Key points for Ciguatera risk assessment in the European Coasts:

Máster Universitario en Zoonosis y Una Sola Salud

Curso 2016-2017

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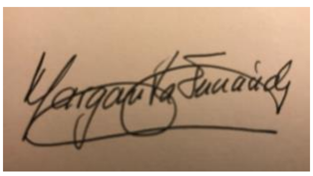

1. Index

Ciguatera Fish Poisoning (CFP), a global health problem

2. Abstract

In the recent years, different cases of Ciguatera Fish Poisoning (CFP) appeared in the European coastlines. CFP is produced by two dinoflagellate genus called *Gambierdiscus* and *Fukuoya* and recently, both insolated in the coastlines of Greece and Macaronesia islands due the new distribution change of these species induced by the Climate Change effects. Due the important repercussions that this could have in human and environmental health, economical and food safety in the European coastlines, the European Food Safety Authority (EFSA) started to create risk assessment and prevention plans against CFP. In this review we wanted to resume all the key points that we think are necessary to know about the biology, epidemiology, and environmental factors that affects these dinoflagellate genera and that are important for a good risk and prevention plans assessment. As the results show a great variability among the species that compounds the genus in environmental factors, biological characteristics and even in toxicity depending on the geographical region they are isolated, the new tendencies about modeling the potential risk of CFP points to a more localized risk and preventions plans, being assessed in function of the characteristic of the species and the environment present in the target area and not big extrapolations can be made.

3. Introduction

Ciguatera Fish Poisoning (CFP) is the most common non-bacterial disease caused by fish consumption in the tropical and sub-tropical areas of the planet (Kibler et al., 2012). This disease affects globally, between 25000 and 50000 people annually, although the incidence could be higher due the misreporting cases. (Berdalet, et al., 2017). This intoxication is caused by consumption of contaminated fish containing ciguatoxin (CTX), a potent thermo and cryo resistant toxin that produce gastrointestinal and, in some cases, cardiac and neurological symptoms in between 24-48h after consuming the contaminated fish. There’s no storage, preparation or cooking procedure that can destroy the toxin. (Friedman et al., 2017).

This toxin is produced by dinoflagellates of the genera *Gambierdiscus* and *Fukuoya* which produce the precursor agent and that one, accumulates and activates through the food web till arrives to the human consumption.

There are three types of CTX detected nowadays, Pacific CTX (P-CTX), Caribbean CTX (C-CTX) and Indian CTX (I-CTX), being the first one the most powerful and toxic (Bravo et al., 2015).

The effects of CFP are beyond the human health in the endemic areas.

Typically, the areas assumed endemic for CFP were the tropical and sub-tropical regions of the planet, where fishing and fish consumption were and are heavily important. These areas are also known by the heavy affluence of tourists and the role of tourism in the regional economics (Berdalet et al., 2017). These two points are the two big problems of CFP in this areas, forcing the communities to change their alimentary habits to avoid it and facing the repercussions on tourism by CFP outbreaks.

In the recent years this traditional point of view could change drastically, due the new CFP cases reported in more temperate areas of the planet, included the European coasts. Although imported cases of CFP were common in temperate areas, due the international seafood trade and travel, in 2004 the first autochthonous outbreak of CFP in the European coasts was reported. The outbreak occurred in Macaronesia islands, exactly in the Canary Islands, by consumption of Amberjack fish (Pérez-Arellano et al., 2005.) After this first autochthonous outbreak, others followed in the same European region.

To understand why this phenomenon is happening in the traditionally considered non-endemic areas (Boada et al 2010), it is necessary to focus on the first responsible of CFP, the genera *Gambierdiscus* and *Fukuoya.,* producers of CTX.

The biology, ecology and epidemiology of these dinoflagellate species depends in a great measure on the environmental factors occurring in the waters they live. It has been proved that Climate Change and other environmental events change their range of distribution globally and it is also proved that it could increase in the incoming years.

These changes in the geographical distribution of the CTX producers, turns CFP in a more important and global problem.

4. Objective

With this perspectives, the aim of this study is to make a compilation, filtration and synthetization of information related with the environmental factors that affect these dinoflagellates species, as well as to know and put together some points of their biology, ecology and epidemiology to create a background for the future risk characterization and assessment plans to prevent and predict new cases of CFP in the European coasts.

Due to the wide global distribution that CFP is going to take in the incoming years and the complexity and quantity of different factors that play a role in this intoxication by fish consumption, CFP requires to be assessed through a multidisciplinary way, treating it as a One Health Problem.

5. Materials and methods

The methodology used for this document consisted in conducting a systematic review of different scientific articles and reports related to CFP starting from the 40's and 50's, when CFP investigations began, to the most actual ones, 2017, following the criteria of the European Food Safety Authority (EFSA) for a systematic search of the appropriate information. Examples of these EFSA criteria are exposed in the Table 1, Table 2 and Table 3:

Table 1:
EFSA points to asses

Points to asses:

1. Studying the population dynamics of *Gambierdiscus* spp. and *Fukuyoa* spp.
2. Predicting blooms of toxin-producing microalgae and outbreaks of CFP.
3. Distinguishing the relative importance of local (production of ciguatoxins by local populations of *Gambierdiscus* spp. and transfer of toxins through the food webs) and imported (migration of ciguateric fish to these waters from distant ciguatera areas) contribution.
4. Understanding the ecological mechanisms leading to ciguatera toxins accumulation in fish.

Table 2:

EFSA questions to answer

Questions to answer:

1. What is the geographical distribution of *Gambierdiscus* and Fukuyoa species in the world?
2. Which are the temperatures in the places where these dinoflagellate species are present?
3. Which are other environmental variables that may determine the presence of these dinoflagellate species, their toxicity, the bioaccumulation to herbivores and carnivores and the possibility of an outbreak in the Macaronesia and the Mediterranean Sea?
4. Which is the seasonality in the different geographical areas worldwide for the presence and for the abundances of these dinoflagellate species in the different geographical areas worldwide?
5. Which are the fish and shellfish species that have been reported to contain ciguatoxins in the world?
6. Are these fish species present in European waters and where?
7. Which information is available on the trophic dynamics in the food web and the bioaccumulation process?
8. Which are the relevant modelling tools for predicting purposes?

Table 3

EFSA information search methodology

Methodology:

1. The protocol for the systematic literature searches will include the following keywords (individually or grouped) using the Boolean terms “and” and “or”: **ciguatera, CFP, fish poisoning, food poisoning, Gambierdiscus, Fukuyoa, ichthyotoxic**.

The information research has been done using the search portals PubMed and Google Scholar, always using the EFSA criteria statements for the paper selection.

The objective of the present document is to provide answers to most of the previous questions with the purpose of clarifying different aspects that remain unclear in reference to the biology, ecology and epidemiology of this toxic disease

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6. Results: The key points for Ciguatera risk assessment in the European coasts

6.1 CFP global distribution

6.1.1 Endemic distribution

CFP has a wide global distribution nowadays, but for many years this disease related to consumption of toxic fish was only described to the tropical and sub-tropical areas of the planet. The traditional distribution of CFP englobes from the Atlantic coasts of the USA, passing by the Gulf of Mexico and the Caribbean and the French Polynesia as a one of the biggest endemic areas of CFP in the world.

In exception of the USA coasts and certain parts of the Gulf of Mexico, most of the endemic areas correspond to islands.

In these islands, but as well of some coasts in the Gulf of Mexico and USA, fishery and fish consumption is on the most important activities, as much as for subsistence as an economically resource. Another reliable activity islands support is tourism and CFP affects directly to these main activities of these islands and coastlines: 1) Safety of daily meals in this areas is compromised, forcing these populations to change their protein source to new imported ones and 2) decreasing the tourism revenue. But not only, other problems associated to CFP are: 3) the reduction of the productivity due to illness, 4) due the insecurity, fisheries resources are underutilized, 5) the imported new protein is more expensive than local fish and finally, 6) the costs of a good prevention and control plans of the disease are expensive. (Berdalet et al., 2017). That's why CFP is an important problem in these endemic regions, and maybe that's the reason why investigation and study of CFP prevention are so extensive in those areas.

6.1.2 Non-endemic distributions

The cases reported in the temperate areas of the planet were, since recently, imported cases. These cases normally come from travelers who visit endemic areas (Mak et al., 2013, Bravo et al., 2015) or due the trade and travel of seafood and fish products from those to non-endemic ones (Kohli et al., 2015).

In Europe, different cases were reported between 2000 and 2013 in Germany and France (Epelboin et al., 2013, Mattei et al., 2014), but none from eating fish from the coastlines of continental Germany and France.

New distribution of CFP is changing worldwide.

This new CFP distribution to non-endemic areas includes the Eastern Atlantic Ocean of the northern hemisphere and the Mediterranean Sea (Bravo et al., 2015) and in consequence, the European coastlines.

In 2004 the first autochthonous species of *Gambierdiscus* are detected in the Macaronesia islands (Aligizaki et al., 2008), till then to 2012 cases of confirmed autochthonous CFP outbreaks appeared in those European islands by consumption of Amberjack fish fished near the Portugal island of Madeira (Pérez-Arellano et al., 2005, Bravo et al., 2015).

Other autochthonous CFP outbreaks may occur as well in the Greek coastlines since Aligizaki et al., 2008 have detected the presence of the toxic species in Greek waters.

These new autochthons CFP outbreaks in European areas were not the only ones. Nowadays CFP outbreaks are mainly reported from Australian, Indonesian and Japanese coastlines among others.

Maybe, in the recent years and the incoming ones, temperate areas of the planet need from the knowledge accumulated in the endemic ones to made their own prevention and protective plans.

6.2 CFP producers: dinoflagellates from the genera *Gambierdiscus* and *Fukuyoa*

6.2.1 Taxonomy and ecology

The genus *Gambierdisucs* was characterized in 1979 by Fukuyo and Adachi in the Gambier Islands, French Polynesia (Adachi and Fukuyo, 1979).

This marine dinoflagellate usually grows in tropical and subtropical waters as an epiphyte on macro algae in coral reefs, mangrove systems, and on artificial surfaces, sand or detritus (Caillaud et al., 2010, Friedman et al., 2017). These substrates provide protection and stability, protecting them from light and providing a bending system that permits these dinoflagellates to interact with other species and groups of the marine plankton, even bacteria.

As it will be commented later, the preference water conditions of these dinoflagellates to live are the calm, transparent, low depth and warm waters of the tropical and sub-tropical coastlines but they had been isolated in other different and more adverse conditions.

These dinoflagellates together with the recently erected *Fukuoya* genus are the producers of the precursor toxin that finally creates CTX and are linked to the CFP outbreaks worldwide.

In 1979, Adachi and Fukuyo described the first *Gambierdiscus spp.* as *Gambierdiscus toxicus*.

Previously, in 1977, Yasumoto et al. hypostatized the relationship of dinoflagellates with the production of CTX and consequently, to be the cause of CFP (Tester et al., 2014).

The genus *Gambierdiscus* remained as an only one specie genus till 1995 when Faust et al. proposed the multi species theory and isolating *G. belizeanus* in the Belize coastlines. Other studies availed this theory describing the variability of morphology, toxicity, physiological characteristics and differences in ribosomal RNA (rRNA) genes (Kohli et al., 2015).

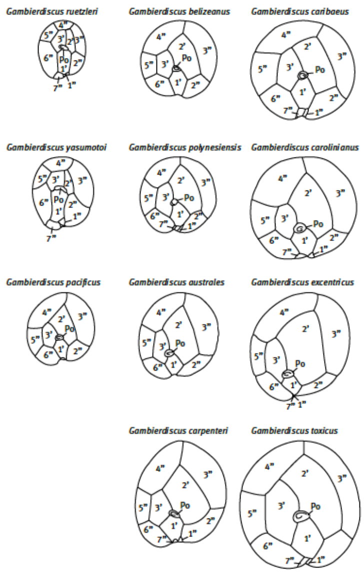

Figure 1. Morphology of the major species of *Gambierdiscus*. Modified from Litaker et al., 2009

After that, the diversification of the *Gambierdiscus* species grows exponentially, reaching to the actual number of 15 *Gambierdiscus* species, 5 genetically described as phylotypes and 3 *Fukuoya* species distributed worldwide.

The genus *Fukuoya* was introduced by Gomez et al. in 2015 when they realized that the morphological characteristics of *G. yasumotoi* and *G. ruetzleri* were found to be different enough to erect another dinoflagellate genus. The globular structure of this organisms in comparison to the anterio-posteriorly compressed one of the other *Gambierdiscus* species confirms that theory.

Nowadays, these two species of the previously *Gambierdiscus* genus are named *Fukuoya yasumotoi* and *Fukuoya rueltzleri* respectively and the present *Gambierdiscus* and *Fukuoya* species described are resumed in Table 4.

The morphology of the major species of those genera is resumed in figure 1, *Fukuoya* species are not included in figure 1, this figure was published by Litaker et al. in 2009.

Finally, the discoveries of new *Gambierdis*cus species has continued to the actuality, 2017. Species like *G.lapillus* discovered in the Great coral reef in Australia in 2016, *G. honu* in the Cook islands in 2017 by Rhodes et al., 2017, *G. balechii* in the Celebes Sea by Fraga et al. 2016 or *G.cheloniae* by Smith et al. 2016 and *Fukuoya paulensis* in 2015 by Laza et al. in the Balearic Islands are the newest discovered. This discoveries show that it may be that not all *Gambierdiscus* and *Fukuoya* species have been discovered yet, and if we add the great inter and intra variability among species and strains that this genus present in the recent laboratory studies the global distribution of *Gambierdiscus* could be more extensive than the one given by the studies in the field.

Taxonomic identification of different species of *Gambierdiscus spp.*

Species
Globular species
F. yasumotoi (1998)
F. ruetzleri (2009)
F. paulensis **(2015)**
Anterio-posteriorly compressed species
G. belizeanus (1995)
G. pacificus (1999)
G. excentricus (2011)
G. australes (1999)
G. caribaeus (2009)
G. carpenteri (2009)
G. toxicus (1979)
G. polynesiensis (1999)
G. carolinianus (2009)
G. balechii **(2016)**
G. cheloniae **(2016)**
G. honu **(2017)**
G. lapillus **(2017)**
G. silvae (2014)
G. scabrosus (2014)
Genetically described phylotypes
Gambierdiscus ribotype 1
Gambierdiscus ribotype 2
Gambierdiscus sp. type 1
Gambierdiscus sp. type 2
Gambierdiscus sp. type 3

Table 4. *Gambierdiscus* and *Fukuoya* actual species.
Summarized from Kohli et al., 2015.

Finally, as Berdalet et al. 2017 present in their study, reliable taxonomy is fundamental for efficient monitoring systems to know the real distribution, toxicity and to interpret the environmental influences of the *Gambierdiscus* population dynamics and, as Tester et al 2014 stated: “It should be noted that in an active area of research, it is not unexpected that taxonomic revisions will be necessary.”

6.2.2 Global distribution

Due to the great diversity of the species, the distribution of these dinoflagellate genera is worldwide. Typically, the main distribution was in the tropical and sub-tropical areas and at low latitudes (~35 °N–35 °S), where the climatology and water conditions are more favorable to that kind of microalgae (Pérez-Arellano et al., 2005, Kibler et al., 2015, Kohli et al., 2015). Recently this has changed, due 1) the change of the climate and water conditions in great part cause the Climate change and 2) the identification of new species of both genera in more temperate areas of the world, possibly due the first factor.

Actually, the genera *Gambierdiscus.* and *Fukuoya* are distributed globally, affecting several coastlines worldwide.

Some species as *G. caribaeus*, *G. carpenteri* and *G. belizeanus* are distributed widely, present in both Atlantic and Pacific Oceans and in several other coastlines around the world due their great adaptability to the different conditions of environmental factors that affect their survival in the environment (Litaker et al., 2010, Berdalet et al., 2017).

On the other hand, some species are less adaptable to the changes in the ranges of environmental factors. Their distribution is more closely related to some punctual and individual places, were the water and environmental conditions play a better role to their necessities.

It's not unusual that more than one species of these dinoflagellates coexist in the same coastlines.

Geographic distribution of different *Gambierdiscus spp.* species

G. toxicus	Tahiti, French Polynesia, Mexican Caribbean, New Caledonia, Reunion Island, Indian Ocean, Nha Trang -Vietnam
F. belizeanus	Belize, Florida, Mexican Caribbean, Malaysia, Pakiostan, Queensland, Australia, St. Barthelemy Island – Caribbean
F. yasumotoi	Singapore, Japan, Mexican Caribbean, Queensland, Australia, Nha Trang – Vietnam
G. australes	French Polynesia, Japan, Cookislands, Hawaii, USA, Pakistan
G. pacificus	French Polynesia, Marshall Islands & Society Islands, Micronmesia, Kota Kinabalu and Sipandan Island, Nha Trang – Vietnam
G. polynesiensis	French Polynesia, Canary Islands, Pakistan, Nha Trang - Vietnam
G. caribaeus	Florida, Belize – Caribbean, Tahiti, Palau, Hawaii, Flower Gardens – Gulf of Mexico, Osho Rios –Jamaica, Bahamas, Grand Caymam Islands, Tol-truk Micronesia, Jeju Island Korea
G. carolinianus	North Carolina, USA, Atlantic ocean, Bermuda, Mexico, Puerto Rico, Flower Gardens – Gulf of Mexico, Osho Rios – Jamaica, Crete – Greece
G. carpenteri	Belize, Guam, Fiji, Hawaii, dry Tortugas – Florida, Flowers Gardens – Gulf of Mexico, Osho Rtios – Jamaica
G. ruetzleri	North Carolina, USA, Belize – Caribbean
G. excentricus	Canary Islands
Gambierdiscus ribotype 1	Belize – Caribbean
Gambierdiscus ribotype 2	Belize – Caribbean, Martinique – Caribbean, Puerto Rico
Gambierdiscus sp. type 1	Japan
Gambierdiscus sp. type 2	Japan
Gambierdiscus sp. type 3	Japan

Table 5. Major Gambierdiscus species and their global location. Summarized from Gurjeet S. Kohli et al., 2015

The coastlines affected worldwide goes from the Atlantic and Pacific ones to the Australian, Indonesian, Japanese, Red Sea coastlines and the Europeans coasts of Greece and Macaronesia islands.

The actual distribution of the major *Gambierdiscus* and *Fukuoya* species is resumed in table 5 and figure 2. The new species *G. balechii*, *G. cheloniae*, *G. honu*, *G. lapillus* and *F.paulensis* are not included in figure 2 since they were described very recently.

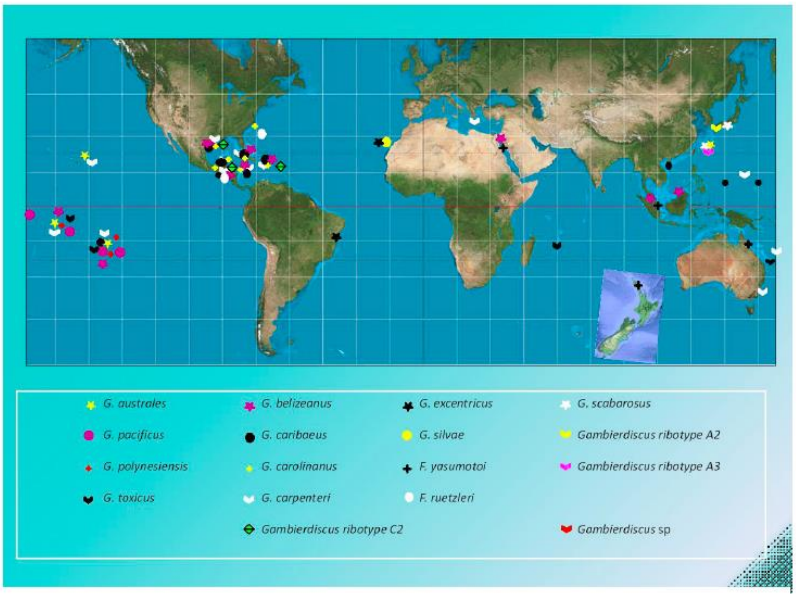

Figure 2. Global distribution of the major *Gambierdiscus* and *Fukuoya* species. Summarized from Tester et al., 2014.

This actual global distribution of the two dinoflagellate genera is just a temporal picture. Recent studies show that due the Climate change and the change of environmental factors that affect these species, a new redistribution of the species from both genera could occur in the incoming years. (Kibler et al., 2015, Berdalet, et al., 2017).

Different studies affirmed that this change is not going to happen as an invasion of tropical and subtropical species to more temperate waters, instead as a specie displacement from one ecological niches to others. An example is the one that Kibler et al., 2015 explains in their study, the prevalent species in the tropical regions of the Great Caribbean and Gulf of Mexico are going to change their own distribution.

As the Caribbean region change their seawater temperatures (SST) in 1-2 °C, the species that tolerates higher water temperatures are going to become more prevalent in this concrete region. Likewise, in the coasts of the Gulf of Mexico and the Atlantic coasts of USA where water temperatures are lower in annual average, species more resistant to low and mild temperature are going to increase their range of activity.

In the case that water temperatures will be not low enough due the Climate Change then the coexistence of diverse species in the same coasts will be the result, incrementing the risk of CFP outbreaks in these more temperate areas, as it is explained below.

This redistribution pattern of *Gambierdiscus* and *Fukuoya* species explains why new species are appearing in the considered non-endemic areas of the planet.

6.2.3 Toxicity

CFP is produced by the ingestion of toxins of the type CTX. This kind of toxin are sodium channel activators that affect the voltage sensitive channels located along the nodes of Ranvier (peripheral nerve cells) (Tester et al., 2014). There are three major families of CTX: 1) P-CTXs (Pacific Ocean), 2) C-CTXs (Caribbean region) and 3) I-CTX (Indian Ocean) (Mak et al., 2013).

The intoxication produces gastrointestinal symptoms in the first 24-48h, but not only, neurological symptoms and, in severe intoxication, cardiovascular ones could appear.

Control of CTX is difficult, this toxin is thermostable, cryostable and no cook or preparation methods can destroy it (Kohli et al., 2015).

It's formed by a cyclic polyether ladders which are lipophilic and that's why different studies affirm that the body parts with more CTX concentration in fish are the viscera instead of flesh of fish. It plays an important role in the bio magnification and accumulation trough the food web (Haro et al., 2013).

MTX or maitotoxin is another toxin produced by the genus *Gambierdiscus* and *Fukuoya* (Tester et al., 2014). This toxin is considered the largest non-proteinous and highly toxic natural products known, but their role with CFP is not yet clarified. It's a highly potent calcium channel inhibitor but their complete mode of action and primary target in mammalian cells have not yet been fully elucidated (Kohli et al., 2015).

As CTX, there's different kinds of MTX discovered in various areas of the world, P-MTX (Pacific Ocean) and C-MTX (Caribbean ocean) and it seems that the biophysical mechanisms of each one are quite different.

Despite that, they normally have less tendency to accumulate in fish flesh and more to the stomach and intestines so, as CTX, in the fish viscera (Kohli et al., 2015).

The relation of CTX/MTX and *Gambierdiscus.* and *Fukuoya* species is clear.

CTX and MTX had been isolated from different species of *Gambierdiscus.*

An example could be *G.excentricus*, considered the major producer of CTX in the Caribbean region (Berdalet et al., 2017). However, the definition: "in the Caribbean region" is maybe the important thing here, no consensus is already accepted in reference to which species of *Gambierdiscus* are more or less toxic, more CTX productive or where they could be in the planet.

The handicap about that is that the major studies in toxicology were made when *G. toxicus* was considered the only specie known, so no distribution among species could be assessed by those studies (Kohli et al., 2015).
Another theory about that phenomena are related to the huge inter specie variability and the low toxicity studies available. But not only, some authors refer that the intra specific variability that could show some strains of the same *Gambiersdiscus* specie could lead to be more toxic ones than others (Litaker et al., 2010), making this process to elucidate the real toxicity of the species more complex.
This could explain why some species in a concrete area of the planet could be considered high toxigenic and in other areas they are not.
At least, more studies are needed to clarify the toxin profile of all of the species and genotypes of the *Gambierdiscus* and *Fukuoya* now known. That's an important matter in terms of create good and personalized risk assessment and prevention plans in the European coastlines.

6.3 Food web relations

How CFP arrives to the human consumption or his food web relation had been discussed since the beginning of CFP cases. The most accepted theory is the simplest one. *Gambierdiscus* and *Fukuoya* grows in dead and live coral reef. Herbivorous fish searching food in those zones became toxic by ingestion of CTX precursor toxin, that this one at his time, suffers a biotransformation to CTX inside them (Bravo et al., 2015). Carnivorous and omnivorous fish who eats herbivorous finfish accumulates the toxin in their bodies, mostly in the head, liver or viscera due the high lipophilic character of that toxin (Haro et al., 2013). When this major toxic fishes are fished and eaten by humans, CFP cases appear.
These food web relations are showed in Figure 3. Realize that in the low stratum natural disturbances and human disturbances are showed, that's because these disturbances are one of the major causing of *Gambierdiscus* population proliferation, as we will discuss later.

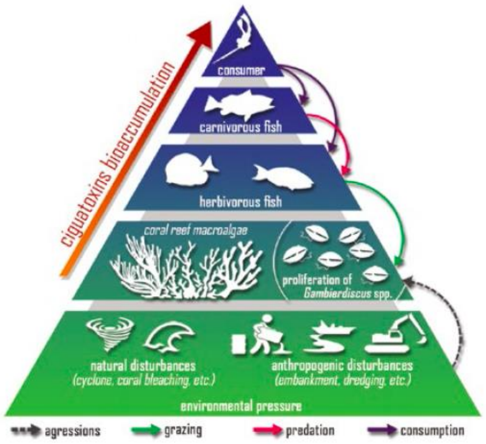

Figure 3. Food web relations. Modified from Chinain et al., 2016

Nowadays, thanks to recent studies, we know that these relations are not as simple as it seems. The complexity of the food web relations of CFP and how it arrives to the human consumption comes for many reasons.

CFP toxicity in a determinate fish depend mostly in their alimentary habits, their efficiency of assimilation, depuration rate and growth capacity. (Mak et al., 2013, Gaboriau et al., 2014).

These factors had been long studied in different endemic zones and there's no general consensus yet. Some studies affirm that toxin depuration by fish could not exist or exist in low rates, but others show that differences between fish families had been documented in terms of toxin assimilation, metabolizing and excretion. (Gaboriau et al., 2014).

It is true that there are some fish families considered to be high risk for CFP intoxication and they are resumed in table 6, but lots of studies show that to ensure which specie could be a risky one, more local studies had to be done. However, it's true that there's no evidence that we can generalize for all the fishes in the world, mostly due these inter-specie differences.

Another accepted theory was that big fishes had more possibilities to be CTX toxin positive.

Studies made in the endemic region of French Polynesia had shown that even the small reef herbivory fish can accumulate amounts of CTX enough to cause CFP to an adult person. (Gaboriau et al., 2014).

Moray eel (*Muraenidae*)
Barracuda (Sphyraenidae)
Grouper (Serranidae)
Jacks (Carangidae)
Amberjack (Carangidae, g. *Seriola*)
Snapper (Lutjanidae)
Surgeon fish (Acanthuridae)
Parrot fish (Scaridae)
Wrasses (Labridae)
Hogfish (Labridae, g. *Lachnolaimus*)
Narrow barred mackerel (Scombridae, g. *Scomberomorus*)
Spanish mackerel (Scombridae, g. *Scomberomorus*)
Trevally (Carangidae, g. *Caranx*)
Triggerfish (Balistidae)

Table 6. Common ciaguatoxigenic fish species. Modified from Friedman et al., 2017.

As same happens with the theory of high risk family fishes, length and weight of fish is not good criteria to select fishes for consume, and no advantages had reported in the endemic areas where applied these methodologies as a prevention one (Gaboriau et al., 2014, Friedman et al., 2017).

Also, we have to remark that not only fishes could be toxic for human consumption. Clams, mollusks and even octopus had shown amounts of CTX in their bodies enough to cause CFP, and some of them, due filtration, an accumulation of *Gambierdiscus* species that can produce CTX (Roue et al., 2016). This phenomenon is called CSP and it makes visible that not only fishes are risky for human consumption and that food web relation for CFP intoxication could be more complex than we think (Mak et al., 2013, Kohli et al., 2015).
That's why in this study we propose that the best way to asses that toxic consumption problem is to propose more studies trying to bring some light to which can be risky fishes and toxic seafood in a local area. We are not capable of make generalizations in terms of fish toxicity and the studies had to be more localized together with an effort to develop new and more efficient mechanisms to CTX detection in ways to help investigations and even veterinarians to detect as quickly as possible new potential cases of CFP in the commercial points of fish distribution.

6.4 Environmental key points

Environmental factors rule a fundamental role in the biology, ecology and epidemiology of *Gambierdiscus* and *Fukuoya* species.
As we commented before, since 1995, the genus *Gambierdiscus* remained as a unique specie genus, and most of the studies trying to understand the influence of these factors are not useful.
In the last years, new studies had tried to figured out who much factors as temperature, salinity or irradiance, among others, affects this genus global distribution.
The two first factors mentioned had been established as the most important ecological drivers of this dinoflagellate biology (Kibler et al., 2012, Sparrow et al., 2017).
The results, show a great diversity among species, as it could be supposed, but not only, the intra-specie variability that these dinoflagellate genus presents, could play a vital role in the future prevention plans instauration.
The ongoing theory is that these plans will need to be ideated thinking in the local species that habits in an area, more than being considered as a global prevention plans. (Xu et al., 2016).

Nevertheless, in this study we try to show the latest results available in the bibliography about the environmental effects and biological limits of the *Gambierdiscus* and *Fukuoya* species. The description of the biological limits is going to be done using the following nomenclature: **Xo**: lower limit for growth, **Xu**: maximum limit for growth, **Xm**: maximum growth levels, **Xop**[1]: optimum growth range for parameters Salinity (**S**), Irradiance (**I**) and Temperature (**T**).

[1]This value shows the optimal parameter when growth rate is > or equal to 0,8 μmax,

6.4.1 Salinity

Salinity had been described by the bibliography as the second most important parameter which affects the biological distribution of the *Gambierdiscus* genus.
The general biological parameters are: 25-35 for **Sm,** which coincide with the normal salinity ranges in the most oceans of the world. From 14 to 21 and 41 or even more for the **So** and **Su** respectively. And finally from 22 to 36 in the range for **Sop.** Even so, these biological parameters are showed in table 7. for different species.

Species	S_m	S_{opt}	S_0	S_u
G. australes	32.1	20.4–38.6	15.0	>41
G. belizeanus	28.4	22.4–36.7	18.3	>41
G. caribaeus	35.0	20.9–39.4	14.5	>41
G. carolinianus	30.3	25.7–36.0	20.9	>41
G. carpenteri	27.3	19.6–39.1	<14	>41
G. pacificus	29.9	23.7 to >41	<18	>41
Gambierdiscus ribotype 2	30.5	24.7–35.1	17.8	39.4
G. ruetzleri	24.7	19.6–35.7	15.9	>41

Table 7. Salinity biological parameters of different *Gambierdiscus* species. Modified from Kibler et al., 2012.

Different studies show that the wide range in salinity parameters that *Gambierdiscus* species can resist goes from 16 to 41. (Xu et al., 2016, Tawong et al., 2016).
This great variation in the salinity biological ranges is due the inter-specific differences that these dinoflagellate species show (Tawong et al., 2016).
It's worth it to comment that normally salinities in bellow 20 dinoflagellate populations decrees dramatically in the most of the species, but as we saw, **So** can reach levels till 14, corroborating a great difference in between resistances (Kibler et al., 2012).
The same can be appreciated for **Su**, laboratorial studies affirmed that some species of this dinoflagellate genus can reach till 43 or even 50 in salinity range resistance.

This different resistances and tolerances to different ranges of salinity among species are showed in figure 4.

This phenomenon could be one of the reasons of the great diversity and expansion of different species in diverse coastlines around the world, and why some species are distributed more widely (Kibler et al., 2012, Tawong et al., 2016).

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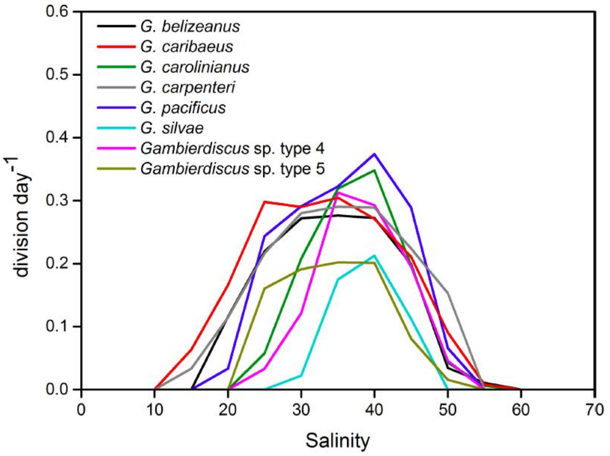


Figure 4. Inter-specific differences in growth over different salinity conditions. Modified from Xu et al., 2016.

6.4.2 Irradiance

As an ephitic photosynthetic dinoflagellate genus, irradiance have an important effect in the growth of *Gambierdiscus* and *Fukuoya* in the normal habit of these dinoflagellate. The average irradiance that these microalgae is exposed normally is of 10% as suggested by Kibler et al., 2012.

The information that irradiance can give to us is useful to determinate the vertical distribution of this genus (Xu et al., 2016). The same author cited before stipulated that in the normal habits of the dinoflagellate these can reach deeps of 75m and in laboratorial conditions even can be found till 125-150m deep where only blue and violet light arrives, the needed for photosynthesis to be done.

In general, the biologicals parameters stipulated for irradiance tolerances are in between 2,5 – 10% for **Im** that equals to 49– 231µ mmol photons m^2. s^1 and the minimum grow rates or **Io** reaches 0,2 and 0,7% of surface irradiances that is equal to 6 and 17 mmol photons m^2. s^1. These values are consistent among all the species, resumed in table 8.

Nevertheless, for **Iu,** differences between species are observed. The average threshold limit tolerance for irradiation in the most of *Gambierdiscus* species is 690 mmol photons m^2. s^1 but *F. ruetzleri* can reach irradiances above 1000 mmol photons m^2. s^1, a lethal irradiance for the rest of the species.

These irradiances are much superior to the 10% stablished by Kibler et al., 2012 as a maximum growth threshold.

Berdalet et al., 2017 explains in his study that mucus production, shadowing strategies using the substrates this dinoflagellate genus is attached at and symbiotic relations with other microalgae to increase cell densities, helps *Gambierdiscus* cells to protects themselves from high irradiation. That's why high irradiations are better accepted than lower ones (Richlen et al., 2011, Parsons et al., 2012).

Finally, returning to vertical distribution, non consensus has arrived when the laboratories results are compared to the field studies results.

Kibler et al., 2012 assumes that *Gambierdiscus* cells could be found till 75 m deep, as we comented before, but, other authors declare that in French Polynesia is more common to find them in 2-3 m deep than in 10-15m. In Johnston Atoll in the Pacific Ocean, 13m deep is usual and in the US Virgin island *Gambierdiscus* could form blooms in between 10 to 20m deep (Richlen et al.,2011, Yoshimatsua et al., 2014, Xu et al., 2016).

[2] these profundities are only speculations in functions of the laboratorial results, the field samples never reach the 30 or 40 meters.

Species	I_m (µmol photons m^{-2} s^{-1})	I_{opt} (µmol photons m^{-2} s^{-1})	I_0 (µmol photons m^{-2} s^{-1})	I_u (µmol photons m^{-2} s^{-1})
G. australes	49	24–108	9	293
G. belizeanus	89	40–216	6	>700
G. caribaeus	101	46–243	12	>700
G. carolinianus	88	58–115	15	156
G. carpenteri	151	55–388	9	>700
G. pacificus	156	108–205	17	294
Gambierdiscus ribotype 2	89	43–185	12	691
G. ruetzleri	231	70 to >700	12	>700

Table 8. Irradiance biological parameters of different *Gambierdiscus* species. Modified from Kibler et al.,2012.

6.4.3 Temperature

Temperature is considered the main environmental factor that explains the actual redistribution of the *Gambierdiscus* and *Fukuoya* species to the non-endemic areas of the planet.

This redistribution is due the increment of the Sea Surface Temperatures (SST) due the Climate Change (Kibler et al., 2015).

The differences in the biological limits for temperature among the different species are resumed in table 9. The average general parameters are in between 25– 31 C^o for **Tm** and the upper and lower thermal limits reaches in between 31-34 C^o and 15-21 C^o respectably for **Tu** and **To**. However, different studies affirmed that some species are capable to survive in temperatures in below 11-14 C^o in laboratorial assays and above the 35 C^o in some regions of the Red sea and the Pacific. This is explained due the inter and intra-variability presented by these dinoflagellate genus species (Kibler et al., 2012, Tawong et al., 2016).

As we commented before, the Climate change is expected to increase the SST in the Great Caribbean Region (GCR) in 1-2 C^o in the next decades (Kibler et al., 2015) and in different studies, the shapes of the temperature vs. growth curves indicated that even small differences of 1 or 2 C^o notably affected growth potentials of this genus.

These discoveries explain and define the recent and incoming redistribution of the *Gambierdiscus* species in the endemic areas and consequently the increases of CFP outbreaks in certain areas.

As well, this phenomenon could explain the apparition of CFP cases in the more tempered areas of the planet, the potential new endemic areas, as the European coastlines. It is supposed to happen not as an endemic areas species invasion but as a new adaption of autochthonous *Gambierdiscus* species that remand in minority due the lower temperatures. This theory is proposed by Rodríguez et al., 2017 in his study about the new cases of CFP in the Canary Islands.

Species	T_m (°C)	T_{opt} (°C)	T_0 (°C)	T_u (°C)
G. belizeanus	28.1	24.7–30.4	19.7	32.7
G. caribaeus[a]	31.1	29.2–32.4	16.8	33.6
G. carolinianus	26.5	23.8–28.7	15.0	31.7
G. pacificus	26.9	23.2–30.2	19.6	32.6
Gambierdiscus ribotype 2	27.2	24.0–29.1	19.8	31.1
G. ruetzleri	29.0	26.0–31.2	16.4	33.1

Table 9. Temperature biological parameters of different *Gambierdiscus* species. Modified from Kiblet et al., 2012

6.4.4 Intra-specific variations

The studies that describe the biological levels of that three main environmental factors describe too an intra-specific variation among the *Gambierdiscus* species.

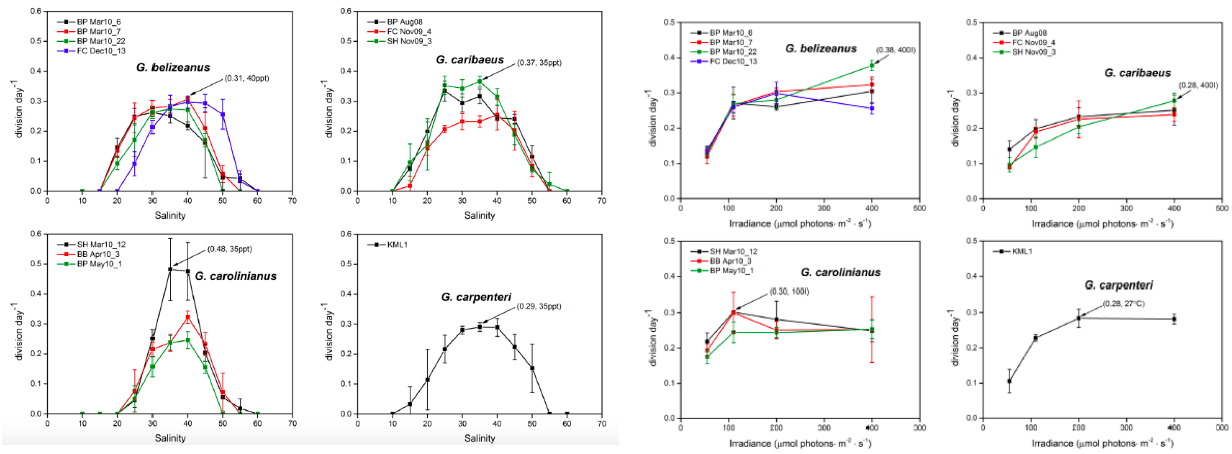
In salinity levels, Sparrow et al., 2017 expose intra-specific variation resistances to this environmental factor from strains of *G. carpenteri*. in the great coral reef of Australia and in irradiation ones, Yoshimatsua et al., 2016 described different growth responses of *Gambierdiscus* under various light conditions that may vary between strains of a given species such as *G. australes*.

These intra-specific variations are normally seen in the extremes of the environmental factors tolerances and can drive some species to be more tolerant to one or another factor in depend on the regionality they are.

These makes strong the theory proposed in this study that the prevention and risk assessment plans have to be done in a local or regional are, studying the spices presents in there and the strains that compound them, as well as the local ranges of environmental factors tolerances (Richlen et al.,2011).

Finally, as Yoshimatsu et al., 2014 said in his study: "growth characteristics of each species/phylotype have to be investigated using several strains for each species/phylotype, because growth characteristic and toxicity potential can vary between strains within a species".

Figures 5. and 6 illustrates that intra-specific variations among strains in the extreme adaptability levels.



Figures 5 and 6. Intra-specific variations among different *Gambierdiscus* species in Salinity and Irradiance growth rates. Modified from Xu et al., 2016.

6.4.5 Nitrates

Nitrates can play an important role as a limiting factor for cell growing.
No only, some studies show that could be an important factor affecting toxin production. The low concentrations of nitrates in the environment normally decrease Gambierdiscus populations, but as the results of the study published by Lartigue et al., 2008, show, some strains of G.toxicus can increment their toxin production when nitrates are limiting.
This toxin production increase normally occurs during the stationary phase, not into the exponential one. However, the toxin that Lartigue et al., 2008 results show to are MTX.
Another interesting discover is that not all the nitrate substrates can be used by *Gambierdiscus*, species, for example putrescine and free amino acids (FAA) are only used when these dinoflagellate is in combination with bacteria and urea is totally negative correlated with growth.
Other interesting characteristics of nitrates on *Gambierdiscus* growth is that these genus is capable to do future growth retaining amounts of nitrates in the days before. That's why growth rates are better if nitrates are delivered in pulse. This could allow the genus to continue growing in the field instead the nitrates are over (Lartigue et al., 2008).
Due this affirmation, we ca conclude that nitrates are not an environmental factor that affects so much in the distribution of these dinoflagellate, at least no as salinity, irradiance or temperature does.

6.4.6 Macro algae substrate

As we know Gambierdiscus and Fukuoya. are ephitic dinoflagellates, but it has been demonstrated that no all macro algal hosts are preferred to them, they are conditional ephitic organisms (Parsons et al., 2012, Rains, 2015).
Known witch kind of macro algal hosts is preferred by the species attachment and which ones can stimulate growing it's important to understand where and why these dinoflagellates are.
The main macro alga's substrates that Gambierdiscus uses in the endemic areas are resumed in figure 7 together with it's effects on growth cell densities.
Little is known about how micro algae substrates interacts whit this genus, but some studies confirm that, as we had commented, there are some kind of macro algal who could stimulate

the growth of this dinoflagellates meanwhile others can suppress it (Richlen et al.,2011, Rains, 2015).

Other important factor is the ephitic behavior, attachment or not attachment of the dinoflagellates to the macro algal structure. Studies as the one presented by Rains, 2015 had studied these relationships with the main common macro algae that *Gambierdiscus* uses in the main endemic areas, the same resumed in Figure 7.

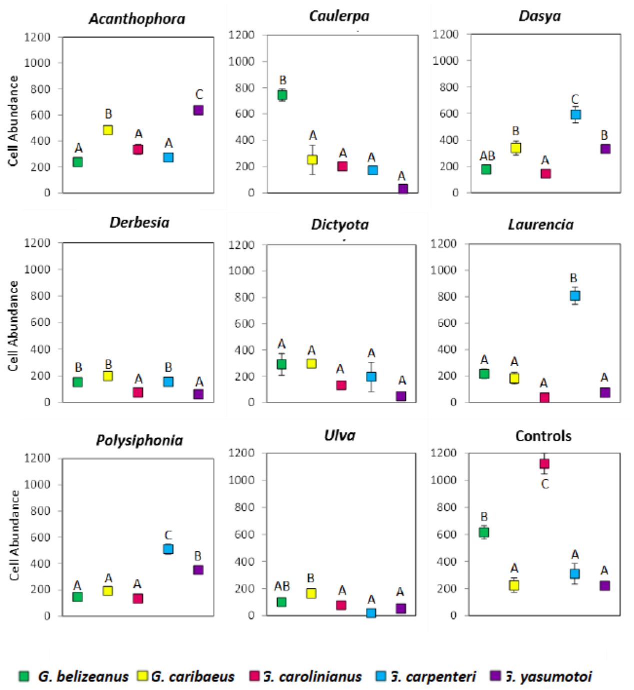


Figure 7. Growth densities in different macro algal substrates. Modified from Rains, 2015.

The results show that in growth rates, there’s a high variability among *Gambierdiscus* species and different macro algal substrates.

The different species grows more or less in cell densities among the different substrates.

However, the macro algal substrate that shows to provide more growing among the different species is *Acanthophora* from the phyla Rhodophyta and the species *G. caribeaus* the one that grew faster in mostly all macro algal substrates.

In the other hand, species as *G. carolinianus* are completely inhibited in all macro algal substrates. This could happen because this specie could be a low competitor for nutrients or because his absorbing rates are lower than the other species. Related to ephitic behaviour, attachment vs non attachment results shows that macro algae *Polysiphonia* (Rhodophyte) and *Derbesia* (Chlorophyte) from different phyla but with the same

Species	Overall average attachment	Host with highest average attachment	Host with highest growth (# cells at end)
G. belizeanus	15.74 %	*Polysiphonia,* 33.43 %	*Caulerpa* 744
G. caribaeus	19.21 %	*Polysiphonia,* 35.93 %	*Acanthophora* 479
G. carolinianus	20.40 %	*Polysiphonia,* 44.50 %	Control 1022
G. carpenteri	16.51 %	*Derbesia,* 50.03%	*Laurencia* 807
G. yasumotoi	4.56 %	*Derbesia* 12.29%	*Acanthophora* 638

Table 10. Attachment rates of *Gambierdiscus* species in different macro algal substrates. Modified from Rians, 2015.

filamentous structure provides better attachment than other macro algal substrates with different structure, although, they provide less substrate than others, as resumed in table 10. This suggest that for these dinoflagellate genus, attachment depends more in the structure than the nutrients provided (Nakahara et al., 1996).
Overall, in many cases, the hosts with the lower attachment percentages were the ones that showed the most *Gambierdiscus* cell growth. This may suggest that conditions of the microenvironment around host algae like nutrient levels, stimulatory or inhibiting metabolites, etc. may be influencing without physical attachment to that host.
As a related characteristic, these studies also had showed that palatability of the macro algal substrates is an important factor in the risk to make pass CTX trough the food web.
Finally, we have to comment that these macro algal relationships are studied with the ones found typically of the endemic zones. To really know who this can affect the European coastlines, studies with the local macro algal substrates had to be made.

6.4.7 Seasonality

Seasonality is an important point to keep in mind in the risk assessment plans. This phenomenon is linked to the water temperatures and directly related to the distribution of the *Gambierdiscus* species.
Two main ideas had to be commented about seasonality. The distribution of dinoflagellate among winter and summer seasons, or cold and hot seasons, and the high variability that this can cause depending on the profundity of the water.
General consensus had arrived in the fact that the areas where hot and cold season are very differentiated, the changes in dinoflagellate species distribution due the water temperature changes is marked. Recent studies show that normally the CFP cases outbreaks are happening at the end of the hot season, that’s explained because areas where the temperature is too low in the winter season to permit cell growing become more prevalent in the hot season due the temperature increase of the SSTs (Kibler et al., 2015). Another factor that makes the end of the hot season more prevalent of CFP outbreaks is the great effluence of tourism and travelers that visit the endemic areas in that season.
This diversification of *Gambierdiscus* species and consequently, due the variability in toxicity, the high risk to induce CFP outbreaks had seen more marked in the low depth coastlines, where the distance between the surface of the water and the sand is lower.

In the other hand, in the areas of the planet where the difference between summer and winter is less marked, like in the European coastlines of the Canary Islands, this diversity of dinoflagellates is more stable, but unfortunately, this can produce more proliferation of the present *Gambierdiscus* species, increasing the risk of creating blooms (Rodríguez et al., 2017).

6.4.8 Environmental phenomena

The major environmental phenomena affecting the worldwide distribution of *Gambierdiscus* and *Fukuoya* species and consequently, the new epidemiology of CFP, is the Climate Change. Their effects both into the endemic and non-endemic areas are going to be discussed later, but Climate Change is not the unic environmental phenome that has been described affecting these dinoflagellate biology, but maybe it's the causing about some of them.
Others environmental factors affecting *Gambierdiscus* species biology are:

- **Coral Bleaching:** Coral bleaching is the previous stage to the death of a coral reef. It happens when the water temperature exceeds in 1-2 Cº the normal temperatures for at least four weeks. When it happens, the coral reef suffers a biological shift, macro algal substrates invade the skeletons of the death coral, providing to *Gambierdiscus* substrate to be attached at and growth. (Cheal et al.,2010, Sparrow et al., 2017).
  It's true that this phenomenon is linked to Climate Change, but the consequences in the proliferation of *Gambierdiscus* and to the different species of the genus, can affect the coastlines around the world (Hales et al.,1999).
- **Hurricanes and tropical storms**: Another environmental factor linked to the Climate change. Different studies had linked this kind of environmental disaster to the increase of *Gambierdiscus* cell densities and in some cases the CFP outbreaks in a period of time around 13-17 month after the disaster occurrence. This is called outbreak lag., or the time between that the environmental conditions change and the first CFP outbreaks or *Gambierdiscus* blooms appear.
  It's true that intensive amount of water delivered in a short period of time into a concrete zone can decrease the salinity of the water, and the high disturbances that

hurricanes creates in the water movement can decrease the dinoflagellates communities, but maybe this is the reason why these phenomena produce CFP outbreaks in a large time period (Chateau-Degat et al., 2005).
Decreased salinity could select different species of *Gambierdiscus* more resistant to it, and even more toxic than the previous ones, and the water motions together with the low salinities are the managers who make possible the elimination of the previous dinoflagellate species.

- **Human disturbances:** These non-environmental disturbances are not linked to the Climate change, the human being can affect the biology of the *Gambierdiscus* species due the destruction of the marine environment, pollution and overfishing (Sparrow et al., 2017). These human effects produce the same consequences as could produce coral bleaching, providing macro algal substrate to the dinoflagellate genus (Lartigue et al., 2008).
Even so, as happens in coral bleaching no direct rations with increases of CFP outbreaks had been stipulated, but positives correlations to the increment of cell densities of different *Gambierdiscus*. and *Fukuoya* species, a potential risk for CFP outbreaks.

No direct relations between cell densities increments and CFP outbreaks exists because lots of factors play a role on it. Among them, the kind of *Gambierdiscus* species and his variable toxicity, the toxin production in the stable phase and not in the exponential phase of growing and finally the complex food web relations that allows a CFP outbreak (Rains, 2015).

7. Discussion

7.1 The effects of Climate Change on CFP

Climate change is the major environmental phenomena that nowadays affects the distributions of this dinoflagellate genus. This phenomenon increases the SSTs of the oceans and seas due the hibernacula effect and increases the frequency and probability of natural disasters, as hurricanes, tropical thunders or coral bleaching phenomena (Kibler et al., 2015).
The effects of this event are global, but the consequences of it are different among endemic and non-endemic areas in CFP terms.

7.1.1 Endemic areas

In the endemic areas, *Gambierdiscus* and *Fukuoya* had a traditional distribution among the Gulf of Mexico coasts, the USA southeast coastlines and the Caribbean in the Atlantic and among the French Polynesia in the Pacific.

The majority of the recent studies had been turned heavily into the knowledge of how this environmental phenomenon could affect that dynamics in that endemic areas.

As the same Kibler et al., 2015 said, and we commented before, Climate change mainly affects in a new redistribution of the *Gambierdiscus* species into new ecological niches and increasing the risk of new CFP outbreaks around the endemic coastlines. For example, in the more temperate areas of the Gulf of Mexico and the southeast USA coasts, the average number of species is going to increase, this is due to the SSTs increases, and allows more thermal resistant species to survive there instead as the cryo resistant ones only (Tester et al., 2010). More concretely the typical species of these areas, *G.carolinianus* and *G. ribotype 2* are going to decrees in prevalence meanwhile more warm-tolerant species like *G. belizeanus*, *G. caribaeus* and *F. ruetzleri*, more typical in the Caribbean sea, will increase their range distribution and growth rates.

In the other hand, into the Caribbean Sea, where SSTs normally are higher, the climate change will have an inhibitory role into *Gambierdiscus* species. Only the most high temperature resistant species as the same *G. caribaeus* and *F.ruetzleri* will survive there (Kibler et al., 2015).

In terms of CFP incidence, it's believed that in the coastlines of Gulf of Mexico and USA, the incidence is going to increase, meanwhile in the Caribbean Sea, will remain stable or decrees lightly.

These predictions are only supposed because this could change due the variability in the toxicity of the species and the environmental factors that Climate Change can bring, as more incidence of hurricanes, tropical thunders or coral bleaching (Tester et al., 2010, Kibler et al., 2015).

Finally, all the studies conclude that only when toxicity of each species had been fully characterized, the real incidences could be assessed (Kohli et al., 2015).

This example can be applied in the other endemic areas of the planet and of course into the new geographic latitudes that can become new endemic.

7.1.2 The European coasts as a new endemic area

The European coastlines that are starting to be considered as potential new endemic areas for CFP are the Greek coastlines and the coast of the Macaronesia islands.

In this study the affords had been focused in the second one.

In the Macaronesia islands, different studies had been made to understand why could be an important new endemic area in Europe since outbreaks of 2004 had not cased to actuality. Some studies defend that the orography of these island is perfect for the *Gambierdiscus.* species development and that these new cases are due autochthonous species that were less prevalent but still existing in these area (Rodríguez et al., 2017). These makes sense with the theory that redistributions of *Gambierdiscus* species is not done by the invasions of tropical species but due the increases of prevalence of species that since now had not enough cell densities and environmental conditions to develop CFP cases or maybe, the ones given where so low that were not reported. Now due the increases of SSTs by the climate change CFP starts to outbreak.

One example of this theory could be the *Gambierdiscus* specie *G.silvae*, that is only present in these temperate Atlantic Ocean areas (Fraga et al., 2014).

Another important factor that these European coastlines have are the low difference between the winter and summer season. This makes more stable the *Gambierdiscus* populations, increasing the probabilities that if the tendency is still ongoing, the cases of CFP outbreaks will increase in these areas in the incoming years (Fraga et al., 2014).

Finally, as well as we commented in the endemic areas, the new CFP outbreaks not only depends on the cell densities of the *Gambierdiscus* species in a determinate area, tropicalitzation of the finish and water environments in these regions could play and important role on it (Rodríguez et al., 2017), as well as the increment on the environmental phenomena linked to the same great big problem, the Climate change.

7.2 CFP as a One Health problem

As we had been discussed in this study, CFP is not a simple problem, and it requires a multidisciplinary point of view to be successful in the modeling of prevention and risk assessment plans.

The big quantity and the complexity of the factors that involve that problem makes this idea stronger. Multi specialist collaboration is needed to:

- Asses the principal problem, to better known the relations between this dinoflagellate genus and the environment that involves them. More studies focusing, in the specie specific toxicity, the local food web relations and the local environmental factors that affects in a determinate area are necessary.
- Better understanding and preventions of the human CFP cases. Good information and knowledge about the distribution and symptoms of the CFP cases by human physicians is needed. As well as a good awareness to the endemic areas populations about the high risk that it involves.
- Better and efficient identification tools that allows to detects CTX and if possible species of *Gambierdiscus* present in certain areas or in the finfish sold in the local and big markets over the endemic areas.
- And finally, the big economics impacts that these intoxication problem can bring to the endemic societies and the good education to the population about the real risk and how to avoid that is a huge job that has to be done by sociologists and economists.

8. Conclusions

8.1 Modelling and risk assessment proposals

As we had discussed along all this bibliographical study, the major conclusion found in the biography is that risk assessment and prevention plans will need of different actions to be accomplished:

- The plans will be assessed depending on the species in the regional areas. However not only the species had to be studied regionally, the orography, water conditions and environmental phenomena in a certain area had to be studied properly to accomplish a great risk assessment plan. As well, food web relations depend in the fish and dietary habits that exist in a certain area, regional studies in that field had to be done.

- New and better species and toxin detectors had to be developed if an accurate risk and prevention plan has to be made. These detectors will help to determinate the species in a certain area and the kind of toxicity they can show. Even so, these detectors will help the veterinary services to detects CTX toxins in the finfish that is going to be sold in the markets.

- Finally, more studies destined to develop new techniques to depurate and grow *Gambierdiscus* species in laboratorial conditions had to be proposed, as much accurate the isolation of *Gambierdiscus* cells could be and more similar to the natural environments the laboratorial experiments are, better responses to the real interactions these dinoflagellates suffer in their own environments and better understanding of the real epidemiology of CFP will be.

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