comprehensive understanding of distribution. For example, Kohli et al. (2014b) demonstrated the importance of seasonal sampling in their study on *Gambierdiscus* populations in New South Wales (NSW), Australia. They reported the seasonal abundance of *Gambierdiscus carpenteri* in temperate Merimbula and Wagonga Inlets for the first time, noting that the populations peaked between April and May 2012/13 at water temperatures of 16.5–17 °C. From June to December, these populations were either undetectable or present at low abundances. This seasonal variability highlights the need for periodic sampling to accurately assess the presence and potential risks posed by *Gambierdiscus* populations. Such comprehensive temporal and spatial sampling are crucial for understanding the conditions that lead to the proliferation of these dinoflagellates and in turn the risk of CP.

A study from St. Thomas (USVI) and the Florida Keys (USA) by

Table 1
Methods used to identify, detect, monitor, and/or quantify *Gambierdiscus* and *Fukuyoa* (referred to as G&F within the table) species. Methods are assessed qualitatively on their sensitivity, specificity, throughput, technical knowledge required, speed and overall cost (set-up and screening).

Detection method	Brief overview	Advantages	Disadvantages	Sensitivity	Specificity	Throughput	Technical knowledge	Speed	Cost
Morphological methods									
Traditional microscopy and cell counting	Visual examination of G&F under a microscope to identify morphologically differences and/or determine abundance in a sample	Provides detailed information on cell structure, assesses cell viability, estimating cell density and monitoring cell growth	Time-consuming, labour intensive and limited species identification capability	✓	✓	✓	✓✓✓	✓	✓✓
Scanning Electron Microscopy (SEM)	High-resolution imaging technique that reveals fine morphological details of G&F	Provides high-resolution images that enable precise species-level identification and detailed morphological characterisation	Expensive, time-consuming, requires specialised equipment and expertise, not practical for routine monitoring	✓	✓	✓	✓✓✓	✓	✓✓✓
Molecular methods									
Polymerase Chain Reaction (PCR)	Amplification of specific DNA sequences from G&F spp., allowing for the identification of targeted organisms	Sensitive and specific, enabling rapid and accurate detection of target species at low concentrations	Prior knowledge of the target DNA sequence required, limiting its applicability to known species and potentially missing unknown species	✓✓	✓✓	✓✓	✓✓✓	✓✓	✓✓
Quantitative PCR (qPCR)	Uses fluorescent probes or DNA-binding dyes to detect and quantify the presence of specific DNA sequences from G&F spp. in environmental samples	Highly sensitive and specific, allowing quantification of target DNA in real-time.	Susceptibility to inhibition from environmental contaminants or complex sample matrices, which can affect the accuracy and reliability of the quantification results	✓✓✓	✓✓✓	✓✓	✓✓✓	✓✓	✓✓
Digital PCR (dPCR)	Partitions the sample into thousands of individual reactions for absolute quantification of target G&F DNA	High precision and accuracy, less affected by inhibitors, useful for low-abundance targets	Higher cost, lower throughput than qPCR, requires specialised equipment	✓✓✓	✓✓✓	✓	✓✓✓	✓✓	✓✓✓
Lateral flow assay (LFA)	Also known as a rapid test or strip test, is an immunological technique used to detect G&F spp. in environmental samples by detecting specific target proteins or toxins	Rapid and user-friendly, allows for on-site detection without the need for complex laboratory equipment or extensive technical expertise	Lower sensitivity compared to other molecular techniques, which may limit its ability to detect at low concentrations	✓✓	✓✓	✓	✓	✓✓✓	✓
Fluorescence in situ hybridization (FISH)	Employs fluorescently labelled DNA probes to directly detect and visualise specific target sequences, allowing for their direct detection and visualisation	Provides direct visualisation and identification of G&F spp. at the cellular level, allowing for precise localisation and characterisation of the organisms within complex environmental samples	Requirement for prior knowledge of specific target sequences or probes, limiting its applicability to known G&F spp. and potentially missing unknown species	✓✓	✓✓	✓	✓✓	✓	✓✓✓
Metabarcoding (environmental or dietary)	Utilises high-throughput sequencing of genetic markers to detect multiple G&F spp. present in samples, enabling rapid and comprehensive	High-resolution and species-specific identification, enabling accurate and efficient monitoring of G&F spp.	Reliance on reference databases, which may be incomplete or lacking representation of all G&F spp., leading to potential misidentification or underestimation	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓

(*continued on next page*)

Table 1 (*continued*)

Detection method	Brief overview	Advantages	Disadvantages	Sensitivity	Specificity	Throughput	Technical knowledge	Speed	Cost
	assessment of diversity and relative abundance								
Transcriptomics and biomarkers	Analysis of RNA molecules from bulk RNA cell populations or in a specific cell. Can be utilised to detect and analyse gene expression patterns	Allows identification of specific genes associated with environmental response and toxin production. Can enable early detection and prediction of areas associated with CP	Requires a reference transcriptome or genome for accurate functional annotation; while not necessary for sequencing, the lack of pre-existing data may limit assigning functions to detected genes	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓
Biosensors	Analytical devices that incorporate biological elements, such as antibodies or nucleic acid probes to selectively recognise and bind to specific target species, generating a measurable signal indicating their presence	Provides rapid, specific, and sensitive detection of target G&F spp.	Need for specific recognition elements or antibodies, which may limit their applicability to known species. Only possible to process one sample at a time.	✓✓	✓✓	✓	✓✓	✓✓✓	✓✓
CRISPR-Cas9/12/13*	Can target specific genetic sequences and provide a rapid and precise means of identifying and characterising dinoflagellate species or genetic markers	High specificity, allowing for precise identification of target genetic sequences	Technology in its infancy and requires validation tests. Not yet used to detect G&F	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓
Other methods									
Flow cytometry	Uses laser-based technology to count, sort, and analyse microscopic particles such as G&F cells by suspending them in a stream of fluid and passing them through a detection apparatus	Allows for rapid, high-throughput analysis of G&F at a single-cell level, can process large volumes	Requires expensive equipment and trained personnel. May require specific dyes or markers for different G&F species	✓✓✓	✓✓	✓✓	✓✓✓	✓	✓
Microfluidic-based technologies	Utilises micro-scale fluidic channels to manipulate and analyse small volumes of fluids, allowing for the integration of multiple laboratory functions on a single chip	High sensitivity, reduced sample and reagent volumes, potential for high-throughput screening of G&F, portable and compact	Development and fabrication of microfluidic devices can be complex and costly. Requires specific equipment and trained personnel	✓✓✓	✓✓✓	✓✓✓	✓✓	✓✓	✓✓
Modelling and forecasting									
Machine learning	The development and application of computational models and algorithms that can analyse large datasets, identify patterns, and learn from data to accurately classify and predict the occurrence of HAB events	The ability to process large volumes of data and identify complex patterns, enabling more accurate and timely detection, prediction, and monitoring of HAB events	The requirement for extensive training data and the potential for model bias or limitations	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓

* Not yet established and tested on *Gambierdiscus* and *Fukuyoa* species.

Richlen et al. (2024) identified deeper locations and specific macrophytes, particularly *Dictyota* (a genus of brown seaweed), as important factors influencing *Gambierdiscus* abundance and toxin transfer dynamics. The widespread distribution of *Dictyota* and its colonisation by *Gambierdiscus silvae* highlight the role of macrophytes as critical habitats and potential vectors for toxin accumulation. This underscores the significance of including detailed field sampling strategies that account for habitat preferences, depth gradients, and substrate interactions when studying *Gambierdiscus* spp. Such strategies not only enhance our understanding of dinoflagellate ecology but also provide essential data for assessing CP risk and implementing effective management and mitigation strategies in affected regions.

Integrating field sampling strategies with advanced methods such as molecular techniques, immunological assays, or toxin analysis is essential for comprehensive study. For instance, samples intended for DNA analysis should be preserved to maintain the integrity of genetic material, while those for toxin analysis may require different preservation methods. Given the epiphytic nature of *Gambierdiscus* and *Fukuyoa* spp., a common field sampling approach involves collecting macro-algae and then shaking off or sieving the water to isolate the cells (Funaki et al., 2022; Parsons et al., 2024; Smith et al., 2017). This method helps avoid collecting larger organisms, making it easier to isolate *Gambierdiscus* and *Fukuyoa* cells for morphological analysis. It also facilitates easier extraction protocols with less interference from larger organisms in molecular approaches.

In conclusion, selecting the appropriate field sampling strategy for *Gambierdiscus* and *Fukuyoa* spp. depends on specific research goals, environmental context, and downstream analysis methods is a crucial element. Often, a combination of techniques is necessary to capture the full complexity of these dinoflagellate populations and their dynamics.

3.2. Morphological methods

Morphological methods for the detection and monitoring of dinoflagellates have played a crucial role in the identification and monitoring of these microscopic organisms. These methods encompass conventional techniques such as microscopy and cell counting. There have been great advancements in technology for morphological analysis, such as scanning electron microscopy (SEM), enabling high-resolution imaging capabilities, which are essential for precise species-level identification. Although often used in combination, each approach possesses distinct methodologies and serves unique purposes. By employing these methods, scientists have been able to successfully identify dinoflagellates, track their population dynamics, and gain insights into their ecological significance.

3.2.1. Microscopy and cell counting

Microscopy allows visualisation of the structure, morphology, and behaviour of *Gambierdiscus* and *Fukuyoa* spp. at a cellular or subcellular level (Arteaga-Sogamoso et al., 2021; Chinain et al., 1999). Cells of these species range from 20 to 100 µm (µm) in diameter (Arteaga-Sogamoso et al., 2021; Bravo et al., 2014), and their identification has traditionally relied on microscopic examination to identify taxa. This technique remains valuable, albeit dependent on skilled taxonomists. SEM can reveal fine morphological details that are not visible with light microscopy. Recent developments in electron microscopy have provided higher resolution for characterising morphological attributes, facilitating differentiation through detailed observation (Pitz et al., 2021). For example, Nascimento et al. (2015) used electron microscopy to describe the sulcal region of *Gambierdiscus excentricus*, a crucial morphological feature for taxonomic identification.

Cell counting is a quantitative technique used to determine the number of cells present in a sample. It involves identifying and counting individual cells under a microscope or using automated cell counting devices such as coulter counters or flow cytometers (Fischer et al., 2020; Gibson and Thomas, 1995). This method helps assess cell viability, estimate cell density, monitor cell growth, and analyse population dynamics. Traditional methods for collecting benthic toxic dinoflagellate (BTD) cells, such as using macrophyte hosts, have been criticised for introducing biases. Tester et al. (2014) demonstrated that using methods such as deploying artificial substrates could standardise cell collection, reducing such biases.

However, these morphological methods have limitations. They can be time-consuming, require expensive setup costs, and depend heavily on the interpretation of morphological features. Some species of *Gambierdiscus* and *Fukuyoa* exhibit no detectable morphological differences, making it challenging to distinguish closely related species (Holmes et al., 1991; Sparrow et al., 2017; Tester et al., 2022). Additionally, cell counting methods may struggle to detect species present in low numbers, and manual counting can introduce human error or observer bias.

3.3. Molecular methods

The development of highly sensitive and high-throughput molecular detection methods is becoming extensively used, particularly in ecological management of harmful algal species (Ginés et al., 2021; Sullivan et al., 2019). Molecular techniques, such as metabarcoding through high-throughput sequencing, targeted qPCR, the utilisation of entire RNA molecules through transcriptomics, and the use of enzymes and biomarkers, have emerged as valuable tools for the identification and monitoring of HABs within the environment (Durán-Vinet et al., 2021; Jacobs-Palmer et al., 2021; Langlois et al., 2021; Murray et al., 2020; Wu et al., 2020).

3.3.1. Genes utilised for Gambierdiscus and Fukuyoa species detection

Prior to employing molecular approaches for taxa identification, it is essential to comprehend and select the most suitable gene and region tailored to the specific taxa under investigation. The utilisation of nuclear ribosomal genes such as the small subunit of the ribosomal RNA gene (18S rRNA), large subunit rRNA (28S rRNA), the internal transcribed spacer (ITS) region and the 5.8S rRNA genes are used most frequently in studies that monitor *Gambierdiscus* and *Fukuyoa* species. Each gene region offers advantages and limitations, and the choice of target gene can be dependent on the research objectives.

18S rRNA

The 18S rRNA gene is well-characterised and contains nine highly variable regions; V1 to V9 (Ki, 2012). Although this gene is occurring in every living cell, there are some distinct differences within its sequence between eukaryotic and prokaryotic organisms. For example, there is an absence of V6 within eukaryotes and higher variability within V4 and V9 regions for eukaryotes (Hadziavdic et al., 2014; Ki, 2012). To reveal the distribution of BTDs such as *Gambierdiscus* and *Fukuyoa* spp. the 18S rRNA V4 region has often been used (Funaki et al., 2022; Ki, 2012). Although, Kohli et al. (2014b) found low read abundance obtained for BTDs due to the universality of the primer. Funaki et al. (2022) attempted to design a primer pair more specific to *Gambierdiscus* species, targeting the V8 and V9 regions, which was successful in identifying multiple *Gambierdiscus* species, but still only represented a small number of the total read count.

There have been a significant number of studies using different regions of the 18S rRNA gene for taxonomic, phylogenetic, and single species PCR detection of *Gambierdiscus* and *Fukuyoa* species (Funaki et al., 2022; Kohli et al., 2014b; Kretzschmar et al., 2017). The V4 and V9 regions of the 18S gene are more suitable compared to other regions for achieving species-level identification, assessing genetic variations within a species, and are recommended as prime candidates for *Gambierdiscus* and *Fukuyoa* spp. assays. These regions prove valuable in the detection of HABs communities and are well-suited for conducting phylogenetic analyses. In addition, there are many 18S sequences available on public repositories because of its popular use (Mordret et al., 2018).

28S rRNA

Another extensively characterised gene is the 28S rRNA gene. By examining specific regions within the 28S rRNA gene such as the D1 and D2 region Gaiani et al. (2022) successfully developed a range of genus (*Gambierdiscus* and *Fukuyoa*) specific and species (*G. australes* and *G. excentricus*) specific primers utilising regions of the 28S gene. They applied these primers through recombinase polymerase amplification with detection via hybridization, which doesn't require an initial denaturation step and can be completed within 15–30 min. This provided a rapid detection method which could be useful/applied for early warning systems for CP.

The 28S gene has been successfully utilised for species-specific primers. However, when developing universal primers to detect *Gambierdiscus* and *Fukuyoa* spp. communities, like the 18S gene, the challenge lies in ensuring that the chosen region possesses enough variability to accurately distinguish between species while remaining selective for the *Gambierdiscus* and *Fukuyoa* genera (Edvardsen et al., 2003). Thus, combining the 28S gene with other genetic markers such as 18S can provide a more comprehensive picture of *Gambierdiscus* species composition and in determining unique species. This was shown by Kretzschmar et al. (2017) on clonal cultures of *Gambierdiscus* collected off Heron Island, Australia. DNA sequencing based on both 18S and 28S regions elucidated variants in the phylogenetic relationships of *Gambierdiscus* spp., leading to the identification of a novel species within a distinct clade, now recognised as *Gambierdiscus lapillus*. This demonstrates the advantage of using multiple markers that utilise different genes to gain a better representation of both species detection and determining differences between closely related taxa.

5.8S rRNA and the Internal Transcribed Spacer (ITS) regions

The 5.8S ribosomal RNA gene (rRNA) and the adjoining internal transcribed spacer (ITS1 and ITS2) reside between the 18S and 28 s gene within the eukaryotic genome. These three regions evolve more rapidly than both the 18S and 28S genes, meaning these regions exhibit greater genetic diversity and can be useful for the development of species-specific primers in dinoflagellates (Yoshida et al., 2003). Species-specific primers using these regions have been developed to target seven *Gambierdiscus* and *Fukuyoa* species (Sup. Table S8), but the region has not been targeted for the development of universal primers due to lack of conserved regions.

3.3.2. Environmental DNA methods

Environmental DNA (eDNA) is intracellular and/or extracellular DNA (i.e., urine, mucus, faeces, gametes, cells) that is released into the environment (i.e., water, soil, air) by an organism, that can be extracted and analysed (Harper et al., 2018). When combined with certain molecular techniques, such as sequencing, PCR, and quantitative PCR (qPCR), it provides a non-invasive, highly sensitive, rapid approach, that has revolutionised the detection and monitoring of species (Bruce et al., 2022; Deiner et al., 2020). When using such approaches for BTDs such as *Gambierdiscus* and *Fukuyoa* spp., the aim is to directly collect these microorganisms from environmental samples and, as they are usually attached to their macroalgal hosts, the collection of the algae as the sampling substrate is frequently used (Funaki et al., 2022; Smith et al., 2017a). After the collection of environmental samples, certain molecular techniques (i.e., PCR, quantitative PCR, next generation sequencing, biosensors) are applied to detect traces of organisms or the organisms themselves (Thalinger et al., 2021; Yates et al., 2021). Both non-specific or community-based approaches and single species or targeted approaches exist for *Gambierdiscus* and *Fukuyoa* spp. When developing assays to target either *Gambierdiscus* and *Fukuyoa* spp. communities or a specific species it is important to investigate which genes are most suitable to be utilised in assay development for a given study.

3.3.3. Metabarcoding

DNA metabarcoding (or just metabarcoding) is the technique used when applying universal primers (Sup. Table S7) with individual DNA barcodes to identify multiple organisms, enabling bulk identification of whole communities within environmental or dietary (guts content/faecal) samples (Steyaert et al., 2020). Subsequently, these samples are sequenced and identified using high-throughput sequencing (HTS) technologies, allowing for huge amounts of data output (Smith et al., 2017a). The sequenced data can be analysed using reference databases, enabling taxonomic assignments, and allowing specific taxa detection, diversity estimates, community structures and abundance estimates (Smith et al., 2017a; Steyaert et al., 2020). Metabarcoding is now a popular and well-established method and has been shown to outperform traditional methods of assessing levels of diversity (Keck et al., 2022; Steyaert et al., 2020).

DNA metabarcoding has the advantage of being able to uncover taxa that are not known or are unexpected to be in an area and can reveal community structures and interspecific/intraspecific interactions (Bruce et al., 2021; Jacobs-Palmer et al., 2021). This was highlighted in a study by Jacobs-Palmer et al. (2021) within the Hood Canal in Washington, USA, that spanned over 19 months. The study used eDNA metabarcoding on water samples to discover new and cryptic species of harmful algae related taxa, including a cold-water variant from the genus *Alexandrium* which produces highly potent neurotoxins (saxitoxins) and is associated with paralytic shellfish poisoning (PSP).

Applying metabarcoding to dietary samples of potential toxin vectors is crucial in understanding trophic transfer of CP (Kobari et al., 2021). Homma et al. (2022) observed the diets of two herbivorous parrotfishes the blue humphead parrotfish *Scarus ovifrons* and the Japanese parrotfish *Calotomus japonicus* that inhabit the same reefs around Japan using metabarcoding. *Scarus ovifrons* is known to cause severe food poisoning where *Calotomus japonicus* is not. Dietary metabarcoding revealed *Gambierdiscus* reads were found in high abundance in some *S. ovirfrons* diets, where they were rarely detected in *C. japonicus*. In Japan *Gambierdiscus* spp. have been reported to attach abundantly on red algae, which is a preferred diet of *S. ovifrons*. This study highlights the importance of using dietary metabarcoding to identify which species are at risk of accumulating harmful CTXs. DNA metabarcoding offers many advantages but also faces several challenges and limitations. One key issue is the universality of eukaryotic primers, which can result in few sequences and low read counts for *Gambierdiscus* and *Fukuyoa* species compared to other taxa in environmental samples (Funaki et al., 2022; Kohli et al., 2014b). Since these species don't need to bloom to be toxic (Kohli et al., 2015; Wang et al., 2022), detecting them when they are in lower abundance is crucial. Increasing sequencing depth can enhance detection but can be costly and may increase data on non-target taxa (Zhan and MacIsaac, 2015). Additionally, applying metabarcoding to complex samples like gut or faecal matter presents challenges such as DNA degradation, PCR inhibitors, and host DNA amplification (De Sousa et al., 2019; Devloo-Delva et al., 2018). While advances in blocking primers have improved target group amplification, difficulties persist, particularly in aquatic environments where non-invasive sample collection is often unfeasible (Homma et al., 2022; De Sousa et al., 2019). Careful primer selection and validation are essential to overcoming these technical obstacles and improving metabarcoding accuracy.

Moreover, it has been demonstrated that sampling strategies significantly impact the efficacy of metabarcoding. For instance, research from the Balearic Islands by Antich et al. (2021) highlighted the benefit of directly sampling from benthic substrates, which provided a more accurate representation of benthic community composition. Optimal DNA yields from benthic environments, particularly BTDs such as *Gambierdiscus* and *Fukuyoa* spp., are often obtained by directly sampling algae from the benthos, shaking to dislodge dinoflagellates, and then adding them to preservatives or directly filtering before DNA extraction (Funaki et al., 2022; Smith et al., 2017). This approach contrasts with common water eDNA samples, which may not effectively monitor complex benthic communities.

In summary, while metabarcoding is a powerful tool for assessing biodiversity and detecting *Gambierdiscus* and *Fukuyoa* spp., it is not without limitations. The choice of primers, sampling strategies, and sequencing depth are critical factors that influence the effectiveness of this method. Therefore, a combination of broad metabarcoding approaches followed by targeted methods can offer a more comprehensive understanding and accurate detection of *Gambierdiscus* and *Fukuyoa* species.

3.3.4. Targeted primer approaches

A targeted approach typically uses species-specific primers with conventional PCR, quantitative PCR (qPCR) or droplet digital PCR (ddPCR) to assess presence-absence and/or abundance of a single species (Harper et al., 2018; Langlois et al., 2021; Vandersea et al., 2012). Species-specific primers and qPCR approaches are the gold standard and are more sensitive when detecting a single species (Bruce et al., 2021; Thalinger et al., 2021). There is currently one developed assay to identify the overall presence of *Gambierdiscus* and *Fukuyoa* genera using qPCR (Smith et al., 2017), although it is not possible to differentiate between the species, which is important due to differences species toxicity. There are also qPCR assays for 18 *Gambierdiscus/Fukuyoa* species/types/ribotypes (Sup. Table S8).

An illustrative case is that of *Gambierdiscus lapillus*, first described by Kretzschmar et al. (2019) off Heron Island in the Great Barrier Reef, Australia. Genetically it is closely related to *G. balechii, G. belizeanus, G. pacificus, G. scabrosus* and *G. toxicus,* although when tested for CTXs it possessed a different CTX peak that did not match any of the available standards. Thus, it was important to establish its distribution around Heron Island using a qPCR assay that was developed within this study. The assay provided high sensitivity detecting as low as 0.05 cells and establishing the known presence of *G. lapillus* around the island. Such findings not only lay the groundwork for further investigation but also offer insights crucial for the establishment of effective monitoring programs.

Another study by Smith et al. (2017) developed a genus-level qPCR assay to detect all species from both genera, facilitating rapid screening of environmental samples for potentially toxic species. The assays were used to analyse 31 samples from New Zealand and the Kingdom of Tonga. Fourteen samples were positive for *Gambierdiscus/Fukuyoa*. High-throughput sequencing (HTS) of these samples identified *Gambierdiscus* species, including the first confirmed identification of *G. polynesiensis*, a known ciguatoxin producer, in New Zealand waters. This discovery underscores the importance of targeted primer approaches in monitoring and managing the risks associated with these dinoflagellates.

The study's qPCR assays demonstrated high specificity and sensitivity, enabling rapid determination of *Gambierdiscus/Fukuyoa* presence in environmental samples and facilitating targeted characterization. The HTS metabarcoding approach further enhanced species-level identification, although its current cost and turnaround times limit routine use. Nevertheless, the genus-level qPCR assay remains a valuable tool for monitoring programs and taxonomic surveys worldwide, allowing for rapid identification of potentially risky sites and prioritisation for more detailed investigations.

Emerging digital PCR (dPCR) methods, such as droplet digital PCR (ddPCR), offer promising alternatives. A study by Yarimizu et al. (2020), demonstrated the efficiency of dPCR in accurately quantifying ribosomal RNA (rRNA) gene copy numbers across a range of phytoplankton species, including *Gambierdiscus* sp. By partitioning samples into thousands of droplets or reaction chambers, dPCR provides absolute quantification without the need for reference standards and is less susceptible to inhibition by environmental contaminants compared to qPCR. Given its enhanced sensitivity and precision, dPCR represents a valuable addition to current monitoring strategies for *Gambierdiscus* and *Fukuyoa* species, offering the potential for more accurate and reliable detection and quantification in environmental samples.

3.3.5. Lateral flow assay (LFA)

A nucleic acid lateral flow assay (LFA) in a dipstick format was developed for the duplex detection of *G. australes* and *G. excentricus*, two species that have shown CTX-like toxicities and could be associated with CP (Ginés et al., 2021). This innovative method exhibited high sensitivity and specificity, effectively distinguishing between the targeted species while avoiding cross-reactivity with closely related *Gambierdiscus* species (Ginés et al., 2021). This research was conducted in a controlled laboratory, and although it showed promising results, further validation in the field on environmental samples must be applied to ensure the approach is successful and able to advise future monitoring programs.

3.3.6. Fluorescence in situ hybridization (FISH)

Fluorescence in situ Hybridization (FISH) is a molecular technique that utilises fluorescently labelled probes to bind to specific nucleic acid sequences within intact cells, enabling the visualisation and identification of target organisms or genetic material under a fluorescence microscope (Medlin and Orozco, 2017; Pitz et al., 2021). FISH has emerged as a powerful tool for the specific detection and monitoring of HAB species such as *Gambierdiscus* and *Fukuyoa.* Pitz et al. (2021) designed FISH probes targeting seven *Gambierdiscus* species, including two known toxin producing species: *G. australes* and *G. excentricus*, prevalent in the Caribbean Sea and Pacific Ocean. These probes facilitated the detection and quantification of *Gambierdiscus* cells in field samples through epifluorescence microscopy, allowing for the accurate assessment of community composition and abundance. The study successfully applied the FISH technique to field samples from the Florida Keys and Hawaii, USA, revealing co-occurrence patterns and quantifying seasonal shifts in community composition. The FISH approach not only provided a means to distinguish individual species but also enabled the tracking of species dynamics over time, linking them to environmental parameters. However, despite its advantages, FISH does have limitations. While it allows for species-specific detection and monitoring, it may be constrained by the need for species-specific probes, and challenges associated with environmental samples, including potential interference from detritus, enzymes, and other organisms. Despite these limitations, the application of FISH in detecting and characterising *Gambierdiscus* and *Fukuyoa* spp. holds immense promise for advancing our understanding of CP risks and facilitating effective environmental management (Cuadrado et al., 2022; Medlin and Orozco, 2017; Pitz et al., 2021).

Most molecular methods rely on nucleic acid detection, which usually involves filtration, centrifuge, and DNA extraction and can be time consuming. Portable methods that don't rely on time consuming laboratory processes are still in their infancy with sensitivity levels an issue (Ginés et al., 2021). Methods such as PCR, qPCR and dPCR are widely used in molecular-detection of specific-taxa (Ginés et al., 2021; Bruce et al., 2021) but portable versions of such methods would allow real-time application that could be invaluable to prevent CP in the future.

3.3.7. Transcriptomics and biomarkers

Transcriptomics, initially used to identify genes associated with toxin synthesis in HAB species, has evolved into a powerful tool for biomarker development. This approach is crucial for taxa with complex genomes lacking full sequencing (Wang et al., 2021). HAB species, characterised by large genomes and diverse functionalities, have been extensively studied using transcriptomes, notably through projects like the Marine Microbial Eukaryote Transcriptome Sequencing and Tara Ocean projects (Keeling et al., 2014; Tara Ocean Project, 2022).

Many transcriptome studies explore how HAB species adapt to nutrient changes, critical in the context of global eutrophication (Gobler, 2020; Wang et al., 2021). Gene expression studies have identified biomarkers associated with cell growth, energy production, and toxin biosynthesis pathways, providing early warning signals for HAB formation (Gong et al., 2017).

For instance, targeting the expression of polyketide synthase (PKS) genes, which are implicated in the production of ladder-like polyether's including ciguatoxins, can provide insights into toxin production. In a study by Van Dolah et al. (2020), transcriptome analysis was used to examine the expression of PKS genes in two *Gambierdiscus* species: *G. polynesiensis* and *G. pacificus*. Although the study did not find significant differences in PKS expression between the toxic and non-toxic species, it highlighted the potential for using targeted gene expression studies to understand toxin biosynthesis pathways better.

However, the complexity and size of the genomes of *Gambierdiscus* and *Fukuyoa* species pose a significant challenge (Kohli et al., 2015), hindering the complete sequencing and accurate assembly of their transcriptomes. This makes it difficult to identify and annotate all relevant genes involved in toxin production and other critical metabolic pathways. This approach emphasises the need for more focused research on the biosynthetic machinery responsible for toxin production in

Gambierdiscus and *Fukuyoa* dinoflagellates.

3.3.8. Biosensors

Biosensors use biological molecules such as nucleic acids, enzymes and antibodies that combine with a signal transducer to detect specific targets (Chin Chwan Chuong et al., 2022). Among various biosensors, electrochemical and optical sensors are the most widely used for HAB species detection due to being highly sensitive, easy to use and because they offer real-time and label free detections (Chin Chwan Chuong et al., 2022; McPartlin et al., 2017).

Gaiani et al. (2022) provided a significant advancement in this area by developing a multiplex detection system for the toxin-producing species *Gambierdiscus australes* and *Gambierdiscus excentricus*. This system used species-specific PCR primers modified with short oligonucleotide tails and capture probes immobilised on maleimide-coated magnetic beads (MBs). This approach allowed simultaneous detection on a dual electrode array and significantly improving the detection sensitivity to as low as 10 cells for both species. Furthermore, a rapid DNA extraction technique using a portable bead beater system and MBs reduced the extraction time from several hours to just 30 min. This innovative method was not only efficient but also facilitated the first detection of *G. excentricus* in the Balearic Islands, highlighting its practical application in field conditions.

Additionally, biosensors have been developed to detect CTXs in fish. A highly sensitive tool for detecting low levels of CTXs in fish samples was demonstrated by Leonardo et al. (2020) through the development of an electrochemical immunosensor that facilitated the detection of CTXs. Such approach provides a rapid and cost-effective strategy for ciguatera risk assessment, aiming to ensure seafood safety. However, despite these advancements, the high specificity and sensitivity of these biosensor systems necessitate continuous optimisation and validation to address various environmental and sample matrix challenges. As technology advances, it is anticipated that these limitations will diminish, making biosensors an increasingly vital tool in monitoring and managing CP.

3.3.9. CRISPR based methods

A promising development for DNA based biosensing is clustered regularly interspaced short palindromic repeats and Cas proteins (CRISPR/Cas). CRISPR-Cas is a powerful gene editing tool and recent CRISPR-Cas technologies have provided facile molecular mechanisms for editing cells and has been exploited to manipulate genomes in animals, plants, and microorganisms. The first application of CRISPR-Cas to detect species from environmental samples came from a study by, in detecting Atlantic salmon *Salmo salar* in Ireland. This method used Cas12a, which is an RNA-guided endonucleases to develop highly specific and sensitive molecular nucleic acid diagnostic platforms. They were able to show detection at a single temperature of 37 °C, non-detection of closely related species, and rapid detection rates (<2.5 hr). The next step is field validation to determine if this could be used an effective tool for monitoring.

A review conducted by Durán-Vinet et al. (2021) examined the application of CRISPR/Cas technology in monitoring harmful algal blooms. The review emphasises that although CRISPR methods are still in their early stages of development for biomonitoring species, initial research has showcased their potential. The use of CRISPR platforms such as Cas9, Cas12, and Cas13, can serve as target-activated fluorescence indicators capable of detecting extremely low levels of concentration. This unique characteristic enables their testing under relaxed conditions, even in the presence of inhibitors that might hinder other molecular methods like qPCR. Ultimately, CRISPR-based approaches may offer an alternative detection method that is portable, specific, cost-effective, and capable of providing rapid, real-time results.

Currently, there are no examples of CRISPR/Cas applications specifically for *Gambierdiscus* or *Fukuyoa*. However, given the promising nature of CRISPR/Cas technology, we believe it holds potential as a future direction for detecting these species.

3.4. Other areas of research for detection and monitoring of Gambierdiscus and Fukuyoa species

3.4.1. Flow cytometry

Flow cytometry is a powerful technique for the detection and enumeration of HABs in environmental samples. This method involves passing cells through a laser beam and detecting the light scattering and fluorescence signals emitted by each cell. These signals provide information on cell size, complexity, and the presence of specific fluorescent markers (Dashkova et al., 2017; McKinnon, 2018). Flow cytometry offers several advantages, including rapid analysis and the ability to process thousands of cells per second, making it highly efficient for monitoring programs. The incorporation of fluorescently labelled antibodies or dyes that bind specifically to target dinoflagellates enhances the method's specificity and sensitivity. This allows for the discrimination of *Gambierdiscus* and *Fukuyoa* species from other phytoplankton and debris in mixed samples (Bravo et al., 2014; Dashkova et al., 2017; McKinnon, 2018).

Studies have demonstrated the utility of flow cytometry in monitoring *Gambierdiscus* populations. Bravo et al. (2014) utilised flow cytometry to observe the morphological changes in *Gambierdiscus* nuclei during cell division, revealing insights into their cell cycle phases. This study highlighted the capability of flow cytometry to distinguish between different stages of cell division based on nuclear morphology, which can be crucial for understanding population dynamics and growth rates.

Wang et al. (2018) further illustrated the application of flow cytometry in co-culture experiments with *Gambierdiscus* spp. and quorum-sensing (QS) bacteria. By using flow cytometry to determine bacterial densities, the study investigated the impact of QS bacteria on algal proliferation and toxin production. The findings indicated that certain QS bacteria significantly influenced the growth and toxicity of *Gambierdiscus*, underscoring the method's potential for exploring interactions between dinoflagellates, their associated microbiota and the potential use of using the detection of QS bacteria as a monitoring system for CP.

However, flow cytometry requires sophisticated instrumentation and expertise, which may limit its accessibility in some regions. Sample preparation and the choice of appropriate fluorescent markers are crucial for obtaining accurate and reproducible results. Despite these challenges, flow cytometry's ability to provide detailed, quantitative data on dinoflagellate populations makes it a valuable tool in the study and monitoring of CP.

3.4.2. Microfluidic-based technologies

Microfluidic-based technologies represent a cutting-edge approach for the detection and monitoring of HABs such as *Gambierdiscus* and *Fukuyoa species*. These technologies involve the manipulation of small volumes of fluids in microscale channels, allowing for the miniaturisation and automation of laboratory processes. Microfluidic devices can integrate various detection methods, including optical, electrochemical, and immunological assays, into a single platform (Maguire et al., 2018). Recent advances in microfluidic technology have enabled the development of highly sensitive and rapid assays for the detection of cells and various toxins produced by HABs, such as ciguatoxins, saxitoxins and brevetoxins (Weng and Neethirajan, 2018; Zhang et al., 2015).

Microfluidic systems are being developed for the quantification of HAB cells by integrating processes like cell concentration, lysis, RNA extraction, and quantitative RNA detection within a single platform. For example, a portable microfluidic system has been designed for the detection of the toxic dinoflagellate *Karenia brevis* cells, utilising on-chip RNA amplification and real-time fluorescence detection, which demonstrates the technology's potential for in situ cell quantification (Tsaloglou et al., 2013).

While microfluidic detection methods show great promise for both toxin and cell detection, it also has some challenges that need to be addressed, such as the need for standardisation and optimisation of assays (Reverté et al., 2016). Overall, microfluidic detection methods offer an effective tool for the detection and monitoring of HABs such as *Gambierdiscus* and *Fukuyoa*, and their further development and application should be pursued.

3.4.3. Modelling and forecasting

Statistical models, such as regression analysis, can identify relationships between environmental variables and CP occurrences. Ecological niche modelling helps predict the potential distribution of toxic dinoflagellates based on environmental conditions. Bayesian networks can integrate various data types and quantify the uncertainty in predictions, providing a probabilistic framework for CP risk assessment (Ralston and Moore, 2019).

One study by Zheng et al. (2020) utilised cross-correlation analysis and auto-regressive integrated moving-average (ARIMA) modelling to develop predictive models of monthly CP incidence in Cook Islands and French Polynesia, using the latest epidemiological data. The results revealed significant time-lagged associations between monthly CP incidence rates and several indicators related to sea surface temperature (SST). Specifically, SST was found to be a strong positive predictor of increased ciguatera incidence for both countries. If these time-lags are further validated, health authorities could take pre-emptive actions to limit or avoid epidemic risks, especially under high-risk climate scenarios. However, the reliance on SST as a predictor may oversimplify the complex ecological interactions involved, and more comprehensive models incorporating multiple environmental factors are necessary for robust predictions.

It is important to consider the effectiveness of such models depends on the availability and quality of data, highlighting the need for continuous monitoring and data collection efforts. Additionally, integrating diverse datasets and addressing potential biases in data sources are critical for developing robust and transferable predictive models. Ralston and Moore (2019) provide a detailed review on modelling HABs in a changing climate that explores different modelling and forecasting techniques for HABs including *Gambierdiscus* spp.

3.4.4. Machine learning

Forecasting HABs and predicting areas vulnerable to future habitat shifts of HAB species is a crucial and important step in preventing certain HAB related syndromes (Wen et al., 2022). Machine learning enables the development of statistical algorithms that can learn from environmental parameters, along with water quality data and historical observations of HABS to help predict areas with a high likelihood of future harmful algal bloom events. This, in turn, assists in making informed decisions regarding seafood harvesting and aquaculture practices in a particular location (Cruz et al., 2021). It has been demonstrated that this approach is effective in managing shellfish-related syndromes (Cruz et al., 2021; Wen et al., 2022), which is partly attributed to the fact that the contaminated species are sedentary.

However, CP presents a more complex challenge since fish can transport CTXs to different locations and the responsible dinoflagellates do not need to bloom to become toxic (Farrell et al., 2017; Kohli et al., 2015). Consequently, the data used to predict CP incidences must consider both the factors that facilitate the presence and growth of toxic dinoflagellates and life history traits of the vectors responsible for carrying the toxin. Therefore, there is a need for further research on CTXs, their sources, and the vectoral nature of the toxin to fully make the most of such methods.

4. Overview of detection methods for Ciguatoxins (CTXs)

While this review primarily focuses on the detection and monitoring of *Gambierdiscus* and *Fukuyoa* species, it is important to briefly summarise the methods used for detecting ciguatoxins. It is worth noting that many of these methods overlap in their approach and application.

4.1. Biological assays and immunological assays

Biological assays, such as the mouse bioassay (MBA) and cell-based assays such as neuroblastoma (N2a), are traditional methods for detecting CTXs. The MBA involves administering extracts of suspected toxic material to mice and observing for symptoms of toxicity. While effective, the MBA raises ethical concerns and lacks specificity and sensitivity (Farrell et al., 2017; Pasinszki et al., 2020). Cell-based assays, which use cultured cells to detect the presence of CTXs by their cytotoxic effects, offer an alternative that is more humane and can be more specific and sensitive. Limitations are that cell-based assays such as N2A only provide CTX like activity and need analytical methods to confirm toxins (Pasinszki et al., 2020).

Immunological assays, such as enzyme-linked immunosorbent assays (ELISA), use antibodies specific to CTXs to detect and quantify these toxins. These assays balance sensitivity, specificity, and practicality, making them suitable for routine monitoring. However, developing high-affinity antibodies is challenging, and cross-reactivity can affect accuracy (Pasinszki et al., 2020).

Tsumuraya et al. (2018) developed highly sensitive fluorescent sandwich ELISAs using monoclonal antibodies (mAbs) that detect four major CTX congeners (CTX1B, 54-deoxyCTX1B, CTX3C, and 51-hydroxyCTX3C) with a detection limit of <1 pg/mL. These ELISAs are sensitive enough to detect CTX1B-contaminated fish at the FDA guidance level of 0.01 ppb and evaluate extraction efficiency and matrix effects, showing great promise as a valuable CTX detection tool.

4.2. Analytical chemistry methods

Analytical chemistry methods, including liquid chromatography-mass spectrometry (LC-MS) and high-performance liquid chromatography (HPLC), are widely regarded as the gold standard for CTX detection (Gika et al., 2014; Caillaud et al., 2010; Pasinszki et al., 2020). These methods provide high sensitivity and specificity, allowing for the precise quantification and identification of different CTX congeners. However, these techniques require sophisticated instrumentation and expertise, limiting their accessibility in some regions (Loeffler et al., 2021; Pasinszki et al., 2020). Though in recent years, there have been significant advancements in MS technology, with portable instruments being developed and tested for on-site rapid detection of CTXs. These advancements could be a breakthrough in the future of CTX detection, as they have the potential to provide faster, more convenient, and cost-effective analysis of CTXs. Studies by Jager et al. (2020) and Mielczarek et al. (2020) have shown promising results in the development of portable MS instruments for the detection of CTXs. Until now, the integration of the liquid chromatography (LC) interface into a portable device has not been achievable. As a result, portable instruments lack the same level of sensitivity as laboratory-based LC-MS methods.

4.3. Functional assays

Functional assays, such as the receptor binding assay (RBA), measure the ability of CTXs to bind to specific receptors, providing an indication of their toxic potential. Rat brain synaptosomes must be isolated and incubated, which contain Na+ channels with a fixed amount of tritiated PbTx-3 ([3H]-PbTx-3). PbTxs will then present in the samples with radio-labelled [3H]-PbTx-3 on the sodium channel receptor (Hardison et al., 2016; Caillaud et al., 2010). PbTxs and CTXs bind to the same sodium cannel receptor area, meaning the PbTx assay can measure CTXs in fish tissues (Hardison et al., 2016). RBA is not as sensitive as the CBA-N2a, but it only requires three hours to complete after the extraction process compared to nearly three days of CBA-N2a (Hardison et al., 2016; Pasinszki et al., 2020).

In summary, the detection of CTXs is critical for managing the risks associated with CP. While traditional methods like biological assays provide foundational insights, modern techniques such as LC-MS, immunological assays, and functional assays offer improved sensitivity, specificity, and practicality. Each method has its own advantages and limitations, and the choice of method often depends on the specific requirements of the monitoring program, available resources, and expertise. For a more detailed discussion of these methods, readers are referred to comprehensive reviews such as Caillaud et al. (2010), Oshiro et al. (2024), and Pasinszki et al. (2020).

5. Conclusions and future recommendations

Ciguatera poisoning poses an increasing global concern, and there is a pressing need on the development of efficient techniques for detecting and monitoring the dinoflagellates responsible for producing the toxins. To advance the field of CP research several key recommendations should be prioritised.

Firstly, achieving a comprehensive understanding of the distribution and life cycles of *Gambierdiscus* and *Fukuyoa* species is essential. Research should focus on exploring their geographic range and addressing unknown aspects of their life cycles, such as if they have a resting cyst stage. This foundational knowledge is crucial for effective monitoring and management strategies.

Additionally, the impact of climate change on the distribution of these dinoflagellates must be investigated. With evidence of their emerging presence in temperate regions, understanding how climate changes may influence their distribution, toxicity and the associated risk of CP is imperative. Implementing long-term monitoring programs and employing machine learning algorithms in order to identify relationships between environmental variables and CP occurrences and help predict potential shifts in the distribution of both the dinoflagellates and ciguatoxin vectors.

The application of molecular methods, such as DNA metabarcoding, is promising for detecting *Gambierdiscus* and *Fukuyoa* communities. Although, there is a need for the development of more targeted assays, and comprehensive reference databases, to aid in accurately identifying these species. Furthermore, integrating sensitive, targeted approaches with real-time, portable detection instruments could enable early detection and prevention of CP. Notably, CRISPR-based technologies, though still in the early stages of development, offer considerable potential for portable real-time application. While there are currently no CRISPR-based applications for *Gambierdiscus* or *Fukuyoa*, the technology's potential in rapid detection and high sensitivity, even in the presence of inhibitors, makes it a promising area for future research.

Finally, advancements in transcriptomics could provide critical insights into the genetic basis of toxin production. Understanding the genes involved in toxin biosynthesis will enhance our knowledge of CP mechanisms and support the development of targeted management strategies. Integrating these approaches into a comprehensive research framework will significantly improve our ability to manage CP and ensure seafood safety in a changing environmental landscape.

CRediT authorship contribution statement

Joseph C. Perkins: Writing – review & editing, Writing – original draft, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kyall R. Zenger:** Writing – review & editing, Supervision, Funding acquisition. **Yang Liu:** Writing – review & editing, Supervision. **Jan M. Strugnell:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Acknowledgements

We would like to acknowledge the project funders, Food Agility Cooperative Research Centre, and the College of Science and Engineering within James Cook University. Additionally, we would like to thank Melissa Joyce, whose creative talent helped develop the infographics within this review. Finally, the authors would like to thank Professor Alison Robertson and her lab group at the University of South Alabama and Dauphin Island Sea Lab (USA) and Andy Selwood from the Cawthron Institute (New Zealand) for their valuable discussions and inputs on ciguatera research, providing a crucial platform for this review.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hal.2024.102735.

Data availability

No data was used for the research described in the article.

References

Abraham, A., Jester, E.L.E., Granade, H.R., Plakas, S.M., Dickey, R.W., 2012. Caribbean ciguatoxin profile in raw and cooked fish implicated in ciguatera. Food Chem. 131 (1), 192–198. https://doi.org/10.1016/j.foodchem.2011.08.059.

Adachi, R., Fukuyo, Y., 1979. 1979_Bull Jpn Soc Sci Fish_Gambierdiscus toxicus.pdf. Bull. Japanese Society of Sci. Fisheries 45 (1), 67–71.

Accoroni, S., Glibert, P.M., Pichierri, S., Romagnoli, T., Marini, M., Totti, C., 2015. A conceptual model of annual Ostreopsis cf. ovata blooms in the northern Adriatic Sea based on the synergic effects of hydrodynamics, temperature, and the N:p ratio of water column nutrients. Harmful. Algae 45, 14–25. https://doi.org/10.1016/j.hal.2015.04.002.

Agrawal, P., Grab, J., 2022. A Case of Ciguatera Fish Poisoning in South Georgia. *Cureus*. https://doi.org/10.7759/cureus.22728.

Antich, A., Palacín, C., Cebrian, E., Golo, R., Wangensteen, O.S., Turon, X., 2021. Marine biomonitoring with eDNA: can metabarcoding of water samples cut it as a tool for surveying benthic communities? Mol. Ecol. 30 (13), 3175–3188. https://doi.org/10.1111/mec.15641.

Arteaga-Sogamoso, E., Rodríguez, F., Mancera-Pineda, J.E., 2021. Morphological and molecular characterization of Gambierdiscus caribaeus (Dinophyceae), with a confirmation of its occurrence in the Colombian Caribbean Tayrona National Natural Park. Botanica Marina 64 (2), 149–159. https://doi.org/10.1515/bot-2020-0070.

Bagins, R., Chantsau, S., Chungub, E., Hurtiii, M., Yasumoto, T., & Inoue, A. (1980). Origins of ciguatera fish poisoning a new dinoflagellate, gambierdiscus toxicus adachi and fukuyo, defintive'ly involved as a causal agent (Vol. 18).

Berdalet, E.C.L.M.M., Sandra Díaz Cordoba, U., Gerhard Heldmaier Marburg, A., Robert Jackson, G.B., Lange, O.L., Mooney, H.A., Schulze Jena, E.D., & Ulrich Sommer Kiel, G. (2018). Ecological Studies Analysis and Synthesis Volume 232 Series editors. http://www.springer.com/series/86.

Bravo, I., Figueroa, R.I., Fraga, S., 2014. Cellular and nuclear morphological variability within a single species of the toxigenic dinoflagellate genus Gambierdiscus: relationship to life-cycle processes. Harmful. Algae 40, 1–8. https://doi.org/10.1016/j.hal.2014.09.009.

Bruce, K., Blackman, R.C., Bourlat, S.J., Hellström, M., Bakker, J., Bista, I., Bohmann, K., Bouchez, A., Brys, R., Clark, K., Elbrecht, V., Fazi, S., Fonseca, V.G., Hänfling, B., Leese, F., Mächler, E., Mahon, A.R., Meissner, K., Panksep, K., … Deiner, K. (2021). A practical guide to DNA-based methods for biodiversity assessment.

Bruce, K., Thailinger, B., Deiner, K., Rees, H., Sint, D., Blackman, R., Harper, L., Traugott, M., & Goldberg, C. (2022, April 19). eDNA Validation Scale. Https://Edna-Validation.Com/.

Cai, W., Borlace, S., Lengaigne, M., Van Rensch, P., Collins, M., Vecchi, G., Timmermann, A., Santoso, A., Mcphaden, M.J., Wu, L., England, M.H., Wang, G., Guilyardi, E., Jin, F.F., 2014. Increasing frequency of extreme El Niño events due to greenhouse warming. Nat. Clim. Chang. 4 (2), 111–116. https://doi.org/10.1038/nclimate2100.

Cai, W., McPhaden, M.J., Grimm, A.M., Rodrigues, R.R., Taschetto, A.S., Garreaud, R.D., Dewitte, B., Poveda, G., Ham, Y.G., Santoso, A., Ng, B., Anderson, W., Wang, G., Geng, T., Jo, H.S., Marengo, J.A., Alves, L.M., Osman, M., Li, S., Vera, C., 2020. Climate impacts of the El Niño–Southern Oscillation on South America. Nature Rev. Earth Environ. 1 (4), 215–231. https://doi.org/10.1038/s43017-020-0040-3.

Caillaud, A., de la Iglesia, P., Barber, E., Eixarch, H., Mohammad-Noor, N., Yasumoto, T., Diogène, J., 2011. Monitoring of dissolved ciguatoxin and maitotoxin using solid-phase adsorption toxin tracking devices: application to Gambierdiscus pacificus in culture. Harmful. Algae 10 (5), 433–446. https://doi.org/10.1016/j.hal.2011.02.004.

Caillaud, A., De La Iglesia, P., Darius, H.T., Pauillac, S., Aligizaki, K., Fraga, S., Chinain, M., & Diogène, J. (2010). Update on methodologies available for ciguatoxin

determination: perspectives to confront the onset of ciguatera fish poisoning in Europe. In Marine Drugs (Vol. 8, Issue 6, pp. 1838–1907). MDPI AG. 10.3390/md8061838.
Centre for Food Safety: The Government of the Hong Kong Special Administrative Region. (2021). Ciguatera Fish Poisoning.
Chateau-Degat, M.L., Chinain, M., Cerf, N., Gingras, S., Hubert, B., Dewailly, É., 2005. Seawater temperature, Gambierdiscus spp. variability and incidence of ciguatera poisoning in French Polynesia. Harmful Algae 4 (6), 1053–1062. https://doi.org/10.1016/j.hal.2005.03.003.
Chan, T.Y.K., 2015. Ciguatera fish poisoning in East Asia and Southeast Asia. In Marine Drugs. MDPI AG 13 (6), 3466–3478. https://doi.org/10.3390/md13063466.
Chin Chwan Chuong, J.J., Rahman, M., Ibrahim, N., Heng, L.Y., Tan, L.L., Ahmad, A., 2022. Harmful Microalgae Detection: biosensors versus Some Conventional Methods. Sensors (9), 22. https://doi.org/10.3390/s22093144.
Chinain, M., Darius, H.T., Ung, A., Cruchet, P., Wang, Z., Ponton, D., Laurent, D., Pauillac, S., 2010. Growth and toxin production in the ciguatera-causing dinoflagellate Gambierdiscus polynesiensis (Dinophyceae) in culture. Toxicon. 56 (5), 739–750. https://doi.org/10.1016/j.toxicon.2009.06.013.
Chinain, M., Faust, M.A., Pauillac, S., 1999. Morphology and molecular analyses of three toxic species of Gambierdiscus (Dinophyceae): G. pacificus, sp. nov., G. australes, sp. nov. and G. polynesiensis, sp. nov. J. Phycol. 35 (6), 1282–1296. https://doi.org/10.1046/j.1529-8817.1999.3561282.x.
Chinain, M., Gatti Howell, C., Roué, M., Ung, A., Henry, K., Revel, T., Cruchet, P., Viallon, J., Darius, H.T., 2023. Ciguatera poisoning in French Polynesia: a review of the distribution and toxicity of Gambierdiscus spp., and related impacts on food web components and human health. Harmful. Algae 129. https://doi.org/10.1016/j.hal.2023.102525.
Chinain, M., Gatti, C.M.I., Darius, H.T., Quod, J.P., Tester, P.A., 2021. Ciguatera poisonings: a global review of occurrences and trends. Harmful. Algae 102. https://doi.org/10.1016/j.hal.2020.101873.
Chinain, M., Gatti, C.M., Roué, M., Darius, H.T., 2019. Ciguatera poisoning in French Polynesia: insights into the novel trends of an ancient disease. In: New Microbes and New Infections, 31. Elsevier Ltd. https://doi.org/10.1016/j.nmni.2019.100565.
Chinain, Mireille., Gatti, C.M., Roue, Melanie., & Darius, Taiana. (2020). Ciguatera-causing dinoflagellates in the genera gambierdiscus and fukuyoa: distribution, ecophysiology and toxicology.
Cooper, E.D., Bentlage, B., Gibbons, T.R., Bachvaroff, T.R., Delwiche, C.F., 2014. Metatranscriptome profiling of a harmful algal bloom. Harmful. Algae 37, 75–83. https://doi.org/10.1016/j.hal.2014.04.016.
Cruz, R.C., Costa, P.R., Vinga, S., Krippahl, L., Lopes, M.B., 2021. A review of recent machine learning advances for forecasting harmful algal blooms and shellfish contamination. J. Mar. Sci. Eng. 9 (3). https://doi.org/10.3390/jmse9030283.
Cuadrado, A., Sixto, M., Figueroa, R.I., Bravo, I., de Bustos, A., 2022. Comparative FISH mapping of 45S and 5S rDNA in the genus Gambierdiscus advances understanding of the cytogenetic diversity and mitosis of dinoflagellates. Eur. J. Phycol. 57 (3), 264–276. https://doi.org/10.1080/09670262.2021.1964608.
Darius, H.T., Roué, M., Sibat, M., Viallon, J., Gatti, C.M.I., Vandersea, M.W., Tester, P.A., Litaker, R.W., Amzil, Z., Hess, P., Chinain, M., 2018. Tectus niloticus (Tegulidae, gastropod) as a novel vector of ciguatera poisoning: detection of pacific ciguatoxins in toxic samples from nuku hiva island (french polynesia). Toxins. (Basel) (1), 10. https://doi.org/10.3390/toxins10010002.
Dashkova, V., Malashenkov, D., Poulton, N., Vorobjev, I., Barteneva, N.S., 2017. Imaging flow cytometry for phytoplankton analysis. Methods 112, 188–200. https://doi.org/10.1016/j.ymeth.2016.05.007.
De Sousa, L.L., Silva, S.M., Xavier, R., 2019. DNA metabarcoding in diet studies: unveiling ecological aspects in aquatic and terrestrial ecosystems. Environ. DNA 1 (3), 199–214. https://doi.org/10.1002/edn3.27.
Deiner, K., Yamanaka, H., & Bernatchez, L. (2020). The future of biodiversity monitoring and conservation utilizing environmental DNA. 10.3929/ethz-b-000488115.
Devloo-Delva, F., Huerlimann, R., Chua, G., Matley, J.K., Heupel, M.R., Simpfendorfer, C. A., Maes, G.E., 2018. How does marker choice affect your diet analysis? Comparing genetic markers and digestion levels in metabarcoding of reef predator gut content. Marine Freshwater Res. 70, 8–18.
Dickey, R.W., Plakas, S.M., 2010. Ciguatera: a public health perspective. Toxicon. 56 (2), 123–136. https://doi.org/10.1016/j.toxicon.2009.09.008.
Doherty, M.J. (2005). Historical Neurology Captain Cook on poison fish.
Durán-Vinet, B., Araya-Castro, K., Chao, T.C., Wood, S.A., Gallardo, V., Godoy, K., & Abanto, M. (2021). Potential applications of CRISPR/Cas for next-generation biomonitoring of harmful algae blooms: a review. In Harmful Algae (Vol. 103). Elsevier B.V. 10.1016/j.hal.2021.102027.
Edvardsen, B., Shalchian-Tabrizi, K., Jakobsen, K.S., Medlin, L.K., Dahl, E., Brubak, S., Paasche, E., 2003. Genetic variability and molecular phylogeny of Dinophysis species (Dinophyceae) from Norwegian waters inferred from single cell analyses of rDNA. J. Phycol. 39 (2), 395–408. https://doi.org/10.1046/j.1529-8817.2003.01252.x.
Estevez, P., Sibat, M., Leão-Martins, J.M., Costa, P.R., Gago-Martínez, A., Hess, P., 2020. Liquid chromatography coupled to high-resolution mass spectrometry for the confirmation of Caribbean ciguatoxin-1 as the main toxin responsible for ciguatera poisoning caused by fish from European Atlantic coasts. Toxins. (Basel) (4), 12. https://doi.org/10.3390/toxins12040267.
Farrell, H., Murray, S.A., Zammit, A., Edwards, A.W., 2017. Management of ciguatoxin risk in eastern Australia. Toxins 9 (11). https://doi.org/10.3390/toxins9110367.
Faust, M.A., 1995. Observation of sand-dwelling toxic dinoflagellates (dinophyceae) from widely differing sites, including t w o new species1. In J. Phycol 31.
Fischer, A.D., Hayashi, K., McGaraghan, A., Kudela, R.M., 2020. Return of the "age of dinoflagellates" in Monterey Bay: drivers of dinoflagellate dominance examined using automated imaging flow cytometry and long-term time series analysis. Limnol. Oceanogr. 65 (9), 2125–2141. https://doi.org/10.1002/lno.11443.
Friedman, M.A., 2008. Ciguatera fish poisoning: treatment, prevention and management. Mar. Drugs 6 (3), 456–479. https://doi.org/10.3390/md20080022.
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., Quintana, H.A.F., Loeffler, C.R., Weisman, R., Blythe, D., Berdalet, E., Ayyar, R., Clarkson-Townsend, D., … Fleming, L.E. (2017). An updated review of ciguatera fish poisoning: clinical, epidemiological, environmental, and public health management. In Marine Drugs (Vol. 15, Issue 3). MDPI AG. 10.3390/md15030072.
Funaki, H., Gaonkar, C.C., Kataoka, T., Nishimura, T., Tanaka, K., Yanagida, I., Abe, S., Yamaguchi, H., Nagasaki, K., Adachi, M., 2022. Horizontal and vertical distribution of Gambierdiscus spp. (Dinophyceae) including novel phylotypes in Japan identified by 18S rDNA metabarcoding. Harmful. Algae 111. https://doi.org/10.1016/j.hal.2021.102163.
Gaboriau, M., Ponton, D., Darius, H.T., Chinain, M., 2014. Ciguatera fish toxicity in French Polynesia: size does not always matter. Toxicon. 84, 41–50. https://doi.org/10.1016/j.toxicon.2014.03.006.
Gaiani, G., Cucchi, F., Toldrà, A., Andree, K.B., Rey, M., Tsumuraya, T., O'Sullivan, C.K., Diogène, J., Campàs, M., 2022. Electrochemical biosensor for the dual detection of Gambierdiscus australes and Gambierdiscus excentricus in field samples. First report of G. excentricus in the Balearic Islands. Science of the Total Environment 806. https://doi.org/10.1016/j.scitotenv.2021.150915.
Garcia-Soto, C., Cheng, L., Caesar, L., Schmidtko, S., Jewett, E.B., Cheripka, A., Rigor, I., Caballero, A., Chiba, S., Báez, J.C., Zielinski, T., Abraham, J.P., 2021. An Overview of ocean climate change indicators: sea surface temperature, ocean heat content, ocean ph, dissolved oxygen concentration, arctic sea ice extent, thickness and volume, sea level and strength of the AMOC (atlantic meridional overturning circulation). Front. Mar. Sci. 8, 1–45. https://doi.org/10.3389/fmars.2021.642372.
GEOHAB. (2012). GEOHAB Core Research Project: hABs in Benthic Systems. http://hdl.handle.net/1834/12437.
Gibson, C.H., Thomas, W.H., 1995. Effects of turbulence intermittency on growth inhibition of a red tide dinoflagellate, Gonyaulax polyedra Stein. J. Geophys. Res. 100 (C12), 841–846. https://doi.org/10.1029/95jc02256.
Gika, H.G., Wilson, I.D., Theodoridis, G.A., 2014. LC-MS-based holistic metabolic profiling. Problems, limitations, advantages, and future perspectives. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 966, 1–6. https://doi.org/10.1016/j.jchromb.2014.01.054.
Ginés, I., Gaiani, G., Ruhela, A., Skouridou, V., Campàs, M., Masip, L., 2021. Nucleic acid lateral flow dipstick assay for the duplex detection of Gambierdiscus australes and Gambierdiscus excentricus. Harmful Algae 110. https://doi.org/10.1016/j.hal.2021.102135.
Gingold, D.B., Strickland, M.J., Hess, J.J., 2014. Ciguatera fish poisoning and climate change: analysis of national poison center data in the United States, 2001-2011. Environ. Health Perspect. 122 (6), 580–586. https://doi.org/10.1289/ehp.1307196.
Gobler, C.J., 2020. Climate change and harmful algal blooms: insights and perspective. Harmful. Algae 91. https://doi.org/10.1016/j.hal.2019.101731.
Gómez, F., Qiu, D., Lopes, R.M., Lin, S., 2015. Fukuyoa paulensis gen. et sp. nov., a new genus for the globular species of the dinoflagellate Gambierdiscus (Dinophyceae). PLoS ONE 10 (4). https://doi.org/10.1371/journal.pone.0119676.
Gong, W., Browne, J., Hall, N., Schruth, D., Paerl, H., Marchetti, A., 2017. Molecular insights into a dinoflagellate bloom. ISME Journal 11 (2), 439–452. https://doi.org/10.1038/ismej.2016.129.
Hadziavdic, K., Lekang, K., Lanzen, A., Jonassen, I., Thompson, E.M., Troedsson, C., 2014. Characterization of the 18s rRNA gene for designing universal eukaryote specific primers. PLoS. One 9 (2). https://doi.org/10.1371/journal.pone.0087624.
HAIS-UNESCO. (2022). IOC-UNESCO Harmful Algae Information System. Https://Data.Hais.Ioc-Unesco.Org/.
Hales, S., Weinstein, P., & Woodward, A. (1999). Ciguatera (Fish Poisoning), El Niño, and Pacific Sea Surface Temperatures. http://ingrid.ldgo.columbia.
Halstead, B.W. (1965). Poisonous and Venomous Marine Animals of the World: vertebrates.
Han, Q., Liu, D., 2014. Macroalgae blooms and their effects on seagrass ecosystems. J. Ocean Univ. China 13 (5), 791–798. https://doi.org/10.1007/s11802-014-2471-2.
Hardison, D.R., Holland, W.C., McCall, J.R., Bourdelais, A.J., Baden, D.G., Darius, H.T., Chinain, M., Tester, P.A., Shea, D., Quintana, H.A.F., Morris, J.A., Litaker, R.W., 2016. Fluorescent receptor binding assay for detecting ciguatoxins in fish. PLoS ONE 11 (4). https://doi.org/10.1371/journal.pone.0153348.
Harper, L.R., Lawson Handley, L., Hahn, C., Boonham, N., Rees, H.C., Gough, K.C., Lewis, E., Adams, I.P., Brotherton, P., Phillips, S., Hänfling, B, 2018. Needle in a haystack? A comparison of eDNA metabarcoding and targeted qPCR for detection of the great crested newt (Triturus cristatus). Ecology and Evolution 8 (12), 6330–6341. https://doi.org/10.1002/ece3.4013.
Hoegh-Guldberg, O., Jacob, D., Taylor, M., Guillén Bolaños, T., Bindi, M., Brown, S., Camilloni, I.A., Diedhiou, A., Djalante, R., Ebi, K., Engelbrecht, F., Guiot, J., Hijioka, Y., Mehrotra, S., Hope, C.W., Payne, A.J., Pörtner, H.O., Seneviratne, S.I., Thomas, A., Zhou, G., 2019. The human imperative of stabilizing global climate change at 1.5°C. Science (1979) (6459), 365. https://doi.org/10.1126/science.aaw6974.
Holmes, M.J., Venables, B., Lewis, R.J., 2021. Critical review and conceptual and quantitative models for the transfer and depuration of ciguatoxins in fishes. In Toxins 13 (8). https://doi.org/10.3390/toxins13080515.
Holmes, Michael J, Lewis, R.J., Poli, M.A., & Gillespie, N.C. (1991). STrain dependent production of ciguatoxin precursors (gambiertoxins) by gambierdiscus toxicus (dinophyceae) in culture (Vol. 29, Issue 4).

Homma, C., Inokuchi, D., Nakamura, Y., Uy, W.H., Ohnishi, K., Yamaguchi, H., Adachi, M., 2022. Effectiveness of blocking primers and a peptide nucleic acid (PNA) clamp for 18S metabarcoding dietary analysis of herbivorous fish. PLoS. One 17. https://doi.org/10.1371/journal.pone.0266268 (4 April).

Hughes, T.P., Kerry, J.T., Baird, A.H., Connolly, S.R., Dietzel, A., Eakin, C.M., Heron, S. F., Hoey, A.S., Hoogenboom, M.O., Liu, G., McWilliam, M.J., Pears, R.J., Pratchett, M.S., Skirving, W.J., Stella, J.S., Torda, G., 2018. Global warming transforms coral reef assemblages. Nature 556 (7702), 492–496. https://doi.org/10.1038/s41586-018-0041-2.

Jacobs-Palmer, E., Gallego, R., Cribari, K., Keller, A.G., Kelly, R.P., 2021. Environmental DNA metabarcoding for simultaneous monitoring and ecological assessment of many harmful algae. Front. Ecol. Evol. 9. https://doi.org/10.3389/fevo.2021.612107.

Jager, J., Gerssen, A., Pawliszyn, J., Sterk, S.S., Nielen, M.W.F., Blokland, M.H., 2020. USB-Powered Coated Blade Spray Ion Source for On-Site Testing Using Transportable Mass Spectrometry. J. Am. Soc. Mass Spectrom. 31 (11), 2243–2249. https://doi.org/10.1021/jasms.0c00307.

Jiang, L.Q., Carter, B.R., Feely, R.A., Lauvset, S.K., Olsen, A., 2019. Surface ocean pH and buffer capacity: past, present and future. Sci. Rep. 9 (1), 1–11. https://doi.org/10.1038/s41598-019-55039-4.

Keck, F., Blackman, R.C., Bossart, R., Brantschen, J., Couton, M., Hürlemann, S., Kirschner, D., Locher, N., Zhang, H., Altermatt, F., 2022. Meta-analysis shows both congruence and complementarity of DNA and eDNA metabarcoding to traditional methods for biological community assessment. Mol. Ecol. 31 (6), 1820–1835. https://doi.org/10.1111/mec.16364.

Keeling, P.J., Burki, F., Wilcox, H.M., Allam, B., Allen, E.E., Amaral-Zettler, L.A., Armbrust, E.V., Archibald, J.M., Bharti, A.K., Bell, C.J., Beszteri, B., Bidle, K.D., Cameron, C.T., Campbell, L., Caron, D.A., Cattolico, R.A., Collier, J.L., Coyne, K., Davy, S.K., Worden, A.Z., 2014. The marine microbial eukaryote transcriptome sequencing project (MMETSP): illuminating the functional diversity of eukaryotic life in the oceans through transcriptome sequencing. PLoS Biol. (6), 12. https://doi.org/10.1371/journal.pbio.1001889.

Ki, J.S., 2012. Hypervariable regions (V1-V9) of the dinoflagellate 18S rRNA using a large dataset for marker considerations. J. Appl. Phycol. 24 (5), 1035–1043. https://doi.org/10.1007/s10811-011-9730-z.

Kibler, S.R., Litaker, R.W., Holland, W.C., Vandersea, M.W., Tester, P.A., 2012. Growth of eight Gambierdiscus (Dinophyceae) species: effects of temperature, salinity and irradiance. Harmful. Algae 19, 1–14. https://doi.org/10.1016/j.hal.2012.04.007.

Kibler, S.R., Tester, P.A., Kunkel, K.E., Moore, S.K., Litaker, R.W., 2015. Effects of ocean warming on growth and distribution of dinoflagellates associated with ciguatera fish poisoning in the Caribbean. Ecol. Modell. 316, 194–210. https://doi.org/10.1016/j.ecolmodel.2015.08.020.

Kobari, T., Tokumo, Y., Sato, I., Kume, G., Hirai, J., 2021. Metabarcoding analysis of trophic sources and linkages in the plankton community of the Kuroshio and neighboring waters. Sci. Rep. (1), 11. https://doi.org/10.1038/s41598-021-02083-8.

Kohli, G.S., Farrell, H., Murray, S.A., 2015. Gambierdiscus, the cause of ciguatera fish poisoning: an increased human health threat influenced by climate change. Climate Change Marine Freshwater Toxins.

Kohli, G.S., Murray, S.A., Neilan, B.A., Rhodes, L.L., Harwood, D.T., Smith, K.F., Meyer, L., Capper, A., Brett, S., Hallegraeff, G.M., 2014a. High abundance of the potentially maitotoxic dinoflagellate Gambierdiscus carpenteri in temperate waters of New South Wales. Harmful. Algae 39, 134–145. https://doi.org/10.1016/j.hal.2014.07.007.

Kohli, G.S., Neilan, B.A., Brown, M.V., Hoppenrath, M., Murray, S.A., 2014b. Cob gene pyrosequencing enables characterization of benthic dinoflagellate diversity and biogeography. Environ. Microbiol. 16 (2), 467–485. https://doi.org/10.1111/1462-2920.12275.

Kretzschmar, A.L., Verma, A., Harwood, T., Hoppenrath, M., Murray, S., 2017. Characterization of Gambierdiscus lapillus sp. nov. (Gonyaulacales, Dinophyceae): a new toxic dinoflagellate from the Great Barrier Reef (Australia). Journal of Phycology 53 (2), 283–297. https://doi.org/10.1111/jpy.12496.

Kretzschmar, A.L., Verma, A., Kohli, G., Murray, S., 2019. Development of a quantitative PCR assay for the detection and enumeration of a potentially ciguatoxin-producing dinoflagellate, Gambierdiscus lapillus (Gonyaulacales, Dinophyceae). PLoS. One (11), 14. https://doi.org/10.1371/journal.pone.0224664.

Kumar-Roiné, S., Taiana Darius, H., Matsui, M., Fabre, N., Haddad, M., Chinain, M., Pauillac, S., Laurent, D., 2011. A review of traditional remedies of ciguatera fish poisoning in the pacific. In Phytotherapy Res. 25 (7), 947–958. https://doi.org/10.1002/ptr.3396.

Kusche, H., Hanel, R., 2021. Consumers of mislabeled tropical fish exhibit increased risks of ciguatera intoxication: a report on substitution patterns in fish imported at Frankfurt Airport, Germany. Food Control 121, 107647. https://doi.org/10.1016/j.foodcont.2020.107647. May 2020).

Langlois, V.S., Allison, M.J., Bergman, L.C., To, T.A., Helbing, C.C., 2021. The need for robust qPCR-based eDNA detection assays in environmental monitoring and species inventories. Environ. DNA 3 (3), 519–527. https://doi.org/10.1002/edn3.164.

Larsson, M.E., Harwood, T.D., Lewis, R.J., Himaya, S.W.A., Doblin, M.A., 2019. Toxicological characterization of Fukuyoa paulensis (Dinophyceae) from temperate Australia. Phycol. Res. 67 (1), 65–71. https://doi.org/10.1111/pre.12349.

Laza-Martínez, A., David, H., Riobó, P., Miguel, I., Orive, E., 2016. Characterization of a strain of fukuyoa paulensis (dinophyceae) from the western mediterranean sea. J. Eukaryot. Microbiol. 63 (4), 481–497. https://doi.org/10.1111/jeu.12292.

Leonardo, S., Gaiani, G., Tsumuraya, T., Hirama, M., Turquet, J., Sagristà, N., Rambla-Alegre, M., Flores, C., Caixach, J., Diogène, J., O'Sullivan, C.K., Alcaraz, C., Campàs, M., 2020. Addressing the analytical challenges for the detection of ciguatoxins using an electrochemical biosensor. Anal. Chem. 92 (7), 4858–4865. https://doi.org/10.1021/acs.analchem.9b04499.

Lewis, R.J., Holmes, M.J., 1993. Mini review origin and transfer of toxins involved in ciguatera. In Biochem. Physiol 106 (3). Issue.

Litaker, R.W., Holland, W.C., Hardison, D.R., Pisapia, F., Hess, P., Kibler, S.R., Tester, P. A., 2017. Ciguatoxicity of Gambierdiscus and Fukuyoa species from the Caribbean and Gulf of Mexico. PLoS ONE 12 (10). https://doi.org/10.1371/journal.pone.0185776.

Litaker, R.W., Vandersea, M.W., Faust, M.A., Kibler, S.R., Chinain, M., Holmes, M.J., Holland, W.C., Tester, P.A., 2009. Taxonomy of Gambierdiscus including four new species, Gambierdiscus caribaeus, Gambierdiscus carolinianus, Gambierdiscus carpenteri and Gambierdiscus ruetzleri (Gonyaulacales, Dinophyceae). Phycologia 48 (5), 344–390. https://doi.org/10.2216/07-15.1.

Litaker, R.W., Vandersea, M.W., Faust, M.A., Kibler, S.R., Nau, A.W., Holland, W.C., Chinain, M., Holmes, M.J., Tester, P.A., 2010. Global distribution of ciguatera causing dinoflagellates in the genus Gambierdiscus. Toxicon. 56 (5), 711–730. https://doi.org/10.1016/j.toxicon.2010.05.017.

Llewellyn, L.E., 2010. Revisiting the association between sea surface temperature and the epidemiology of fish poisoning in the South Pacific: reassessing the link between ciguatera and climate change. Toxicon. 56 (5), 691–697. https://doi.org/10.1016/j.toxicon.2009.08.011.

Loeffler, C.R., Spielmeyer, A., Friedemann, M., Kapp, K., Schwank, U., Kappenstein, O., Bodi, D., 2022. Food safety risk in germany from mislabeled imported fish: ciguatera outbreak trace-back, toxin elucidation, and public health implications. Front. Mar. Sci. 9. https://doi.org/10.3389/fmars.2022.849857.

Loeffler, C.R., Tartaglione, L., Friedemann, M., Spielmeyer, A., Kappenstein, O., Bodi, D., 2021. Ciguatera mini review: 21st century environmental challenges and the interdisciplinary research efforts rising to meet them. In: International Journal of Environmental Research and Public Health, 18. MDPI AG, pp. 1–27. https://doi.org/10.3390/ijerph18063027.

Longo, S., Sibat, M., Darius, H.T., Hess, P., Chinain, M., 2020. Effects of pH and nutrients (nitrogen) on growth and toxin profile of the ciguatera-causing dinoflagellate gambierdiscus polynesiensis (dinophyceae). Toxins. (Basel) (12), 12. https://doi.org/10.3390/toxins12120767.

McPartlin, D.A., Loftus, J.H., Crawley, A.S., Silke, J., Murphy, C.S., O'Kennedy, R.J, 2017. Biosensors for the monitoring of harmful algal blooms. Curr. Opin. Biotechnol. 45, 164–169. https://doi.org/10.1016/j.copbio.2017.02.018.

Maguire, I., Fitzgerald, J., Heery, B., Nwankire, C., O'Kennedy, R., Ducrée, J., Regan, F., 2018. Novel Microfluidic Analytical Sensing Platform for the Simultaneous Detection of Three Algal Toxins in Water. ACS Omega 3 (6), 6624–6634. https://doi.org/10.1021/acsomega.8b00240.

Mak, Y.L., Wai, T.C., Murphy, M.B., Chan, W.H., Wu, J.J., Lam, J.C.W., Chan, L.L., Lam, P.K.S., 2013. Pacific ciguatoxins in food web components of coral reef systems in the Republic of Kiribati. Environ. Sci. Technol. 47 (24), 14070–14079. https://doi.org/10.1021/es403175d.

McKinnon, K.M., 2018. Flow cytometry: an overview. Curr. Protoc. Immunol. https://doi.org/10.1002/cpim.40, 2018(February), 5.1.1-5.1.11.

Medlin, L.K., & Orozco, J. (2017). Molecular techniques for the detection of organisms in aquatic environments, with emphasis on harmful algal bloom species. In Sensors (Switzerland) (Vol. 17, Issue 5). MDPI AG. 10.3390/s17051184.

Meyer, L., Capper, A., Carter, S., Simpfendorfer, C., 2016. An investigation into ciguatoxin bioaccumulation in sharks. Toxicon. 119, 234–243. https://doi.org/10.1016/j.toxicon.2016.06.007.

Mordret, S., Piredda, R., Vaulot, D., Montresor, M., Kooistra, W.H.C.F., Sarno, D., 2018. dinoref: a curated dinoflagellate (Dinophyceae) reference database for the 18S rRNA gene. Mol. Ecol. Resour. 18 (5), 974–987. https://doi.org/10.1111/1755-0998.12781.

Mielczarek, P., Silberring, J., Smoluch, M., 2020. MINIATURIZATION IN MASS SPECTROMETRY. In: *Mass Spectrometry Reviews*, (Vol. 39, Issues 5–6. John Wiley and Sons Inc, pp. 453–470. https://doi.org/10.1002/mas.21614.

Monserrat, M., Asnaghi, V., Verdura, J., Meroni, L., Lemée, R., Rossi, A.M., Romero, G., Priouzeau, F., Chiantore, M., Mangialajo, L., 2024. From micro to mesoscale: understanding the influence of macroalgal communities on Ostreopsis Schmidt blooms. Harmful. Algae 136. https://doi.org/10.1016/j.hal.2024.102650 (May).

Morin, E., Gatti, C., Bambridge, T., Chinain, M., 2016. Ciguatera fish poisoning: incidence, health costs and risk perception on Moorea Island (Society archipelago, French Polynesia). Harmful. Algae 60, 1–10. https://doi.org/10.1016/j.hal.2016.10.003.

Moshynets, O.V., Baranovskyi, T.P., Iungin, O.S., Kysil, N.P., Metelytsia, L.O., Pokholenko, I., Potochilova, V.V., Potters, G., Rudnieva, K.L., Rymar, S.Y., Semenyuta, I.V., Spiers, A.J., Tarasyuk, O.P., Rogalsky, S.P., 2022. eDNA inactivation and biofilm inhibition by the polymeric biocide polyhexamethylene guanidine hydrochloride (PHMG-Cl). Int. J. Mol. Sci. 23 (2). https://doi.org/10.3390/ijms23020731.

Mudge, E.M., Miles, C.O., Ivanova, L., Uhlig, S., James, K.S., Erdner, D.L., Fæste, C.K., McCarron, P., Robertson, A., 2023. Algal ciguatoxin identified as source of ciguatera poisoning in the Caribbean. Chemosphere 330, 138659. https://doi.org/10.1016/j.chemosphere.2023.138659.

Mudge, E.M., Robertson, A., Uhlig, S., McCarron, P., Miles, C.O., 2024. 3-Epimers of caribbean ciguatoxins in fish and algae. Toxicon. 237, 107536. https://doi.org/10.1016/j.toxicon.2023.107536 (October 2023).

Mullins, M.E., & Hoffman, R.S. (2017). Is mannitol the treatment of choice for patients with ciguatera fish poisoning? In Clinical Toxicology (Vol. 55, Issue 9, pp. 947–955). Taylor and Francis Ltd. 10.1080/15563650.2017.1327664.

Murray, J.S., Nishimura, T., Finch, S.C., Rhodes, L.L., Puddick, J., Harwood, D.T., Larsson, M.E., Doblin, M.A., Leung, P., Yan, M., Rise, F., Wilkins, A.L., Prinsep, M.R.,

2020. The role of 44-methylgambierone in ciguatera fish poisoning: acute toxicity, production by marine microalgae and its potential as a biomarker for Gambierdiscus spp. Harmful. Algae 97. https://doi.org/10.1016/j.hal.2020.101853.
Nascimento, S.M., Melo, G., Salgueiro, F., Dos Santos Diniz, B., Fraga, S., 2015. Morphology of Gambierdiscus excentricus (Dinophyceae) with emphasis on sulcal plates. Phycologia 54 (6), 628–639. https://doi.org/10.2216/15-61.1.
NSW Government Food Authority. (2022). Fish ciguatera poisoning. Https://Www.Foodauthority.Nsw.Gov.Au/Consumer/Food-Poisoning/Fish-Ciguatera-Poisoning.
Oshiro, N., Gago-Martínez, A., Tubaro, A, 2024. Chemistry, toxicology and etiology of marine biotoxins. J. Mar. Sci. Eng. 12 (2). https://doi.org/10.3390/jmse12020236.
Otero, P., Pérez, S., Alfonso, A., Vale, C., Rodríguez, P., Gouveia, N.N., Gouveia, N., Delgado, J., Vale, P., Hirama, M., Ishihara, Y., Molgó, J., Botana, L.M., 2010. First toxin profile of ciguateric fish in Madeira Arquipelago (Europe). Anal. Chem. 82 (14), 6032–6039. https://doi.org/10.1021/ac100516q.
Parsons, M.L., Richlen, M.L., Smith, T.B., Anderson, D.M., Abram, A.L., Erdner, D.L., Robertson, A., 2024. CiguaMOD I: a conceptual model of ciguatoxin loading in the Greater Caribbean Region. Harmful. Algae 131, 102561. https://doi.org/10.1016/j.hal.2023.102561 (December 2023).
Pasinszki, T., Lako, J., Dennis, T.E., 2020. Advances in detecting ciguatoxins in fish. In: Toxins, 12. MDPI AG. https://doi.org/10.3390/toxins12080494.
Pearn, J., 2001. Neurology of ciguatera. J. Neurol. Neurosurg. Psychiatry 70 (1), 4–8. https://doi.org/10.1136/jnnp.70.1.4.
Pitz, K.J., Richlen, M.L., Fachon, E., Smith, T.B., Parsons, M.L., Anderson, D.M., 2021. Development of fluorescence in situ hybridization (FISH) probes to detect and enumerate Gambierdiscus species. Harmful. Algae 101. https://doi.org/10.1016/j.hal.2020.101914.
Pulido, O.M., 2014. Marine algal toxins: seafood safety, human health and beyond. Oceanography: Open Access 02 (04). https://doi.org/10.4172/2332-2632.1000e111.
Quiroz, R.N., Herrera-Usuga, J.C., Osorio-Ospina, L.M., Garcia-Pertuz, K.M., Quiroz, E. N., 2020. Epidemiology and toxicology of ciguatera poisoning in the Colombian Caribbean. In: Marine Drugs, 18. MDPI AG. https://doi.org/10.3390/md18100504.
Rains, L.K., Parsons, M.L., 2015. Gambierdiscus species exhibit different epiphytic behaviors toward a variety of macroalgal hosts. Harmful. Algae 49, 29–39. https://doi.org/10.1016/j.hal.2015.08.005.
Ralston, D.K., Moore, S.K., 2019. Modeling harmful algal blooms in a changing climate. Harmful Algae 91, 101729. https://doi.org/10.1016/j.hal.2019.101729.
Ramilo, I., Figueroa, R.I., Rayón-Viña, F., Cuadrado, Á., Bravo, I., 2021. Temperature-dependent growth and sexuality of the ciguatoxin producer dinoflagellate Gambierdiscus spp. in cultures established from the Canary Islands. Harmful. Algae 110. https://doi.org/10.1016/j.hal.2021.102130.
Ramos-Sosa, M.J., García-álvarez, N., Sanchez-Henao, A., Sergent, F.S., Padilla, D., Estévez, P., Caballero, M.J., Martín-Barrasa, J.L., Gago-Martínez, A., Diogène, J., Real, F, 2022. Ciguatoxin detection in flesh and liver of relevant fish species from the canary islands. Toxins. (Basel) (1), 14. https://doi.org/10.3390/toxins14010046.
Randall, J.E. (1958). A revievv of ciguatera, poisoning, explanation tropical. 212.
Raposo-Garcia, S., Louzao, M.C., Fuwa, H., Sasaki, M., Vale, C., Botana, L.M., 2022. Determination of the toxicity equivalency factors for ciguatoxins using human sodium channels. Food Chem. Toxicol. 160. https://doi.org/10.1016/j.fct.2022.112812.
Raven, J.A., Gobler, C.J., Hansen, P.J., 2020. Dynamic CO2 and pH levels in coastal, estuarine, and inland waters: theoretical and observed effects on harmful algal blooms. Harmful. Algae 91. https://doi.org/10.1016/j.hal.2019.03.012.
Redfield, A.C. (1933). On the proportions of organic derivatives in sea water and their relation to the composition of plankton. https://www.researchgate.net/publication/344709447.
Resiere, D., Florentin, J., Mehdaoui, H., Zakaria, M., Gueye, P., Hommel, D., Pujo, J., Nkontcho, F., Boisnoir, A., Portecop, P., Névière, R., Kallel, H., & Mégarbane, B. (2022). Clinical Characteristics of Ciguatera Fish Poisoning in Martinique, French West Indies-a case series. 10.20944/preprints202204.0037.v1.
Reverté, L., Prieto-Simón, B., Campàs, M., 2016. New advances in electrochemical biosensors for the detection of toxins: Nanomaterials, magnetic beads and microfluidics systems. A review. Analytica Chimica Acta 908, 8–21. https://doi.org/10.1016/j.aca.2015.11.050.
Rhodes, L., Harwood, T., Smith, K., Argyle, P., Munday, R., 2014. Production of ciguatoxin and maitotoxin by strains of Gambierdiscus australes, G. pacificus and G. polynesiensis (Dinophyceae) isolated from Rarotonga, Cook Islands. Harmful. Algae 39, 185–190. https://doi.org/10.1016/j.hal.2014.07.018.
Rhodes, L.L., Smith, K.F., Murray, S., Harwood, D.T., Trnski, T., Munday, R., 2017. The epiphytic genus Gambierdiscus (Dinophyceae) in the Kermadec Islands and Zealandia regions of the southwestern Pacific and the associated risk of ciguatera fish poisoning. Mar. Drugs (7), 15. https://doi.org/10.3390/md15070219.
Rhodes, L.L., Smith, K.F., Sam Murray, J., Nishimura, T., Finch, S.C., 2020. Ciguatera fish poisoning: the risk from an aotearoa/New Zealand perspective. In: Toxins, 12. MDPI AG. https://doi.org/10.3390/toxins12010050.
Richlen, M.L., Horn, K., Uva, V., Fachon, E., Heidmann, S.L., Smith, T.B., Parsons, M.L., Anderson, D.M, 2024. Gambierdiscus species diversity and community structure in St. Thomas, USVI and the Florida Keys. Harmful. Algae 131, 102562. https://doi.org/10.1016/j.hal.2023.102562.
Rongo, T., Bush, M., Van Woesik, R., 2009. Did ciguatera prompt the late Holocene Polynesian voyages of discovery? Journal of Biogeography 36 (8), 1423–1432. https://doi.org/10.1111/j.1365-2699.2009.02139.x.
Rongo, T., van Woesik, R., 2013. The effects of natural disturbances, reef state, and herbivorous fish densities on ciguatera poisoning in Rarotonga, southern Cook Islands. Toxicon 64, 87–95. https://doi.org/10.1016/j.toxicon.2012.12.018.
Satake, M., Murata, M., Yasumoto, T., 1993. The structure of CTX3C, a ciguatoxin congener isolated from cultured Gambierdiscus toxicus. Tetrahedron Lett. 34 (12), 1975–1978. https://doi.org/10.1016/S0040-4039(00)91978-6.
Schulze, A., Erdner, D.L., Grimes, C.J., Holstein, D.M., Miglietta, M.P., 2020. Artificial Reefs in the Northern Gulf of Mexico: community Ecology Amid the "Ocean Sprawl. In: Frontiers in Marine Science, 7. Frontiers Media S.A. https://doi.org/10.3389/fmars.2020.00447. Vol.
Smith, K.F., Biessy, L., Argyle, P., Trnski, T., Halafihi, T., Rhodes, L.L., 2017. Molecular identification of Gambierdiscus and Fukuyoa (Dinophyceae) from environmental samples. Mar. Drugs 15 (8). https://doi.org/10.3390/md15080243.
Smith, K.F., Kohli, G.S., Murray, S.A., Rhodes, L.L., 2017a. Assessment of the metabarcoding approach for community analysis of benthic-epiphytic dinoflagellates using mock communities. N. Z. J. Mar. Freshwater Res. 51 (4), 555–576. https://doi.org/10.1080/00288330.2017.1298632.
Soliño, L., Costa, P.R., 2018. Differential toxin profiles of ciguatoxins in marine organisms: Chemistry, fate and global distribution. In: In *Toxicon*, 150. Elsevier Ltd, pp. 124–143. https://doi.org/10.1016/j.toxicon.2018.05.005.
Soliño, L., Costa, P.R., 2020. Global impact of ciguatoxins and ciguatera fish poisoning on fish, fisheries and consumers. Environmental Research 182, 109111. https://doi.org/10.1016/j.envres.2020.109111. October 2019.
Sparrow, L., Heimann, K., 2016. Key environmental factors in the management of ciguatera. J. Coast. Res. 1 (75), 1007–1011. https://doi.org/10.2112/SI75-202.1.
Sparrow, L., Momigliano, P., Russ, G.R., Heimann, K., 2017. Effects of temperature, salinity and composition of the dinoflagellate assemblage on the growth of Gambierdiscus carpenteri isolated from the Great Barrier Reef. Harmful. Algae 65, 52–60. https://doi.org/10.1016/j.hal.2017.04.006.
Steyaert, M., Priestley, V., Osborne, O., Herraiz, A., Arnold, R., Savolainen, V., 2020. Advances in metabarcoding techniques bring us closer to reliable monitoring of the marine benthos. J. Appl. Ecol. 57 (11), 2234–2245. https://doi.org/10.1111/1365-2664.13729.
Sullivan, T.J., Dhar, A.K., Cruz-Flores, R., Bodnar, A.G., 2019. Rapid, CRISPR-based, field-deployable detection of white spot syndrome virus in shrimp. Sci. Rep. 9 (1). https://doi.org/10.1038/s41598-019-56170-y.
Tara Ocean Project. (2022). Tara Oceans.
Tawong, W., Yoshimatsu, T., Yamaguchi, H., Adachi, M., 2016. Temperature and salinity effects and toxicity of Gambierdiscus caribaeus (Dinophyceae) from Thailand. Phycologia 55 (3), 274–278. https://doi.org/10.2216/15-111.1.
Tester, P.A., Feldman, R.L., Nau, A.W., Kibler, S.R., Wayne Litaker, R., 2010. Ciguatera fish poisoning and sea surface temperatures in the Caribbean Sea and the West Indies. Toxicon. 56 (5), 698–710. https://doi.org/10.1016/j.toxicon.2010.02.026.
Tester, P.A., Litaker, R.W., Soler-Onís, E., F.Z, J., Berdalet, E., 2014. comparison of articifial and natural substrates for Gambierdicus detection. Harmful. Algae.
Tester, P.A., Litaker, R.W., Soler-Onís, E., Fernández-Zabala, J., Berdalet, E., 2022. Using artificial substrates to quantify Gambierdiscus and other toxic benthic dinoflagellates for monitoring purposes. Harmful. Algae 120. https://doi.org/10.1016/j.hal.2022.102351 (August).
Thalinger, B., Deiner, K., Harper, L.R., Rees, H.C., Blackman, R.C., Sint, D., Traugott, M., Goldberg, C.S., Bruce, K., 2021. A validation scale to determine the readiness of environmental DNA assays for routine species monitoring. Environmental DNA 3 (4), 823–836. https://doi.org/10.1002/edn3.189.
Ting, J.Y.S., Brown, A.F.T., 2001. Ciguatera poisoning: a global issue with common management problems. In: European Journal of Emergency Medicine, 8. http://journals.lww.com/euro-emergencymed.
Trick, C.G., Anderson, L., Beausolei, D., Berdalet, E., Cochlan, W.P., Wang, P., & Wells, M.L. (2020). Economic Assessment of Ciguatera Outbreaks 4 An Economic Assessment of Ciguatera Outbreaks-An Island Model.
Tsaloglou, M.N., Laouenan, F., Loukas, C.M., Monsalve, L.G., Thanner, C., Morgan, H., Ruano-López, J.M., Mowlem, M.C., 2013. Real-time isothermal RNA amplification of toxic marine microalgae using preserved reagents on an integrated microfluidic platform. Analyst 138 (2), 593–602. https://doi.org/10.1039/c2an36464f.
Tsumuraya, T., Sato, T., Hirama, M., Fujii, I., 2018. Highly Sensitive and Practical Fluorescent Sandwich ELISA for Ciguatoxins. Anal. Chem. 90 (12), 7318–7324. https://doi.org/10.1021/acs.analchem.8b00519.
Tudó, À., Gaiani, G., Varela, M.R., Tsumuraya, T., Andree, K.B., Fernández-Tejedor, M., Campàs, M., Diogène, J., 2020. Further advance of Gambierdiscus Species in the Canary Islands, with the First Report of Gambierdiscus belizeanus. Toxins. (Basel) (11), 12. https://doi.org/10.3390/toxins12110692.
Van Dolah, F.M., Morey, J.S., Milne, S., Ung, A., Anderson, P.E., Chinain, M., 2020. Transcriptomic analysis of polyketide synthases in a highly ciguatoxic dinoflagellate, Gambierdiscus polynesiensis and low toxicity Gambierdiscus pacificus, from French Polynesia. PLoS ONE 15 (4). https://doi.org/10.1371/journal.pone.0231400.
Vandersea, M.W., Kibler, S.R., Holland, W.C., Tester, P.A., Schultz, T.F., Faust, M.A., Holmes, M.J., Chinain, M., Wayne Litaker, R., 2012. Development of semi-quantitative pcr assays for the detection and enumeration of gambierdiscus species (gonyaulacales, dinophyceae). J. Phycol. 48 (4), 902–915. https://doi.org/10.1111/j.1529-8817.2012.01146.x.
Villareal, T.A., Hanson, S., Qualia, S., Jester, E.L.E., Granade, H.R., 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. https://doi.org/10.1016/j.hal.2006.08.008.
Villareal, T.A., & Morton, S.L. (2002). Use of Cell-Specific PAM-Fluorometry to Characterize Host Shading in the Epiphytic Dinoflagellate Gambierdiscus toxicus.
Wang, B., Yao, M., Zhou, J., Tan, S., Jin, H., Zhang, F., Mak, Y.L., Wu, J., Chan, L.L., Cai, Z., 2018. Growth and toxin production of gambierdiscus spp. Can be regulated by quorum-sensing bacteria. Toxins. (Basel) (7), 10. https://doi.org/10.3390/toxins10070257.

Wang, D., Zhang, S., Zhang, H., Lin, S., 2021. Omics study of harmful algal blooms in China: current status, challenges, and future perspectives. Harmful. Algae 107. https://doi.org/10.1016/j.hal.2021.102079.

Wang, D.Z., Xin, Y.H., Wang, M.H., 2022a. Gambierdiscus and its associated toxins: a minireview. Toxins. (Basel) 14 (7), 485. https://doi.org/10.3390/toxins14070485.

Wang, Z., Liu, L., Tang, Y., Li, A., Liu, C., Xie, C., Xiao, L., Lu, S., 2022b. Phytoplankton community and HAB species in the South China Sea detected by morphological and metabarcoding approaches. Harmful. Algae 118. https://doi.org/10.1016/j.hal.2022.102297.

Wen, J., Yang, J., Li, Y., Gao, L., 2022. Harmful algal bloom warning based on machine learning in maritime site monitoring. Knowl. Based. Syst. 245, 108569. https://doi.org/10.1016/j.knosys.2022.108569.

Organisation, World Health, 2020. Report of the expert meeting on ciguatera poisoning. In: Report of the Expert Meeting on Ciguatera Poisoning. FAO and WHO. https://doi.org/10.4060/ca8817en.

Weng, X., Neethirajan, S., 2018. Paper-based microfluidic aptasensor for food safety. J. Food Safety 38 (1). https://doi.org/10.1111/jfs.12412.

Wu, Z., Luo, H., Yu, L., Lee, W.H., Li, L., Mak, Y.L., Lin, S., Lam, P.K.S., 2020. Characterizing ciguatoxin (CTX)- and Non-CTX-producing strains of Gambierdiscus balechii using comparative transcriptomics. Sci. Total Environ. 717. https://doi.org/10.1016/j.scitotenv.2020.137184.

Xu, Y., Richlen, M.L., Liefer, J.D., Robertson, A., Kulis, D., Smith, T.B., Parsons, M.L., Anderson, D.M., 2016. Influence of Environmental Variables on Gambierdiscus spp. (Dinophyceae) Growth and Distribution. PLoS ONE 11 (4), 1–30. https://doi.org/10.1371/journal.pone.0153197.

Xu, Y., Richlen, M.L., Morton, S.L., Mak, Y.L., Chan, L.L., Tekiau, A., Anderson, D.M., 2014. Distribution, abundance and diversity of Gambierdiscus spp. from a ciguatera-endemic area in Marakei, Republic of Kiribati. Harmful. Algae 34, 56–68. https://doi.org/10.1016/j.hal.2014.02.007.

Yang, Z., Luo, Q., Liang, Y., & Mazumder, A. (2016). Processes and pathways of ciguatoxin in aquatic foodwebs and fish poisoning of 1 seafood consumers 2. www.nrcresearchpress.com.

Yarimizu, K., Sildever, S., Hamamoto, Y., Tazawa, S., Oikawa, H., Yamaguchi, H., Basti, L., Mardones, J.I., Paredes-Mella, J., Nagai, S., 2020. Development of an absolute quantification method for ribosomal RNA gene copy numbers per eukaryotic single cell by digital PCR. Harmful Algae 103, 102008. https://doi.org/10.1016/j.hal.2021.102008.

Yasumoto, T., Bagins, R., Nakajima, I., Adachi, R., 1977. Finding of a dinoflagellate as a likely culprit of ciguatera. In: Nippon Suisan Gakkaishi, 43, pp. 1021–1026. https://doi.org/10.2331/suisan.43.1021. Issue.

Yates, M.C., Derry, A.M., Cristescu, M.E., 2021. Environmental RNA: a revolution in ecological resolution? Trends Ecol. Evol. 36 (7), 601–609. https://doi.org/10.1016/j.tree.2021.03.001.

Yoshida, T., Nakai, R., Seto, H., Wang, M.K., Iwataki, M., Hiroishi, S., 2003. Sequence analysis of 5.8S rDNA and the internal transcribed spacer region in dinoflagellate heterocapsa species (dinophyceae) and development of selective PCR Primers for the bivalve killer heterocapsa circularisquama. Microbes. Environ. 18 (4), 216–222. https://doi.org/10.1264/jsme2.18.216.

Yoshimatsu, T., Tie, C., Yamaguchi, H., Funaki, H., Honma, C., Tanaka, K., Adachi, M., 2016. The effects of light intensity on the growth of Japanese Gambierdiscus spp. (Dinophyceae). Harmful. Algae 60, 107–115. https://doi.org/10.1016/j.hal.2016.10.009.

Zha, K., Silvestri, N.J., 2022. Iatrogenic immunodeficiency-associated lymphoproliferative disorder secondary to mycophenolate mofetil treatment in a patient with myasthenia gravis. In: Muscle and Nerve, 65. John Wiley and Sons Inc, pp. E14–E16. https://doi.org/10.1002/mus.27485.

Zhan, A., MacIsaac, H.J., 2015. Rare biosphere exploration using high-throughput sequencing: research progress and perspectives. Conser. Genet. 16 (3), 513–522. https://doi.org/10.1007/s10592-014-0678-9.

Zhang, Z., Zhang, C., Luan, W., Li, X., Liu, Y., Luo, X., 2015. Ultrasensitive and accelerated detection of ciguatoxin by capillary electrophoresis via on-line sandwich immunoassay with rotating magnetic field and nanoparticles signal enhancement. Anal. Chim. Acta 888, 27–35. https://doi.org/10.1016/j.aca.2015.06.018.

Zheng, L., Gatti, C.M.iti, Garrido Gamarro, E., Suzuki, A., Teah, H.Y., 2020. Modeling the time-lag effect of sea surface temperatures on ciguatera poisoning in the South Pacific: implications for surveillance and response. Toxicon 182(May), 21–29. https://doi.org/10.1016/j.toxicon.2020.05.001.