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Escuela de Ciencias Biológicas e Ingeniería

TÍTULO: Exploratory Analysis of Toxins in Shellfish during Red Tide "Harmful Algal Bloom" (HABs) on the Pacific Ocean Coasts

Trabajo de integración curricular presentado como requisito para la obtención del título de Ingeniero Biomédico

Autor:
Jaime David Sevilla Carrasco

Tutor:
PhD. Renée Constanza Lira Vergara

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DEDICATORIA

Dedico este trabajo a Dios, a mi hermosa familia y a todas aquellas personas que me han acompañado durante mi proceso de formación como persona y profesional.

Jaime David Sevilla Carrasco

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Jaime David Sevilla Carrasco

RESUMEN

"Marea roja" es el término comúnmente utilizado para referirse a las floraciones de algas nocivas (HAB). Este fenómeno se da principalmente en las regiones costeras del Océano Pacífico, provocando grandes cambios en el color del agua debido a que tienen pigmentos con los que captan la luz solar. Las FAN son floraciones de microalgas que generan en su metabolismo sustancias altamente tóxicas conocidas como toxinas marinas, que afectan a humanos y animales. Los principales mariscos afectados por la marea roja son los filtradores, especialmente moluscos como los mejillones (*Mytilus galloprovincialis* o *Mytilus edulis*), las almejas (*Venerupi* ssp.), las ostras (*Ostrea edulis*) o las vieiras (*Pecten maximus*). Todos ellos consumen microalgas que concentran estas toxinas en sus tejidos, convirtiéndolos en alimentos altamente tóxicos. Así, las consecuencias de comer mariscos contaminados con Marea Roja son graves, ya que las toxinas actúan de inmediato y pueden causar la muerte. Además, hay carnívoros, que se alimentan de filtradores, como los locos y los caracoles de mar. Los organismos del fitoplancton y las toxinas que se encuentran en el Océano Pacífico están presentes en las especies de Dinoflagelados (Pyrrhophyta) como componentes de la Marea Roja. Entre los tipos de toxinas se encuentran: Saxitoxina y Gonyatoxinas (PSP, Paralytic Shcellfish Poisoning), Ácido okadoico (DSP, Diarrhetic Shellfish Poisoning), Brevetoxinas (NSP, Neurotoxic Shellfish Poisoning) y Ciguatoxin (CFP, Ciguatera Fish Poisoning). Además, las toxinas presentes en las especies de diatomeas son el ácido domoico (ASP, Amnesic Shellfish Poisoning).

Palabras clave: Floraciones de algas, toxinas, marea roja, enfermedades.

ABSTRACT

"Red Tide" is the term commonly used to refer to Harmful Algal Blooms (HABs). This phenomenon occurs mainly in the coastal regions of the Pacific Ocean, causing large changes in the color of the water because they have pigments with which they capture sunlight. HABs are microalgae blooms that generate highly toxic substances known as marine toxins in their metabolism, which affect humans and animals. The main shellfish affected by Red Tide are filter feeders, especially mollusks such as mussels (*Mytilus* galloprovincialis or *Mytilus edulis*), Clams (*Venerupissp*.), Oysters (*Ostrea edulis*) or Scallops (*Pecten maximus*). All of them consume microalgae that concentrate these toxins in their tissues, converting them into highly toxic foods. Thus, the consequences of eating shellfish contaminated with Red Tide are serious, since the toxins act immediately and can cause death. Furthermore, there are carnivores, which that feed on filter feeders, such as loco and sea snails. The phytoplankton organisms and toxins found in the Pacific Ocean are present in Dinoflagellate species (*Pyrrhophyta*) as components of the Red Tide. Among the types of toxins are: Saxitoxin and Gonyatoxins (PSP, Paralytic Shcellfish Poisoning), Okadoic acid (DSP, Diarrhetic Shellfish Poisoning), Brevetoxins (NSP, Neurotoxic Shellfish Poisoning) and Ciguatoxin (CFP, Ciguatera Fish Poisoning). Also, the toxins present in diatom species are Domoic acid (ASP, Amnesic Shellfish Poisoning).

Keywords: Algal blooms, toxins, red tide, sickness.

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DEFINITIONS

AOAC = Association of Official Agricultural Chemists

ASP = Amnesic Shellfish Poisoning

AST = Amnesic Shellfish Toxin

AZP = Azaspiracid Shellfish Poisoning

BC = British Columbia

CDIAT = Centric diatoms

CFP = Ciguatera Fish Poisoning

CILI = Ciliates

CTX = Ciguatoxins

DA = Domoic acid

DINO = Dinoflagellates

DOM = Dissolved organic matter

DSP = Diarrhetic Shellfish Poisoning

DST = Diarrhetic shellfish toxin

DTX-1 = Dinophysistoxin-1

DTX-2 = Dinophysistoxin-2

DTX-3 = Dinophysistoxin-3

ELISA = Enzyme-linked immunosorbent assay

HAB = Harmful Algal Blooms

HILIC-MS/MS = Hydrophilic interaction liquid chromatography-tandem mass spectrometry

HPLC = High-performance liquid chromatography

ICHA = International Conference on Harmful Algae

ICTC = International Committee for the Control of Cytomegalovirus

IFREMER = French Research Institute for Sea Exploitation

INSIVUMEH = Instituto Nacional de Sismología, Vulcanología, Meteorología e Hidrología

LAESI-HRMS = Laser ablation electrospray ionization-high resolution mass spectrometry

LC = Liquid Chromatography

LC-FLD = Liquid chromatography fluorescence detector

LC-MS = Liquid chromatography-mass spectrometry

LOD = Limits of detection

MAGA = Ministerio de Agricultura Ganadería y Alimentación

MBA = Mouse Bioassay

MIST = Maritime in vitro Shellfish Test

MS = Mass spectrometry

MU = Mouse units

NOAA = National Oceanic and Atmospheric Administration

NOC = Non-toxic

NSP = Neurotoxic Shellfish Poisoning

OA = Okadaic acid

PDIAT = Pennate diatoms

PP1 = Protein phosphatase 1

PP2 = Protein phosphatase 2

PP2A = Protein phosphatase 2A

PSP = Paralytic Shcellfish Poisoning

PST = Paralytic shellfish toxin

PTG = Phytoplankton taxonomic groups

SAGARPA = Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación

SEDESOL = Secretaria de Desarrollo Social

SST = Sea surface temperature

STX = Saxitoxins

STXeq = Saxitoxins equivalent

TOX = Toxic

TTX = Tetrodotoxin

UNIPESCA = Unidad Nacional de Pesca y Acuicultura

UV = Ultraviolet

UVD = UltraViolet Detection

VGSC = Voltage-gated sodium channel

Chapter 1: Introduction

1.1 Introduction

Climate change is one of the world's biggest challenges due to natural causes, mainly human activity. Climate change and sewage discharges into the ocean make harmful algal blooms more common, generally known as red tides (Nicholls et al., 1982; Watson et al., 1999; Søndergaard et al., 2007; Noges et al., 2010; Carey et al., 2012; Francis et al., 2014; Watson et al., 2015). Among the environmental drawbacks attributed to climate change, there is evidence of a disproportionate increase in the number of certain organisms that unbalance the environment. These can be considered a danger to human, animal, or plant health, and we can cite examples of microalgae that cause red tides. This natural phenomenon, and the risks associated with it, maybe misperceived by the public (Centers for Disease Control and Prevention, 2022).

An algal bloom occurs when there are excessive algae cells in the water. Most algae are harmless to humans, animals, and ecosystems, but some pose serious health risks (Lewitus et al., 2012; Manivasagan & Kim, 2015). The main reason for this to develop is when environmental circumstances are just right for a bloom of algae. Typical red tides can be seen along the coast of Ecuador. Despite appearances, these organisms are not algae (Wells et al., 2015). They are a type of bacteria that can take several shapes. The cyanobacterium *Microcystis* produces at least 80 distinct toxin types that can seriously harm or kill humans, pets, wildlife, and livestock. Paralysis and death can occur in both people and animals when exposed to high enough doses of the poison (NJ Department of Environmental Protection, 2021).

HABs have distinct life stages, each with different morphologies, environmental interactions, behaviors and responsibilities at each stage of the life cycle in which Dinoflagellates, *Pseudo-Nitzchia* and Cyanobacteria (nostocales) are found. Asexual reproduction, or a dormant cyst stage, is a successful method of survival and dissemination, allowing species to be carried by ocean currents, fish, or even people (via ballast water discharge) (Azanza et al., 2018).

Among the main toxins present in harmful algal blooms are Saxitoxin known as PSP (Paralytic Shellfish Poisoning), Okadaic acid known as DSP (Diarrheal Shellfish Poisoning),

Brevetoxin NSP (Neurotoxic Shellfish Poisoning), Ciguatoxin CFP (Ciguatera Fish Poisoning) and Domoic acid known as ASP (Amnesic Shellfish Poisoning). The ingestion of shellfish containing these toxins affects the health of the population; however, it should be noted that Ciguatoxin mainly affects fish, which is reflected in a massive mortality. It should be emphasized that biological and biochemical methods are used to detect these toxins, such as mouse bioassay, cell culture assays, liquid chromatography, phosphatase inhibition assay another (Prego-Faraldo et al., 2013).

Thus, several HABs events involving poisoning and death have been recorded. From that point on, the creation of control bodies has been urged, as is the case of the Comisión Nacional de Vigilancia de Marea Roja (Carrillo-Ovalle, 2009; Leiva, 2008). Another country that has experienced this type of event is Canada, which began monitoring and research on the Atlantic and Pacific coasts in 1940 (McKenzie et al., 2021). In the same sense, the United States of America has registered harmful algal blooms; in which human, animal and ecological health have been affected. Ecuador is also another country in which algal bloom events have been recorded due to the fact that oceanographic characteristics play an important role in these events (Cucalón, 1989; Longhurst, 2006; Borbor-Córdoba et al., 2019). Mexico is also part of the group of countries that suffer from HABs, as a result of which intoxications and human losses have been reported due to the consumption of contaminated shellfish (Alonso-Rodríguez et al., 2015; Salcedo-Garduño et al., 2018).

1.2 Statement of the problem

Harmful algal blooms are sometimes called “red tides” (HABs). Large color shifts in the Pacific Ocean’s coastal regions can be attributed to this phenomenon, which occurs when pigments in the water absorb sunlight. Human and animal health can be negatively impacted by HABs, which are microalgae blooms that produce marine toxins in their metabolism, causing illness or even death in some cases. That is why it is critical that even if a hazardous bloom has not been detected in the area, residents should avoid touching any surface scum or severely discolored water. Because depending on how closely one comes into contact with the HABs, they may induce various symptoms, including rashes, gastrointestinal pains, diarrhea, and even liver problems (Lynch, 2018).

1.3 General and Specific Objectives

General Objective

Carry out an exploratory analysis of the most important harmful algal bloom (HABs) in the Pacific Ocean Coasts, with particular reference to Ecuador.

Specific Objectives

- Explore the toxins which are present in the main events of HABs.
- Immunological identification of toxins presents in algal blooms in Ecuador.
- Comparison of toxins present in algal blooms in different countries having coast in the Pacific Ocean.

Chapter 2: Harmful Algal Blooms (HABs)

Contrary to the popular belief, HABs represent an international issue, not unique to any particular country (Band-Schmidt et al., 2010). Thus, in 2000, the International Conference on Harmful Algae (ICHA) began, its precursor was the French Research Institute for the Exploitation of the Sea. This conference covers a wide range of HABs, and brings together professionals and concerned citizens to discuss and evaluate recent scientific findings and developments in the field. In 2016, the conference was hosted in Brazil. In 2016, the conference was hosted in Brazil; in October 2018, it was held in France; Japan will host the 20th annual conference in 2023, specifically in Hiroshima (Fig. 1).

Figure 1. The 20th International conference on Harmful Algae.

However, the cyanobacteria that cause algal blooms that create microcystin are the focus of another international research group at the International Conference on Toxic Cyanobacteria Harmful Algae Blooms. Therefore, the eleventh edition of the conference was held in May 2019 in Krakow, Poland. It is important to emphasize that when microcystin broke out worldwide in the early 21st century, the International Cytomegalovirus Control Committee (ICTC) was formed to deal with it. Finally, HABs can develop no matter the kind of body water, whether it is fresh or salty (Lynch,2018).

Due to the ocean is intrinsically related to human health as a source of nutrients and overall well-being. Harmful algal bloom (HAB) events are of concern to the scientific community. These natural events can grow in response to rising sea surface temperatures, thermocline shoaling, coastal upwelling, and other unknown causes (Borbor-Córdova et al.,

2018; Fleming et al., 2014; McCabe et al., 2016; Moore et al., 2008). For example, phytoplankton proliferation in aquatic habitats is a natural occurrence that occurs worldwide. This represent nutritional foundation of aquatic food webs, and as photosynthesizing creatures, they are also a major contributor to the global carbon cycle. Nonetheless, several species of phytoplankton and benthic microalgae generate large biomass and hazardous cell aggregations known as HABs (Busch et al., 2013; Smayda, 1997). As a result, coastal regions on all continents are reporting large accumulations of phytoplankton, macroalgae, and occasionally colorless heterotrophic protists.

Therefore, the presence of large numbers of these organisms can cause a variety of undesirable effects, including the formation of red, mahogany, brown, or green tides; the floating of scums on the surface; the covering of beaches with biomass or exudates; and the depletion of oxygen levels due to excessive respiration or decomposition (Manivasagan, 2015).

Microalgal and phytoplankton blooms have contributed to “red tides” in coastal seas worldwide (HABs). In addition, HABs are organisms that can significantly lower the oxygen levels in natural water systems while also harming the aquatic life in lakes and coastal areas (Anderson, 2012; Anderson et al., 2017; Borbor-Cordova et al., 2019; Hallegraeff, 1993; Pal et al., 2020). Moreover, HABs indicate an ecosystem that is out of balance, typically brought on by the multiple environmental changes brought on by the expanding global human footprint and climate change. These adjustments can generally be divided into three groups (Fig. 2).

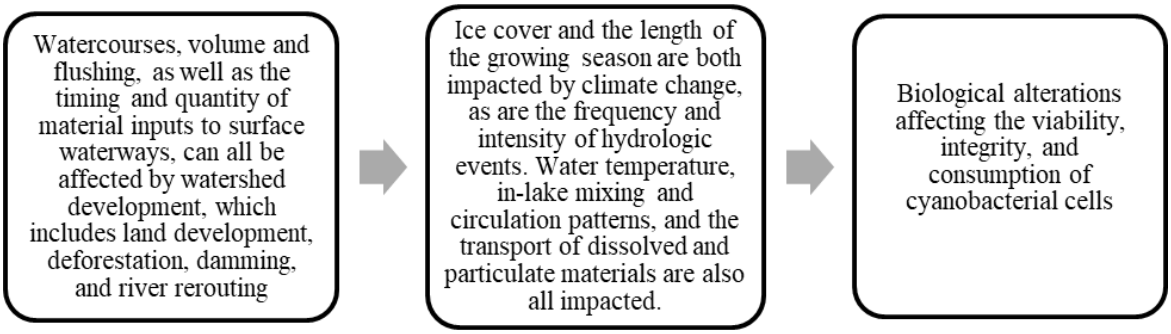


Figure 2. Causes of HABs (Nicholls et al., 1982; Watson et al., 1999; Søndergaard et al., 2007; Noges et al., 2010; Carey et al., 2012; Francis et al., 2014; Watson et al., 2015).

Such events occur in less productive systems, especially those with some degree of human intervention, such as acidification, organic loading, or a history of restorative nutrient reduction. HABs are more severe and frequent in eutrophic and mesotrophic ecosystems,

although they also occur there (Carey et al., 2012; Francis et al., 2014; Nicholls et al., 1982; Nõges et al., 2010; Søndergaard et al., 2007; S. B. Watson et al., 1999, 2015). However, is important consider that certain factors, including upwelling systems and water column stratification brought on by low wind stress and marine heat waves, may limit the prevalence of HABs. Oceanographic and climatic conditions also impact their proliferation (Pal et al., 2020). Diatoms, dinoflagellates, and cyanobacteria are just a few phytoplankton species that produce blooms in freshwater and marine habitats (Pal et al., 2020; Sangolkar et al., 2006, 2009).

HABs are becoming increasingly widespread in coastal locations and have spread worldwide (Glibert et al., 2014; Gobler et al., 2017; Hallegraeff, 2010). Although coastal eutrophication and increased monitoring have had a role in these patterns, there is growing acknowledgment that climate change has also played a role in the worldwide intensification of some HABs (Anderson, 2012; Glibert et al., 2014; Gobler et al., 2017; Wells et al., 2015). Biotoxins that build up in the tissues of bivalve clams can cause most cases of PSP in humans have been observed following ingestion of bivalves (Hégaret et al., 2009; Shumway, 1990). Residents who live near or downstream of blooms may get respiratory problems due to some pollutants becoming aerosolized (Backer et al., 2003; Cheng et al., 2007; Pierce et al., 2003). Toxins generated by HABs endanger marine creatures, fish, seabirds, crabs, and seagrasses (Griffith & Gobler, 2020).

Another factor important of Harmful Algal Blooms is the relationship with the ecological processes are influenced by species individual metabolic capacity and interconnecting metabolic networks. Examples include maritime habitats, where autotrophy greatly affects the carbon cycle on a global scale. Periodic dinoflagellate blooms, which can produce toxins and cause a localized oxygen scarcity, can have severe ecological and economic effects as opposed to having a positive impact on atmospheric carbon (Manivasagan & Kim, 2015; Morey et al., 2011).

A variety of HABs jeopardizes water quality, living resources health, and Pacific coast community's economy. These HABs regularly cross jurisdictional boundaries. Effective HABs control will involve cooperation and coordination on an international and interstate scale (Lewitus et al., 2012; Manivasagan & Kim, 2015). A regionally coordinated effort will be required to understand the causes and effects of west-coast HABs fully.

Chapter 3: HABs species life cycle dynamics

The dynamics and effects of HABs are heavily influenced by the multi-stage life cycles of the phytoplankton species responsible for them. There are different morphologies, environmental interactions, behaviors, and responsibilities at each life cycle stage. Asexual reproduction, or a dormant cyst stage in the life cycle of some HABs species, such as the *Alexandrium dinoflagellates*, allows these organisms to overwinter in sediments and generate blooms in temperate locations. The cyst stage is a successful method of survival and dissemination, allowing species to be carried by ocean currents, fish, or even people (via ballast water discharge). A small number of cysts moved to a new place can serve as the start of a new colony. *Pseudo-nitzschia* diatoms, like many other species, undergo sexuality but do not appear to have a true resting stage. Toxigenic cyanobacteria species that cause blooms in freshwaters, like Nostoc, have evolved a technique for surviving in hostile environments by creating thick-walled resting cells called akinetes (Azanza et al., 2018).

3.1 Dinoflagellate Life Cycle.

Most dinoflagellates, including *Alexandrium*, reproduce asexually by a process called fission. A single parent cell develops and divides into two offspring during this process. When environmental factors, including a lack of nutrients, sunshine, or animal grazing, do not prevent further expansion, hazardous algal populations can build up to stunning but disastrous heights.

Because of a drop in food supply, several animals have shifted to sexual reproduction and a new life stage. The gametes produced by the cells combine to form the diploid, motile zygote (called a planozygote). Planozygotes develop a cyst that lies latent in the ocean floor's sediments. These cysts can stay viable for years, giving a species a fighting chance against nutritional deprivation, harsh winters, or animal consumption. When conditions are right, the cysts will germinate, releasing a wave of photosynthetically active cells into the water column.

For survival and spread, the cyst stage is a smart choice. Every time a bloom enters the cyst stage, it becomes more portable, making it easier to transport it by ocean currents,

fish, or even people (via ballast water discharge) to new bodies of water where it can be deposited as a "seed" population and colonize.

3.2 *Pseudo-nitzchia* Life Cycle.

Diatoms (like *Pseudo-nitzschia*) have both asexual and sexual stages in their life cycles, just as dinoflagellates. One daughter cell is the same size as the parent cell, and the other is somewhat smaller during asexual reproduction.

Most diatoms experience a population decline in mean cell size over time due to asexual reproduction, which can be reversed by engaging in sexual reproduction. When two parent cells align, one will generate four "active" gametes, while the other will produce four "passive" gametes during sexual reproduction. To create an auxospore, the active gametes travel to the site of the passive gametes and merge with them.

With their protective properties, auxospores help cells recover their original dimensions as they grow and divide. Cell division will continue in diatoms that cannot engage in sexual reproduction, but the mean size of the daughter cells will decline until the organisms die. Because of this, diatoms can be tricky to keep alive in culture for extended periods. Although diatoms do not make cysts, some research suggests they may enter a "quiescent phase" that helps them survive in harsh environments (Fig. 3).

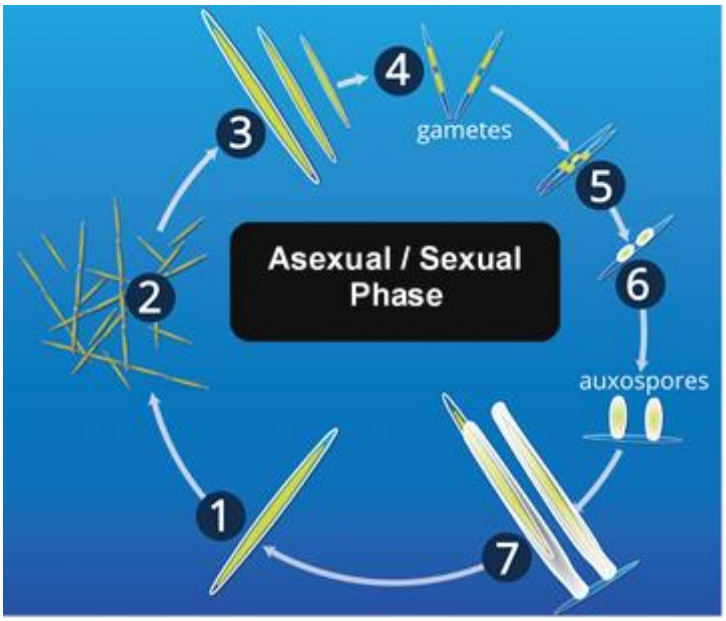


Figure 3. Simplified life cycle of a *Pseudo-nitzchia* (Woods Hole Oceanographic Institution, 2019).

3.3 Cyanobacteria (Nostocales) Life Cycle.

The Life Cycle of Nostoc-Class Cyanobacteria Unicellular to more complex filamentous and colonial structures characterize freshwater cyanobacteria's morphology and cellular organization. Gas-filled vacuoles in many cells propel them to the ocean's surface, where they can make use of sunlight to fuel the photosynthetic process.

Cellular fission is the asexual reproduction mechanism used by cyanobacteria, in which a single parent cell divides into two identical daughter cells. Summer is when cyanobacteria bloom, and their effects are most commonly seen because bloom formation is primarily driven by water temperature and the availability of nutrients like phosphorus and nitrogen.

In nutrient-poor environments, certain species of cyanobacteria can transform into heterocysts, specialized cells that can take in nitrogen by fixing it from the air. Cyanobacteria that develop heterocyst is also making akinetes, which are bottom-dwelling, winter-dormant cells with thick walls. As long as environmental conditions are good, the akinete will secrete a germling cell that will continue photosynthesizing and dividing to form a colony. Thus, the bloom cycle to repeat (Fig. 4).

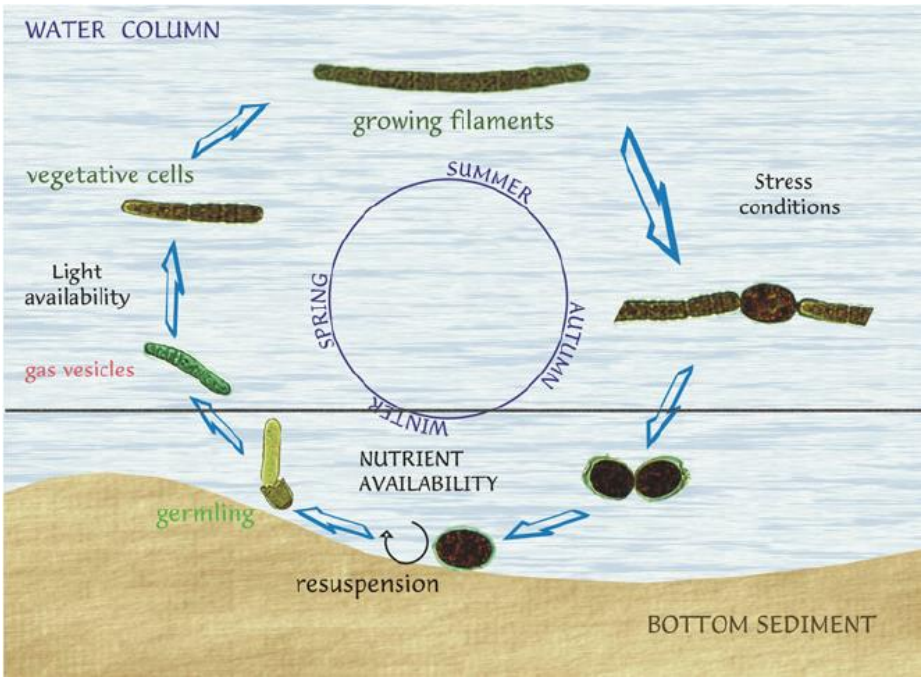


Figure 4. Simplified life cycle of a Cyanobacteria (Nostocales) Life Cycle (Kaplan-Levy et al., 2010; Hense & Beckmann, 2006).

Chapter 4: Main toxins Harmful Algal Blooms (HABs)

4.1 Saxitoxin (PSP, Paralytic Shcellfish Poisoning)

4.1.1 Saxitoxin producing microalgae species.

Some marine dinoflagellates and various kinds of cyanobacteria produce alkaloids called saxitoxins (STXs), also known as paralytic shellfish poisoning (PSP) toxins (Fig. 5). The toxin was first isolated from the butter clam (Saxidermus) and was identified in 1957 by Shantz et al. because it has the name Saxitoxin (STX). They were first discovered in mollusks after human poisonings were traced back to the intake of fish and shellfish

STXs prevent action potentials from spreading along an axon by inhibiting sodium channels in nerve cells. The effects can be devastating, from mild symptoms like tingling or numbness in the tongue or the back of the throat to more serious ones like respiratory failure and death. Tingling and numbness are the results of damage to sensory neurons, while weakening or paralysis of the corresponding muscles results from damage to motor neurons. Numerous PSP instances have provided a detailed description of the outcomes of STX poisoning in humans and animals. An example of how dangerous it can be being whales that have eaten contaminated organisms die after hours of exposure to the toxin.

Regulators are worried about its weaponization and powerful toxicological effects on animals and humans, despite its widespread usage in neurochemical and molecular biology research (Gad, 2014).

Alexandrium catenella, *Gymnodium catenatum*, *Alexandrium cohorticula*, and *Pyrodinium bahamense* are among the saxitoxin-producing microalgae species. The toxic potency of Saxitoxin is derived from tetrahydropurine compounds composed of two guanidine moieties fused in a stable azaketal linkage (Ogongo, 2015). And the freshwater cyanobacteria *Aphanizomenon gracile*, *Cylindrospermopsis raciborskii*, and *Dolichospermum spp.* (Brown et al., 2019; Kellmann & Neilan, 2007; Ongley et al., 2016; Wiese et al., 2010).

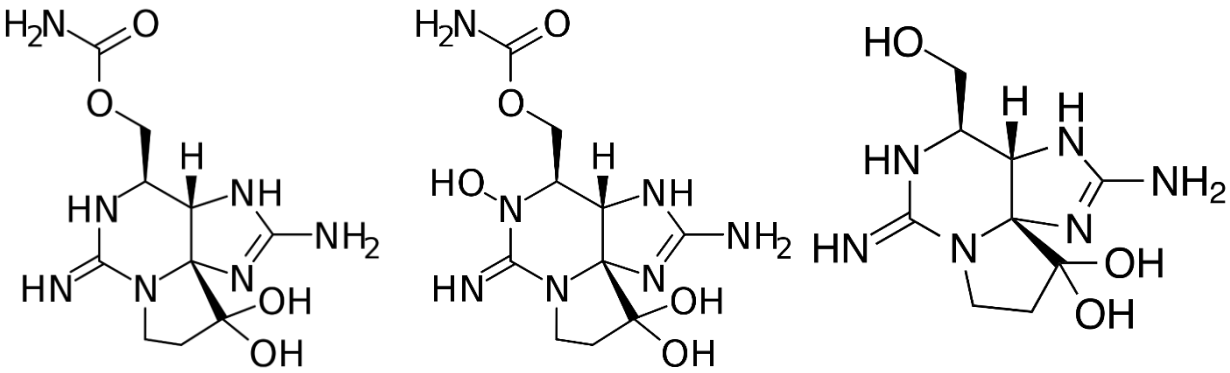


Figure 5. Structure of saxitoxin and its derivatives.

4.1.2 Saxitoxin poisoning and mechanism of action.

The most common way people become exposed to saxitoxin is through eating shellfish that have been contaminated. Some red tide victims have reported breathing issues after being exposed to even minute levels of the toxin in the spray produced by the crashing waves. The Chemical Weapons Convention of 1993 led to the destruction of several weapons that could have contained saxitoxin. The most common sources of saxitoxin are scallops, oysters, mussels, cockles, univalve mollusks, clams, puffer fish, and whelks. Some herbivorous fish and crabs, such as the Atlantic thorny lobster and the Australian xanthid crab, have been discovered to have lower quantities of the toxin (Gab, 2014).

Due to differences in each person's sensitivity, the methods employed to calculate the dose, and the victim's access to quality medical treatment, STXeq doses that resulted in either moderate side effects or death in people varied significantly. The estimated range for an oral dose of STXeq to produce mild symptoms was 120–4128 μg, and the range for a lethal dose was 456–12400 μg. Although eating 300 μg STXeq proved lethal in some instances, administering somewhat greater doses of the toxin has also been associated with the absence of toxic symptoms (World Health Organization, 2019).

Clinical signs of Saxitoxin poisoning can vary depending on the extent of exposure. Consumption of toxins-accumulating fish or shellfish is frequently linked to high exposure levels, as are dinoflagellate blooms (in marine habitats) or cyanobacterial blooms (freshwater environments). Depending on many different factors, a few minutes to 72 hours may pass

between exposure and the start of clinical symptoms. At relatively low exposure levels, moderate paresthesia-often described as a tingling sensation-are experienced in the mouth and on the extremities. After longer exposures, a creeping numbness of the tongue, throat, and limbs develops. High exposure levels may also cause sudden muscle paralysis and respiratory collapse (García et al., 2004; Malik et al., 2020; Miller et al., 2017; Montebruno, 1993; van der Merwe, 2014).

Other physicochemical properties can be seen in Table 1. When ingested orally or through the mucous membranes, saxitoxin is quickly absorbed into the body, and the possible cause is that most STXs are universally hydrophilic, except for those having two sulfate groups. Because of its persistence at low pH and high temperatures, saxitoxin can linger in the body for a long time before being expelled or digested. The after-effects could linger for a few days, but other symptoms, including a lack of muscle strength and coordination, could persist for a few weeks. However, both of these processes are time-consuming (Gad, 2014).

Proporty	**Saxitoxin (STX)**	**Neosaxitoxin (neoSTX)**	**Decarbamoylsaxitoxin (dcSTX)**
CASRN	35523-89-8	64296-20-4	58911-04-9
Chemical formula	$C_{10}H_{17}N_7O_4$	$C_{10}H_{17}N_7O_5$	$C_9H_{16}N_6O_3$
Average molecular weigth[a] (g/mol)	299.292	315.291	256.266
Monoisotpic mass[b] (Da)	299.134	315.129	256.128
K_{ow}[c]	-4.6	-4.3	-4.6

Table 1. The characteristics of typical saxitoxins in their free base form (World Health Organization, 2020).

Saxitoxins are voltage-gated sodium channel blockers that are selective and reversible. Saxitoxins can pass across the blood-brain barrier, and as a result, they can paralyze the central nervous system by blocking sodium channels. Following this, vasomotor nerve inactivation, relaxation of vascular smooth muscle, and possible hypotension may occur. Damage to the muscles that control breathing and heart function can be fatal. In adults, the mortality rate is 5.9%, although it appears to be greater in children (Borison & McCarthy,

1977; Huot et al., 1989; Tarnawa et al., 2007; van der Merwe, 2014; Walker et al., 2012). It has been estimated that 50 fatal dosages can be found in just one clam with contamination.

4.1.3 Transfer of Saxitoxin in the trophic chain.

While the chemical effects of salinity on the toxicity of STX in *Alexandrium* species have been researched for quite some time, more is needed about the biological impacts (Caruana & Amzil, 2018). Environmental factors and genetic makeup contribute differently to their development and the frequency of PSP outbreaks. To better monitor and foresee the emergence of blooms and PSP occurrences, it is helpful to have a firm grasp on the environmental conditions that contribute to STX production. In the following (Fig. 6), it is possible to observe how the cycle makes the tropical charm work.

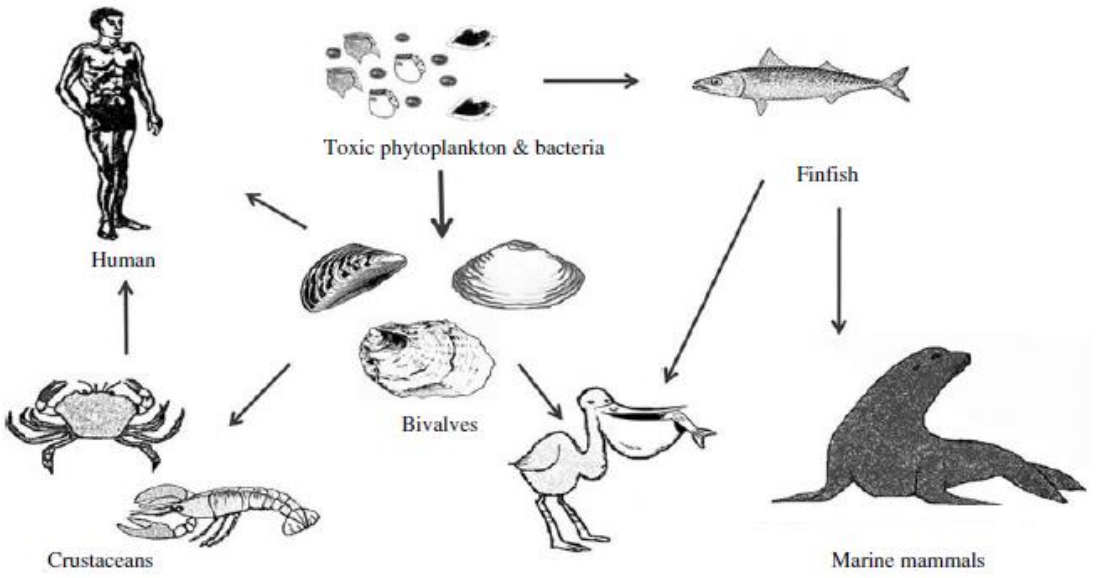


Figure 6. Representation of the interactions between toxic algae and shellfish (James et al., 2010).

4.1.4 Methods of detection and quantification of Saxitoxin.

According to Cusick and Sayler (2013), immunoassays, *in vitro* functional assays, analytical techniques, in vitro cell assays, and in vivo animal bioassays are the five major categories of toxin analysis techniques (ELISA). In addition to approaches for toxin

detection, molecular tools that target toxin-producing organisms are becoming more prevalent.

4.1.4.a Immunoassays

Enzyme-linked immunosorbent assays, or ELISAs, are a class of biochemical experiments that rely on antibodies produced for the target analyte, with detection usually shown as a color shift. Many different assays have been developed for Saxitoxin and its derivatives, including direct and indirect formats, poly- and monoclonal antibodies, and a wide variety of other components (Cusick & Sayler, 2013). Usleber et al. (1991), as cited in Cusick and Sayler (2013), created a microtiter and a test strip assay for Saxitoxin using polyclonal antibodies.

The Ridascreen rapid saxitoxin assay (R-BioPharm), the Abraxis enzyme-linked immunosorbent assay (ELISA) for paralytic shellfish poisoning (Abraxis), and the MaxSignal Saxitoxin ELISA (Promega) are other examples (Bio Scientific). The Ridascreen assay has been developed specifically for detecting shellfish, and it has a very high detection limit (50 versus 0.02 g and 1.2 g/L, respectively). One potential drawback of ELISAs is cross-reactivity among the many compounds, which is of no consequence if such reactions correlate with toxicity. Assay results can indicate true toxicity even though, for example, C-toxins exhibit low cross-reactivity and low toxicity. However, the tests cannot identify the lethal decarbamoyl and N1-hydroxylated versions. This is why ELISAs are better suited for screening tools than quantitative assays (Humpage et al., 2010; Cusick & Sayler, 2013).

4.1.4.b *In* vitro functional assays

The receptor binding assay for toxin identification is an alternative to the MBA for routine monitoring (Cusick & Sayler, 2013). This test relies on the interaction between the toxins and one of their pharmacological targets, site 1 of the sodium channel, and was first introduced in 1984 by Davio and Fontelo, as cited in Cusick and Sayler (2013). Since then, it has been refined and adapted to a microtiter plate format (Vieytes et al., 1993; Doucette et al., 1997; Cusick & Sayler, 2013). The assay involves competition between tritiated Saxitoxin

and unlabeled Saxitoxin (and its derivatives) for a limited number of receptor sites on the voltage-gated sodium channel in a rat membrane preparation (Cusick & Sayler, 2013).

Liquid scintillation counting is used to determine how much-labeled Saxitoxin has been attached to a receptor, and the amount of unbound toxin is determined by subtracting the amount of labeled toxin that has been withdrawn.

By the hierarchy of toxicity shown in the animal bioassay, the receptor binding affinities followed the same pattern: STX > GTX1/4 > neoSTX > GTX2/3 > dcSTX > GTX5. Recent worldwide multi-lab research has shown that the Receptor Binding Assay was able to accurately detect PSTs in the critical range of shellfish toxicities below, near and slight above the regulatory limit in comparison with MBA demonstrating its suitability for routine monitoring of shellfish (Van Dolan et al., 2012).

4.1.4.c Analytical techniques

Toxins can be detected, and their concentrations determined using analytical techniques. One of the earliest analytical approaches developed for saxitoxin detection is high-performance liquid chromatography (HPLC) (Sayfritz et al., 2008; EFSA, 2009; Cusick & Sayler, 2013; Rey et al., 2016), which is commonly used for the separation of organic substances. In the late 1970s, post-column derivatization using a silica-based stationary phase became the basis of the HPLC approach for saxitoxin analysis (Cusick & Sayler, 2013; Rey et al., 2016). Since then, numerous techniques for toxin separation employing pre- and post-column oxidation with various columns have been developed (Sullivan, 1990; Cusick & Sayler, 2013). Converting poisons into fluorescent derivatives helped solve the problem of the toxins poor UV absorption, which had previously hampered their detection (Sullivan, 1990; Cusick & Sayler, 2013). Many developments in sample extraction, column type, eluent composition, and oxidation procedures have improved the separation and detection of numerous congeners since the 1970s (Humpage et al., 2010; Cusick & Sayler, 2013; Rey et al., 2016). A method for quantitative assessment of PSTs in shellfish using pre-chromatographic oxidation was proposed and accepted by the Association of Official Agricultural Chemists (AOAC) in the same year based on a joint study involving 16 laboratories from 12 countries (Lawrence et al., 2005; Humpage et al., 2010). As a result of the developments in HPLC methods and their subsequent approval by monitoring agencies,

an LC-fluorescence detection (LC-FLD) approach has been recognized as an alternative to the MBA in the European Union (Turner & Hatfield, 2012; Cusick & Sayler, 2013).

In order to identify unknown chemicals, quantify known materials, and elucidate molecular structural features, liquid chromatography-mass spectrometry (LC-MS) is a strong approach. It has been advocated as the universal approach for all marine toxins (Humpage et al., 2010; Cusick & Sayler, 2013) due to its widespread application in analyzing PSTs and other marine toxins. Another approach utilized in conjunction with MS is hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC-MS/MS) (Qin et al., 2008; Quintela et al., 2010).

Quilliam laboratory's original creation for most algal toxins, including PSTs, were separated and detected in concentrations ranging from 50 to 1000 and 5-30 nM, respectively, on a single run (Dell'Aversano et al., 2004; Cusick & Sayler, 2013). New advances have allowed excellent linearity throughout concentration ranges of 5-50 nM and 25-200 nM (Halme et al., 2012; Cusick & Sayler, 2013).

4.1.4.d *In vitro* cell assays

The antagonist effects of the channel-blocking PSTs (Fig. 7A) in conjunction with veratridine and ouabine led to the development of multiple cell viability assays simultaneously. These tests were adapted from Kogure et al. (1988), which relied on tetrodotoxin's (TTX) ability to shield veratridine- and ouabine-treated mouse neuroblastoma cells. Ouabine inhibits the activity of Na/K-ATPases, while veratridine opens sodium channels, resulting in cell swelling and final lysis (Fig. 7B). As a result of TTX's actions, the cell is spared from swelling and eventual death caused by an excess of sodium ions. Kogure's technique was based on microscopy; specifically, he counted the number of rounded cells following toxin administration. However, this technique was questioned because of the difficulty in counting which cells were rounded based on sodium uptake and the different morphologies of the mouse neuroblastoma cells (Cusick & Sayler, 2013). Colorimetric endpoints based on direct cell labeling, vital stain uptake, and tetrazolium dye reduction were used in the assays designed by Jellet et al. (1992), Gallacher and Birkbeck (1992), and Manger et al. (1993), allowing for automation and usage in a 96 well plate size. The assays

for this purpose detected 10 ng STX eq/mL extract (2.0 g STX eq/100 g shellfish tissue) (Cusick & Sayler, 2013).

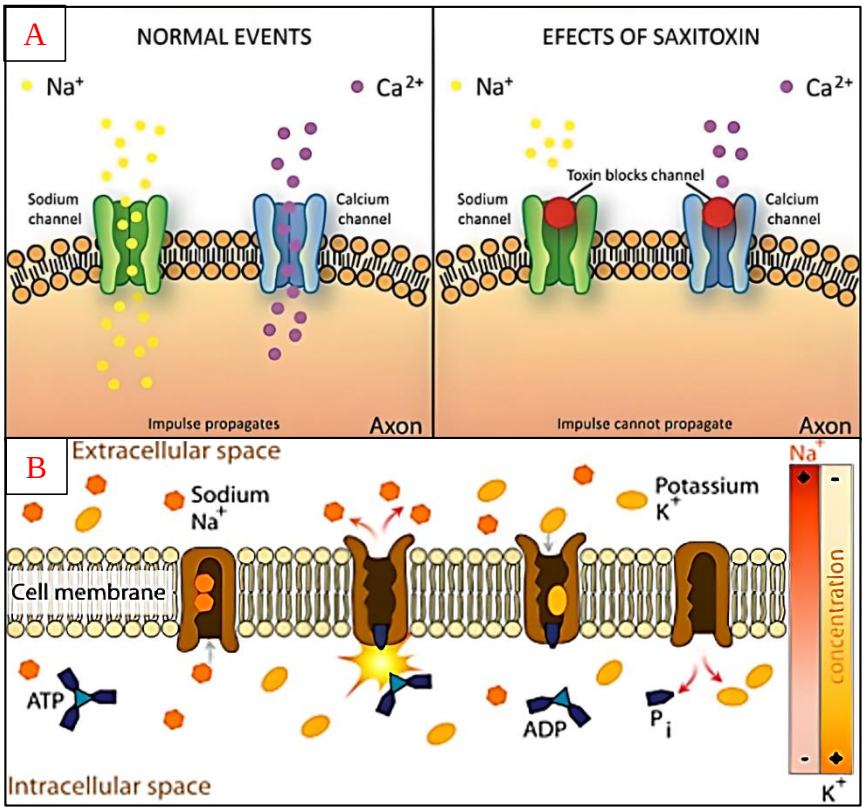


Figure 7. (A) Schematic representation of saxitoxin toxicity mechanism. (B) Ouabine inhibitory activity (Valério et al., 2010; Proteopedia).

This neuroblastoma cell bioassay (Jelletet al., 1992) served as the basis for the commercially available MIST (Maritime *in vitro* Shellfish Test) (Jellet et al., 1998), which is available in several variants that are well-suited to either quantitative or qualitative screening (Cusick & Sayler, 2013).

Toxin-induced membrane depolarization can be detected using voltage-sensitive fluorescent dyes, which has led to the development of a new type of in vitro cell test (Cusick & Sayler, 2013). To measure membrane potential, Nicholson et al. (2000) used the voltage-sensitive fluorescent probe rhodamine 6D in a membrane-fractionated preparation of mouse synaptoneurosomal (Cusick & Sayler, 2013).

In order to evaluate the toxin's block of depolarization due to sodium channel opening, an assay was developed to measure Saxitoxin's ability to inhibit veratridine-

increased rhodamine 6G fluorescence. Antagonizing effects of veratridine and Saxitoxin on membrane potential are similarly at the heart of the experiment created by Louzao et al. (2003), with changes in membrane potential measured by the fluorescent probe bis-oxonol (Cusick & Sayler, 2013). Using the fluorescent probe bis-(1,3-diethylthiobarbituric acid) trimethine oxonol (DiSBAC2(3)), a method was developed for rapid detection of Saxitoxin and its derivatives down to 1 ng STX eq/mL within minutes, with a nearly linear dose-response curve between 1 and 100 ng STX eq/mL (Manger, 2007; Cusick & Sayler, 2013). While these fluorescent probe functional assays show promise for sensitive and quick detection of Saxitoxin and its derivatives, more research is needed to ensure their reliability and widespread use (Humpage et al., 2010; Cusick & Sayler, 2013).

4.1.4.e *In vivo* animal bioassays

Regarding saxitoxin quantification, the mouse bioassay is the current "gold standard" (MBA). The Association of Official Analytical Chemists has worked to standardize and improve the MBA so that it may be used to take measurements rapidly and with sufficient accuracy. Nonetheless, this approach is coming under fire for its reliance on living creatures, and substitutes have either recently been accepted or are undergoing validation testing. The mouse bioassay is commonly used to quantify the efficacy of Saxitoxin, also known as relative toxicity, in the scientific literature. In this assay, a 1 mL test solution is injected (usually intraperitoneally) into a mouse weighing 17-23 g, and the time it takes for the mouse to die after injection is recorded (Cusick & Sayler, 2013).

In terms of mice, the total count is one mouse unit defined as the amount of toxin that will kill a 20 g mouse in 15 min (Hall & Strichartz, 1990); this value is calculated by referring to a standard chart that incorporates the death duration and mouse weight (Cusick & Sayler, 2013). In terms of Saxitoxin, this is equivalent to 200 ng (Hall & Strichartz, 1990). Having too much salt in a sample can reduce its hazardous effects (Schantz et al., 1958); zinc buildup in oysters can kill mice at levels that would not harm humans (Aune et al., 1998; Cusick & Sayler, 2013).

Metals were found to have a suppressive impact on the MBA in subsequent studies. Furthermore, there might be a seven-fold differential in neurotoxins depending on characteristics like gender and mouse strain (Aune et al., 2008; Cusick & Sayler, 2013).

Most nations have an action level of 80 μg STX equivalents per 100 g of shellfish (sometimes written as 400 MU per 100 g in the literature) (Egmond & Apeldoorn, 2004). However, Mexico and the Philippines have dropped this to 30 and 40 g STX eq per 100 g of shellfish, respectively (Humpage et al., 2010; Cusick & Sayler, 2013). Due to the FDA's alert level for Saxitoxin being 80 g/100 g of shellfish meat, commercial shellfish harvesting in the US must be put on hold if elevated levels are found during routine monitoring (Louzao et al., 2001). The MBA has a detection threshold of around. A shellfish with 40 μg STX eq per 100g. Toxins can be screened for with a variant of this assay that employs sublethal markers of toxicity rather than time to death. Acetylcholine levels in mouse blood at predetermined intervals (Cheng et al., 2012) form the basis of this method (Cusick & Sayler, 2013).

The assay's detection limit was lower than the legal threshold for shellfish closures, which is set at 20 nanograms per milliliter (ng/mL) (Cusick & Sayler, 2013).

4.2 Okadaic acid (DSP, Diarrhetic Shellfish Poisoning)

4.2.1 Okadaic acid -producing microalgae species.

Numerous dinoflagellate species belonging to the genera *Dinophysis* and *Prorocentrum*, as well as bacteria belonging to the Roseobacter clade, that interact with these dinoflagellates create these toxins. In the marine sponge genus *Halichondria*, okadaic acid was first identified (Emery et al., 2021; Solter & Beasley, 2013) (Fig. 8).

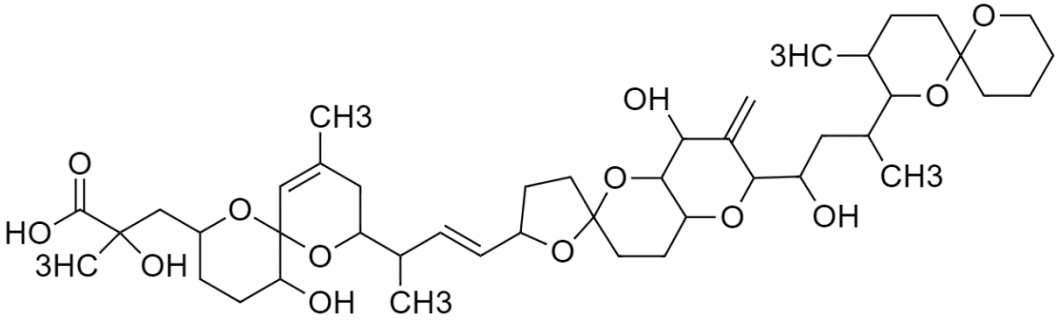


Figure 8. Okadaic acid basic structure.

4.2.2 Okadaic acid poisoning and mechanism of action.

Shellfish accumulate the poisons, which are lipophilic polyethers, in their hepatopancreas. They are strong inhibitors of serine/threonine protein phosphatase 1 (PP1)

and 2A (PP2A) in vulnerable organisms (PP2A). The IC50 values for PP2A range from 0.07 to 0.2nM for okadaic acid, while IC50 values for PP1 range from 3.4 to 19 nM. Myosin and other proteins are phosphorylated at higher levels due to PP1 and PP2A inhibition when ingested—the results in tightening smooth muscles and, perhaps, an increase in intestinal cells' salt secretion. Abdominal cramps and fluid buildup within the digestive tract's lumen are the results (Solter & Beasley, 2013).

DSP, or diarrhetic shellfish poisoning, is Humans commonly used this term for poisoning caused by this class of poisons. Clinical signs and symptoms can range from moderate to severe, including nausea, vomiting, and stomach cramps (Diarrhetic shellfish poisoning (DSP), 1991; Barceloux, 2008; Watkins, 2008; Vilariño et al., 2018). It appears that mortality is rare. Dinophysistoxins are consumed when shellfish like mussels and scallops develop a filter-feeding infection because of the hazardous dinoflagellates (Solter & Beasley, 2013).

4.2.3 Transfer of Okadaic acid in the trophic chain.

The exploration of the processes involved in the accumulation and depuration of OA in marine animals is critical to a better understanding of this biotoxin's influence on ecosystems and the efficient management of toxic episodes, hence limiting their impact on human health. The combination of analytical approaches with the study of sentinel marine organisms has thus far enabled researchers to comprehend the mechanisms of OA within the cell and its transmission across the food chain (Prego-Faraldo et al., 2013) (Fig. 9).

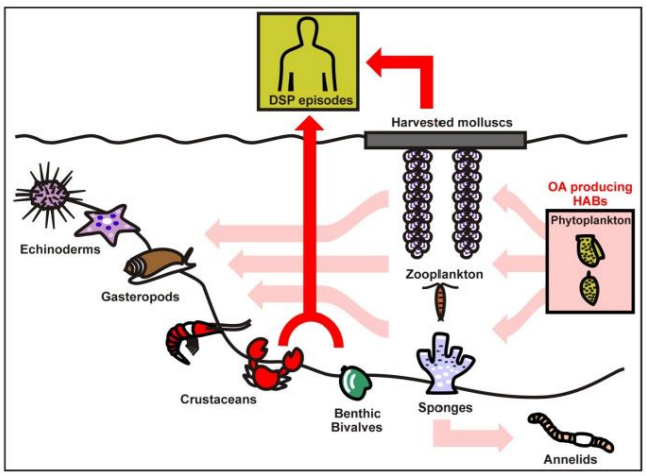


Figure 9. OA transmission schematic diagram (Prego-Faraldo et al., 2013).

In bivalve mollusks, OA is primarily absorbed and stored in the digestive gland, either free or linked with higher densities of soluble lipoproteins (Svensson & Förlin, 2004; Blanco et al., 2007; Duinker et al., 2007; Rossignoli & Blanco, 2008; Rossignoli & Blanco, 2010; Prego-Faraldo et al., 2013). This interaction sequesters OA, restricting its transfer to other organs and impeding its removal from the body. In contrast, free OA is easily transported and rapidly eliminated by diverse passive detoxification pathways, including immediate OA excretion through the digestive tract (Blanco et al., 2007; Svensson, 2003; Prego-Faraldo et al., 2013). Furthermore, active depuration of OA in bivalves has been examined; however independent investigations based on both environmental and endogenous factors eventually ruled it unlikely (Prego-Faraldo et al., 2013). On the one hand, it has been proven that OA depuration control is unresponsive to rapid environmental changes. On the contrary, supplementary reports showed that neither organism length nor neither age played a significant role in the depuration rate of OA (Svensson & Förlin, 2004; Prego-Faraldo et al., 2013).

4.2.4 Methods of detection and quantification of Okadaic acid.

In order to detect and quantify the okadaic acid strategies, it is possible to take the biological, chemical, and biochemical approaches (Fig. 10).

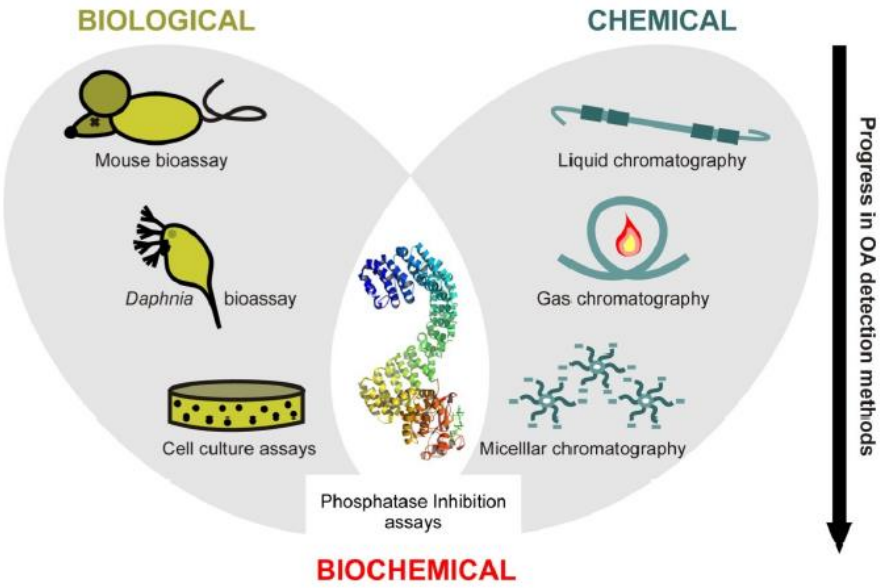


Figure 10. Methods most commonly used for Okadaic Acid (OA) detection and quantification (Prego-Faraldo et al., 2013).

4.2.4.a Biological Methods

The study of OA's toxicological effect on either animals or tissues/cells forms the basis of its biological detection (Prego-Faraldo et al., 2013). Biological parameter-based methods were the first to be developed and are still the most used method for detecting marine biotoxins today. The Mouse BioAssay (MBA) is the gold standard for detecting OA in food samples, and its widespread use (Yasumoto et al., 1978; Prego-Faraldo et al., 2013) makes it stand out among biological approaches. However, the MBA's limited specificity and sensitivity, as well as its reliance on test laboratory animals, presents both ethical and technical challenges in terms of its practical applicability (Vilariño et al., 2010; Prego-Faraldo et al., 2013).

Therefore, authorities have encouraged the development of alternative methods to improve or replace the MBA, such as the development of biological detection methods using alternative test organisms, such as the planktonic crustacean *Daphnia Magna* (Daphnia bioassay), which is a cheap tool that can measure OA levels up to 10 times below the MBA's threshold (Vernoux et al., 1993; Prego-Faraldo et al., 2013). However, the approach is not sensitive enough to fully replace the MBA just yet (Garthwaite, 2000; Prego-Faraldo et al., 2013). In a similar vein, alternative detection procedures based on molecular methodologies, such as cytotoxic assays based on the examination of morphological alterations of cultured cell lines exposed to OA (Croci et al., 1997; Amzil et al., 1992; Tubaro et al., 1996; Prego-Faraldo et al., 2013), have been proposed. As a bonus, these methods eliminate the need to conduct experiments on live animals, allowing for greater sensitivity in diagnosing OA. Overall, the improvements to biological approaches for OA detection will pave the way for exciting new advancements shortly (Prego-Faraldo et al., 2013).

4.2.4.b Biochemical Methods

Although biological techniques are the gold standard for detecting marine biotoxins, they do not lend themselves to quantitative analysis of the chemicals of interest. Chemical detection and quantification approaches based on the chromatographic features of biotoxins (Gerssen et al., 2010; Gerssen et al., 2011) have been developed due to this nuisance (Prego-Faraldo et al., 2013). The most common chemical techniques for OA detection rely on Liquid Chromatography (LC) or High-Performance Liquid Chromatography (HPLC) separation

strategies coupled with Mass Spectrometry (LC-MS), tandem mass spectrometry (LC-MS/MS), FLuorimetric Detection (HPLC-FLD), and UltraViolet Detection (HPLC-UVD) (Prego-Faraldo et al., 2013; Vilariño et al., 2010; Christian & Luckas, 2007; Lee et al., 1987).

Given the main importance of this biotoxin during DSP outbreaks along the European coasts (Falconer, 1993; Gerssen et al., 2010; Nincevic Gladan et al., 2011; Armi et al., 2011; Prego-Faraldo et al., 2013); the development of simple, rapid, sensitive, reproducible, and inexpensive detection methods for OA has been a key priority for some time. As a result of fusing biological and chemical approaches, highly effective biochemical techniques have been developed and are now being used for the detection and quantification of OA (Prego-Faraldo et al., 2013). Inhibition of protein phosphatases is the most common target for these biotoxins detection procedures (Vieytes et al., 1997; Prego-Faraldo et al., 2013).

One biochemical approach for detecting and quantifying OA is the Protein Phosphatase 2A (PP2A) inhibition assay (Tubaro et al., 1996; Prego-Faraldo et al., 2013). As long as some biochemical approaches continue to underestimate the overall amount of toxin in the samples (Morton & Donald, 1996; Prego-Faraldo et al., 2013), the MBA method will continue to be recommended. However, direct labeling ELISA techniques are being developed at the moment (Sassolas et al., 2013), and they are more sensitive to OA than indirect labeling assays (Prego-Faraldo et al., 2013).

4.3 Brevetoxins (NSP, Neurotoxic Shellfish Poisoning)

4.3.1 Brevetoxins -producing microalgae species.

Brevetoxins produced by *Karenia brevis*, formerly known as *Gymnodinium breve* or *Ptychodiscus breve* (Davis, 1948; Gunter et al., 1947), and *Chattonella*, *Fibrocapsa*, and *Heterosigma* are those responsible for PSP and NSP, respectively (Daneshian et al., 2013; Pistocchi et al., 2012; Plakas & Dickey, 2010; Solter & Beasley, 2013) (Fig. 11).

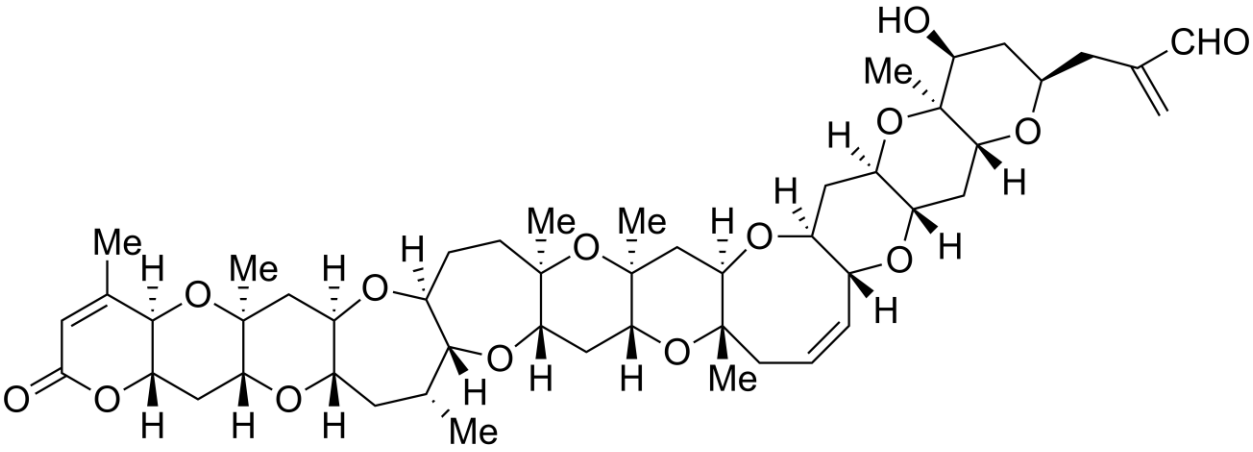


Figure 11. Brevetoxins basic structure.

4.3.2 Brevetoxins poisoning and action mechanism

Brevetoxins permeate cell membranes, including the blood-brain barrier, due to their lipid solubility. Brevetoxins bind to receptor site 5 on the voltage-gated sodium channel (VGSC) with high affinity and cause a channel-mediated Na+ ion influx. Nerve membrane depolarization and spontaneous firing cause neuro excitement. Only the nerve is depolarized in certain situations, although simultaneous nerve and muscle depolarization have been seen. Brevetoxins are quickly absorbed and transported throughout the body before being metabolized in the liver (Baden, 1983, 1989; Cattet & Geraci, 1993; M.-Y. Dechraoui et al., 1999; M.-Y. B. Dechraoui et al., 2005; Huang et al., 1984; Hughes & Merson, 1976; Mark et al., 1990; McFarren et al., 1965; Poli et al., 1986; Sakamoto et al., 1987; Watkins et al., 2008; Wu & Narahashi, 1988).

4.3.3 Transfer of Brevetoxins in the trophic chain.

The data collected in the last decade indicate that trophic transmission of brevetoxins through the food chain is a phenomenon that is significantly more complicated than was previously believed. During a red tide bloom, brevetoxins are first intracellular in *K. brevis*; however, as the cells lyse or die, the toxins become even more widespread in the environment (Blum et al., 2000). During continuous blooms, brevetoxin levels in the environment can rise and damage several animal species in the food web through various routes of exposure, such as vectoring or accumulation of toxins at different trophic levels. The next diagram represent

depicts a generalized method for transferring brevetoxins in the environment (Pierce et al., 1990; Tester, 2000; Landsberg, 2002) (Fig. 12).

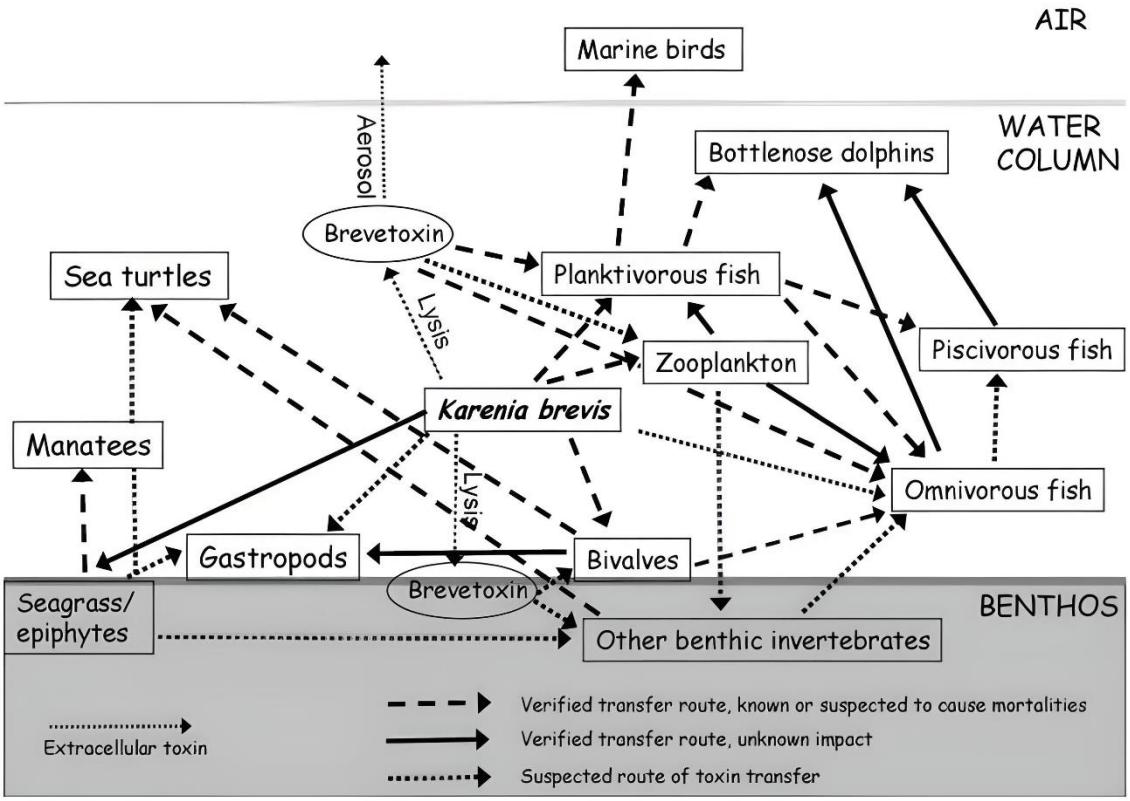


Figure 12. The propagation and impact of brevetoxins in the marine environment (Landsberg et al., 2009).

4.3.4 Methods of detection and quantification of Brevetoxins.

4.3.4.a Mouse bioassay

The toxicity of shellfish exposed to *K. brevis* blooms was measured using a mouse bioassay through history. By a standard protocol (APHA, 1970), the mouse bioassay correlates the time to death with the toxicity per unit dosage (administered i.p.) of crude hazardous substances extracted from shellfish homogenate using diethyl ether (Plakas & Dickey, 2010). The outcomes are reported in mouse units (MU) per 100g of tissue, as determined using dose-response tables. One mouse unit is the crude toxin required to kill 50 percent of test mice within 930 minutes (15.5h) (Plakas & Dickey, 2010).

4.3.4.b Cytotoxicity assay

The cytotoxicity assay is based on the effects of brevetoxins on voltage-gated sodium channels (VGSC). In its conventional formulation (Manger et al., 1993), cells of a known neuroblastoma type (neuro-2a) are pre-treated with veratridine (binds to site 2 of VGSC) to induce migration of VGSC to the open state and ouabain to inhibit sodium-potassium pump function. Brevetoxins bind to the veratridine-activated VGSC and enhance ion flow in a concentration-dependent way (Plakas & Dickey, 2010).

4.3.4.c Receptor binding assay

Receptor binding assay is a pharmacological technique based on brevetoxin's affinity for its sodium channel receptor (Plakas & Dickey, 2010). Composite brevetoxins are typically evaluated in shellfish tissue extracts via binding competition with radiolabeled brevetoxin for receptor sites, utilizing separated membrane preparations from excitable tissues (Trainer & Poli, 2000) or whole-cell preparations (Bottein Dechraoui et al., 2007; Plakas & Dickey, 2010).

4.4 Ciguatoxin (CFP, Ciguatera Fish Poisoning)

4.4.1 Ciguatoxin -producing microalgae species.

Gambierdiscus toxicus has been identified as the primary species responsible for CTX production (Fig. 13). This marine dinoflagellate thrives as an epiphyte on macroalgae on coral reefs, mangrove systems, and artificial surfaces or sand in tropical and subtropical seas. *G. belizeanus, G. yasumotoi, G. polynesiensis, G. pacificus, and G. australes* were later described as additional *Gambierdiscus* species. Several species have been described as toxin production, but their direct involvement in CFP outbreaks has never been proven. Cases ascribed to *G. toxicus*, on the other hand, might have been caused by different *Gambierdiscus* species (Caillaud et al., 2010; Chinain et al., 1999; Faust, 1995; Hales et al., 1999; Holmes, 1998; Rhodes et al., 2010; Villareal et al., 2007).

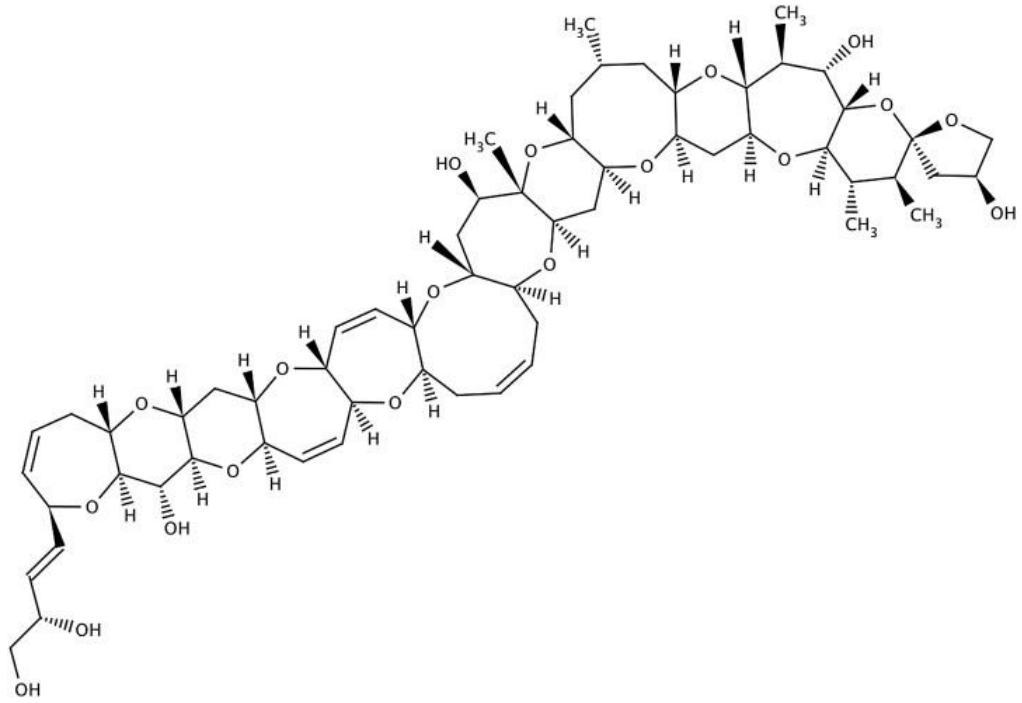


Figure 13. Ciguatoxin basic structure.

4.4.2 Ciguatoxin poisoning and mechanism of action.

CTX is one of nature's most powerful chemicals. Despite being toxic to humans at concentrations as low as 0.08 to 0.1 g/kg, one of the ciguatoxins (P-CTX-1, found in the Pacific Ocean) seldom accumulates to deadly levels in fish. CTX stimulates voltage-gated sodium channels, increasing sodium ion permeability and depolarizing the nerve cell. This depolarization of nerve cells is considered to be the source of the wide range of neurological symptoms associated with CFP (Arias, 2006; Baden et al., n.d; Cameron et al., 1991; Friedman et al., 2008; Lehane & Lewis, 2000; Nicholson & Lewis, 2006).

CTX is reported to have rather high toxicity in humans, causing symptoms such as perioral numbness and paralysis, reversal of temperature feeling, muscle and joint pains, headache, itching tachycardia, hypertension, impaired vision, and paralysis (Daneshian et al., 2013).

4.4.3 Transfer of Ciguatoxin in the trophic chain.

Mills (1956) was the first to offer the food chain hypothesis, which implies that the transmission of poisons along the food web explains CFP migration. Mills proposed that phytoplankton have a role in toxin production. Randall (1958) later supported this

implication. The isolation of a hazardous marine benthic dinoflagellate, later named Gambierdiscus toxicus, provided more proof; it was considered a credible source of CTXs (Yasumoto et al., 1977). Adachi and Fukuyo (1979) verified that both wild and farmed G. toxicus produce CTX precursors (known as *gambiertoxins*) (Satake et al., 1996). It was proposed that the transfer of CTXs along the food web would begin with dinoflagellates, then progress to herbivorous grazing fish, and finally to carnivorous fish that fed on the grazers (Garcon, 1979; Caillaud et al., 2010).

Some of these toxins accumulate in fish tissue and can be converted into forms (e.g., CTX-1B and 51-hydroxyCTX-3C) that are ultimately responsible for human intoxication (Caillaud et al., 2010).

4.4.4 Methods of detection and quantification of Ciguatoxin.

4.4.4.a Bioassays with animals

Attempts to employ a variety of animals to screen ciguatoxicity in fish have been proposed; however, except for mice, animal bioassays have met with minimal success (Banner, 1976). In vivo bioassays involve feeding or exposing animals to fish samples or administering fish extract by injection. Among these assays, mammalian-based approaches utilizing mangoose (Banner et al., 2006) or cat (Bagnis et al., 1985) may elicit symptoms similar to those observed in humans exposed to CTX-containing foods (Caillaud et al., 2010).

4.4.4.b Bioassay utilizing tissues

The contractile activity of CTXs was studied on isolated guinea pig atria and was found to have a prolonged, positive ionotropic impact on electrically stimulated atria (Miyahara et al., 1979; Caillaud et al., 2010). This effect was refined to allow the detection of CTX-containing fish extract using the guinea pig atrium test (Caillaud et al., 2010). The test compared the amplitude of contraction of the guinea pig atrial after the administration of the extract to the amplitude of contraction of the untreated (Kimura et al., 1982; Caillaud et al., 2010). This assay validated ionotropic action in fish extract (Campora et al., 2008; Caillaud et al., 2010) when CTXs were present in high amounts (Caillaud et al., 2010).

4. 5 Domoic acid (ASP, Amnesic Shellfish Poisoning)

4.5.1 Domoic acid -producing microalgae species.

Domoic acid (DA) is currently known to be produced by 11 species of pennate diatoms in the genus *Pseudo-nitzschia* (*P. multiseries, P. pseudelicatissima, P. delicatissima, P. australis, P. seriata, P. fraudulenta, P. pungens, P. turgidula, P. multistriata, P. calliantha, P. cuspidata, and P. galaxiae*) as well as a related species, *Nitzschia navis-varingica* (Bates & Trainer, 2006; Bejarano et al., 2008; Kotaki et al., 2000) (Fig. 14).

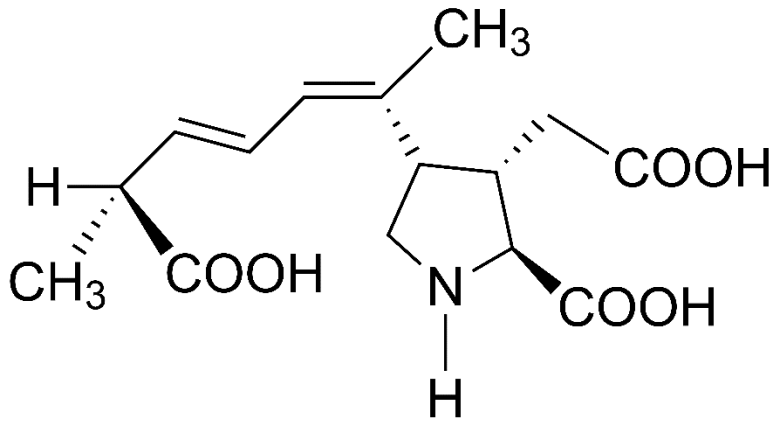


Figure 14. Domoic acid basic structure.

4.5.2 Domoic acid poisoning and mechanism of action.

Domoic acid (DA) harms animals with sophisticated central nervous systems. This biotoxin is a derivative of glutamic acid, an amino acid, and kainic acid, an excitatory neurotoxin. Because of its structural similarities to both substances, DA can bind tightly to glutamate receptors and displace both drugs from binding sites, enhancing its lethal potential. Glutamic acid is a major excitatory neurotransmitter in the central nervous system, found in almost 40% of all neuronal synaptic locations (Bejarano et al., 2008; Coyle & Puttfarcken, 1993).

4.5.3 Transfer of Domoic acid in the trophic chain.

The absorption of DA into sediments could have been enduring effects on the aquatic food web (Trainer et al., 2012). DA is stored by zooplankton, molluscan shellfish, crustaceans, cephalopods, and worms during hazardous *Pseudo-nitzschia* blooms (Bargu et al., 2002; Goldberg, 2003; Maneiro et al., 2005), confirming its steady transmission

across the marine food web. Consumption of infected food products (such as squid, scallops, mussels, and razor clams) is humans most common route of DA poisoning (Trainer et al., 2012; Lefebvre & Robertson, 2010; Kvitek et al., 2008).

4.5.4 Methods of detection and quantification of Domoic acid.

4.5.4.a Liquid chromatography-mass spectrometry with a high level of performance.

Several modified or innovative LC-MS approaches for detecting DOM have been utilized. Lately, laser ablation electrospray ionization-high resolution mass spectrometry (LAESI-HRMS) has been used for DOM measurement with LODs ranging from 0.24 to 1.6 mg DOM/kg tissue. It can identify samples containing less than 5 mg DOM/kg of wet clam tissue, which is one-fourth of the regulation limit (Beach et al., 2016).

4.5.4.b Enzyme-linked immunosorbent assay

ELISA is often used to detect DOM in shellfish (Branaa et al., 1999; Kawatsu et al., 1999), blue mussels (Tsao et al., 2007; Kleivdal et al., 2007), clams (Yu et al., 2004), pacific oysters, king scallops (Kleivdal et al., 2007), bivalve molluscs (Johnson et al., 2016), human bodily fluids (Smith & Kitts, 1994), and seawater (Garthwaite et al., 1998).

ELISA kits have also been utilized to measure dissolved DOM samples from pseudo-nitzschia (LOD of 6.8 ng/L) (Sun et al., 2011; Saeed et al., 2017).

Chapter 5: Main events of HABs

5.1 The United States of America

Each year, several “hot spots” along the West Coast of the United States experience harmful algal blooms. Many marine birds and mammals are susceptible to a neurotoxic produced by some HABS; this toxin is called Domoic acid and can accumulate in shellfish, other invertebrates, and even fish (Lewitus et al., 2012).

The accumulation of algal toxins in shellfish, which can poison humans and animals who eat or absorb contaminated seafood, is one of the most serious repercussions of HABs in the United States (Anderson et al., 2021). Paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP), amnesic shellfish poisoning (ASP), and azaspiracid shellfish poisoning (AZP) are all names given to human poisoning syndromes caused by eating shellfish. Recreationally gathered shellfish pose the biggest threat to human health; commercially harvested shellfish and finfish are strictly regulated to ensure their safety. Paralytic shellfish poisoning-causing *Alexandrium catenella* blooms also occur nearly annually in this area. Sporadic occurrences of additional HABs, especially threatening animal and ecological health, also occur in these areas. Dinoflagellates are responsible for all these diseases except ASP, which is caused by the toxin domoic acid, produced primarily by diatoms of *Pseudo-nitzschia*. In the next figure, it is possible to identify one of the most common hotspots in the United States on the west coast (Anderson et al., 2021) (Fig. 15).

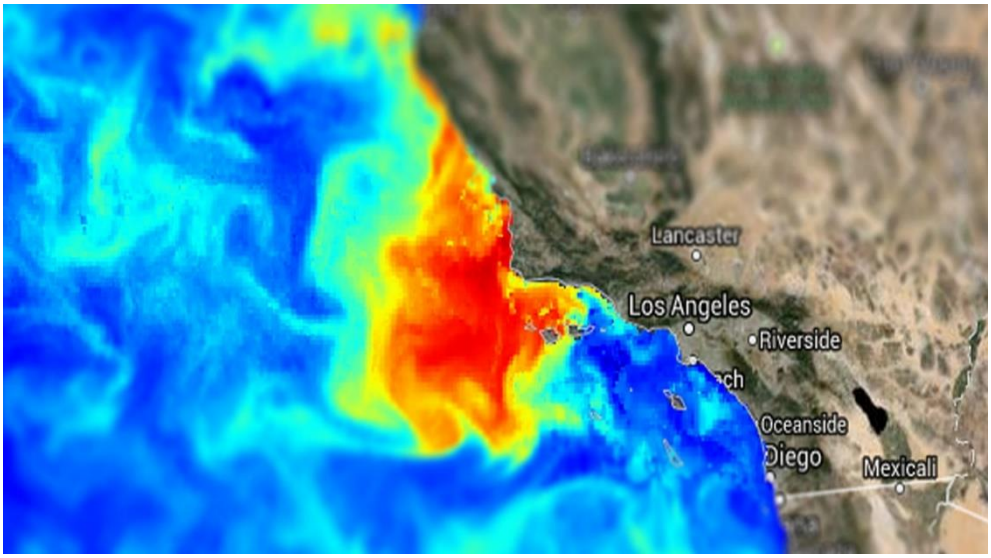


Figure 15. Illustration of a harmful algal bloom off the Californian coast in 2017 (NOAA).

The first verified case of diarrhetic shellfish poisoning in the United States occurred in July 2011; where the Washington Department of Health reported to the Public Health–Seattle & King County that a family's health was compromised when they ate mussels that had been picked for fun, three of the family members symptoms appeared 4, 7, and 14 hours (2, 5, and 45 years old) after eating them (Lloyd et al., 2013). Mussels were collected from a public dock at Washington, Sequim, and Sequim Bay State Park (Lloyd et al., 2013). Towards this event, the dock was converted into a DSP monitoring site where hydrolyzed extracts were evaluated by liquid chromatography-tandem mass spectrometry on August 2, 2011 (European Union Reference Laboratory for Marine Biotoxins, 2011; Lloyd et al., 2013) (Fig. 16).

All analyses were conducted in negative ion mode with multiple-reaction monitoring and acidic chromatographic conditions (Lloyd et al., 2013).

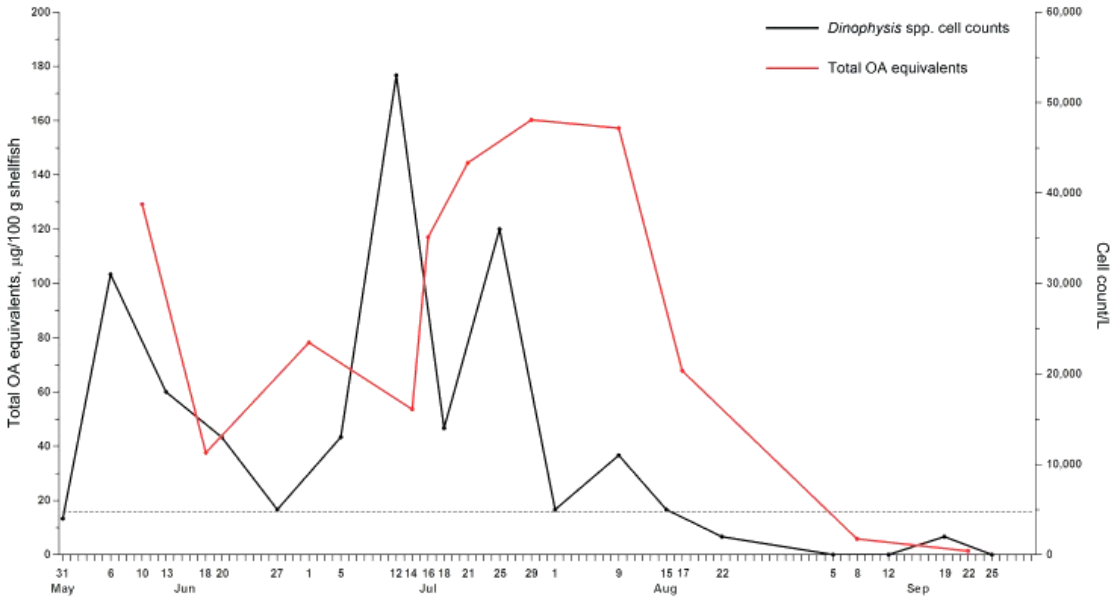


Figure 16. Timeline comparing blooms of *Dinophysis spp*. Dinoflagellates and diarrhetic shellfish poisoning toxin levels were detected in mussels collected in 2011 from Sequim Bay State Park, Sequim, Washington (Lloyd et al., 2013).

The OA equivalents of all OA-related substances were established by calibration using the previously described European standard. Utilizing approved reference standards (OA, DTX-1, and DTX-2) from the National Research Council, retention time and equimolar response were validated above the FDA guideline threshold. DTX-1 was shown to be the primary DSP toxin in every case. These heightened levels of DSP toxins followed the dBy calibrating with the European standard mentioned before; the OA equivalents of all OA-

related compounds were determined. Detection of *Dinophysis* blooms. Since then, shellfish contamination by diarrhetic shellfish toxins (DTX), most notably DTX-1, has resulted in the annual closure of over one hundred commercial and recreational shellfish harvesting locations in Washington State (Lloyd et al., 2013).

Managers assume additional toxins, such as AZAs, are to blame for DSP-like symptoms reported by consumers who ate Puget Sound shellfish from locales with no DTX or *Vibrio* (Kim et al., 2017).

In another study, sediment samples from Puget Sound, Washington, were used to isolate *Azadinium* spp. in a separate investigation. Light and electron microscopy were used to investigate the morphology of many species of *Azadinium*, all of which were conclusively identified. A new azaspiracid toxin was confirmed in situ at low concentrations utilizing a solid phase resin at various areas along the beaches of Puget Sound (Kim et al., 2017).

The following methods detected Azadinium in sediment samples: DNA extraction from sediments and Quantitative PCR (Kim et al., 2017). In this study, they conducted several tests and found some interesting findings. Sediment samples from 15 sites in Puget Sound were collected using a hydraulically dampened Craib corer in January and February of 2016. Until analysis, the top 0–1 cm of sediment from the core or grab samples was maintained in the dark at 4 °C (Craib, 1965).

Then, they conducted a microscopy study. One of these experiments used cells under a stereomicroscope with dark field illumination, an inverted microscope, and a compound microscope with differential interference contrast optics. All the microscopy experiments throw the following results see Table 2.

The detection of *Azadinium* in sediment samples were made by the following methods: DNA extraction from sediments and Quantitative PCR (Kim et al., 2017). Then, they conducted a microscopy study. One of these experiments carried out used cells under a stereomicroscope with dark field illumination, an inverted microscope and a compound microscope with differential interference contrast optics. All the microscopy experiments throw the following results see Table 2.

Strain information (LM-light microscopy; SEM - scanning electron microscopy, LSU - large subunit rDNA, ITS - internal transcribed spacer, ND - not detected; - - not analyzed).

	Strain	Origin Station	Length (µm) **Mean** ±SD (Min-max)	Windth (µm) **Mean** ±SD (Min-max)	I/w ratio **Mean** ±SD	N	Morphological analysis		Antapical spine	Sequence data	Accession number	AZA
A. cuneatum	35C4	Dabob Bay	**13.2**±1.4 (10.5-16.3)	**9.8**±1.1 (7.0-12.4)	**1.36**±0.10	106	LM	SEM	No	LSU,ITS	KY404229	ND
	35A2	Dabob Bay	**14.4**±0.9 (12.9-17.9)	**11.1**±0.9 (9.4-12.8)	**1.30**±0.10	55	LM	SEM	No	LSU,ITS	KY404228	ND
	965F5	Dabob Bay	**14.4**±0.9 (12.4-18.1)	**10.9**±0.7 (9.0-12.8)	**1.32**±0.06	100	LM	SEM	No	LSU,ITS	KY404225	ND
	966G8	Dabob Bay	**14.2**±1.7 (10.3-18.8)	**10.6**±1.5 (7.2-14.3)	**1.34**±0.10	106	LM	-	No	LSU,ITS	KY404226	ND
	968B10	Dabob Bay	-	-	-	-	-	-	-	LSU,ITS	KY404227	ND
A. dalianense	121F6	Center Lynch Cove	**15.6**±1.5 (11.0-18.9)	**11.2**±1.2 (8.4-14.6)	**1.40**±0.11	106	LM	SEM	74%(n=121) 54%(n=100)	LSU,ITS	KY404223	ND
	962B8	Center Lynch Cove	**15.4**±1.4 (12.6-19.7)	**11.3**±1.3 (8.3-14.6)	**1.37**±0.11	106	LM	SEM	20%(n=100) 31%(n=100) 37%(n=100)	LSU,ITS	KY404222	ND
	481F8	Center Lynch Cove	**15.8**±1.5 (12.1-19.8)	**11.0**±1.3 (8.5-13.9)	**1.45**±0.12	108	LM	SEM	72%(n=105)	LSU,ITS	KY404220	ND
	962B3	Center Lynch Cove	**16.9**±1.5 (13.4-20.1)	**12.2**±1.2 (8.8-15.8)	**1.39**±0.09	82	LM	SEM	74%(n=101) 83%(n=100)	LSU,ITS	KY404221	ND
	481F2	Center Lynch Cove	**15.5**±1.2 (11.5-19.2)	**11.2**±1.1 (8.7-16.5)	**1.39**±0.10	107	LM	SEM	No	LSU,ITS	KY404219	ND
A. poporum	967B8	Dabob Bay	**17.1**±1.9 (12.5-21.4)	**12.8**±1.6 (8.7-16.6)	**1.34**±0.09	107	LM	SEM	No	LSU,ITS	KY404215	AZA-59
	967G9	Dabob Bay	**16.4**±1.6 (12.9-21.0)	**11.6**±1.1 (9.0-16.2)	**1.42**±0.10	107	LM	SEM	No	LSU,ITS	KY404216	AZA-59
	968B7	Dabob Bay	**15.2**±1.6 (11.4-20.3)	**11.2**±1.4 (8.0-15.9)	**1.37**±0.11	107	LM	SEM	No	LSU,ITS	KY404217	AZA-59
	121E10	Center Lynch Cove	**15.9**±1.2 (11.3-18.5)	**11.0**±1.1 (8.5-16.6)	**1.45**±0.12	107	LM	SEM	No	LSU,ITS	KY404218	AZA-59

Each row represents spine frequency determined on independent subcultures at different times.

Table 2. Results of the microscopy experiments performed (Kim et al., 2017).

The shellfish time series data gathered by state shellfish safety programs like those in Washington State, Oregon, and California has been extremely helpful in identifying patterns and hotspots. The strong relationship between toxins in species like the Dungeness crab and the razor clam is illustrated by data from a DA time series obtained near Long Beach, Washington. The correlation between rising clam abundance and rising crab abundance demonstrates the importance of razor clams as a food source for crabs. The Monitoring Oregon's Coast for Harmful Algae data set and the California Department of Public Health Marine Biotoxin Monitoring Program monthly report are two more valuable data sets that shed light on environmental factors contributing to DA incidents (Anderson et al., 2021).

The United States has been monitoring almost all harmful algae events all over the country. However, focusing only on the west pacific coast, it is possible to see that Washington has had the majority of cases reported achieving 111(Harmful Algal Information System) (Fig. 17) throughout history, making it an extremely important place to carry a different kind of experiments. The economic impact of H. *akashiwo* blooms in Washington State has ranged from $2 million to $6 million per episode (Anderson et al., 2021).

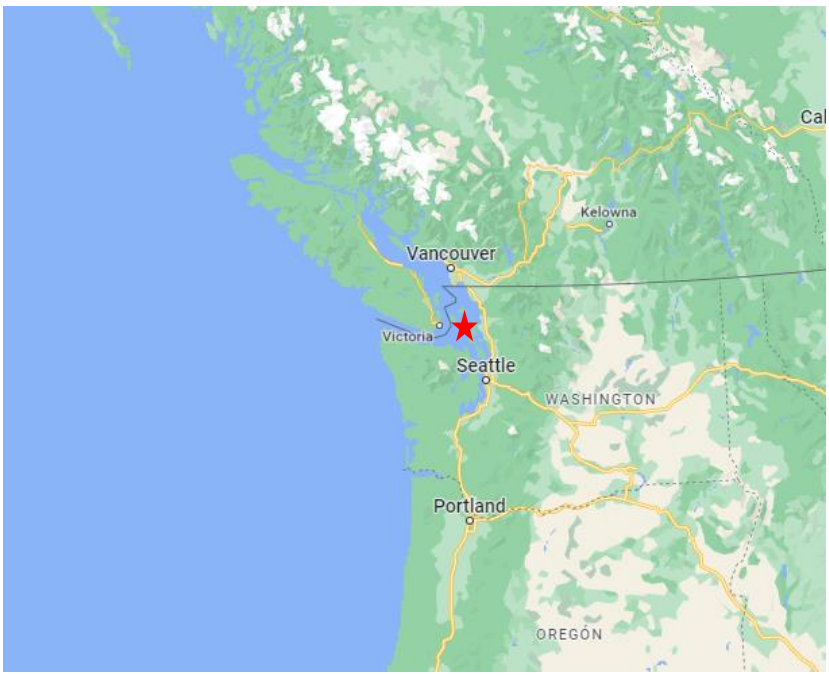


Figure 17. Location of events related to HABs, the West Pacific Coast in Washington.

Another incident was when mussels caught in the waters around Mount Desert Island were recalled in September 2017 by the state of Maine's Department of Marine Resources. For fear of contamination with a marine neurotoxin, thousands of pounds of shellfish from five dealers in Maine were recalled. After a similar algal bloom in 2016, this was the second documented recall in history. Maine's Department of Marine Resources issued the following safety notice:

Those who eat tainted shellfish develop gastrointestinal symptoms within the first 24 hours, such as nausea, vomiting, abdominal cramping, and diarrhea. Some people experience neurological conditions that persist, including dementia. While it is uncommon, the neurotoxic can be lethal, especially in the elderly and kids with diabetes, chronic renal disease, and hypertension (Harmful Algal Information System).

Moreover, because of recent issues with net pen collapse, salmon aquaculture in Puget Sound has been banned as of 2017; there are no longer any records of mortalities attributable to H. *akashiwo*. Aquacultured fish mortality due to H. *akashiwo* has been documented most thoroughly in surrounding Canadian inland streams, which share territory with the USA, where detailed histories of H. *akashiwo's* appearance may be found (Anderson et al., 2021).

5.2 Canada

The history of harmful algae (HA) studies in Canada began with research and monitoring of the Atlantic and Pacific coasts in the early 1940s. However, until the 1980s, the coordinated national Canadian HA program was formally established. Several studies are evaluating the last three decades of harmful algal events. One of the most current takes 500 events from the Harmful Algal Event Database (HAEDAT) reported in Canada between 2000 and 2017 (McKenzie et al., 2021) to compare them (Fig. 18A and 18B).

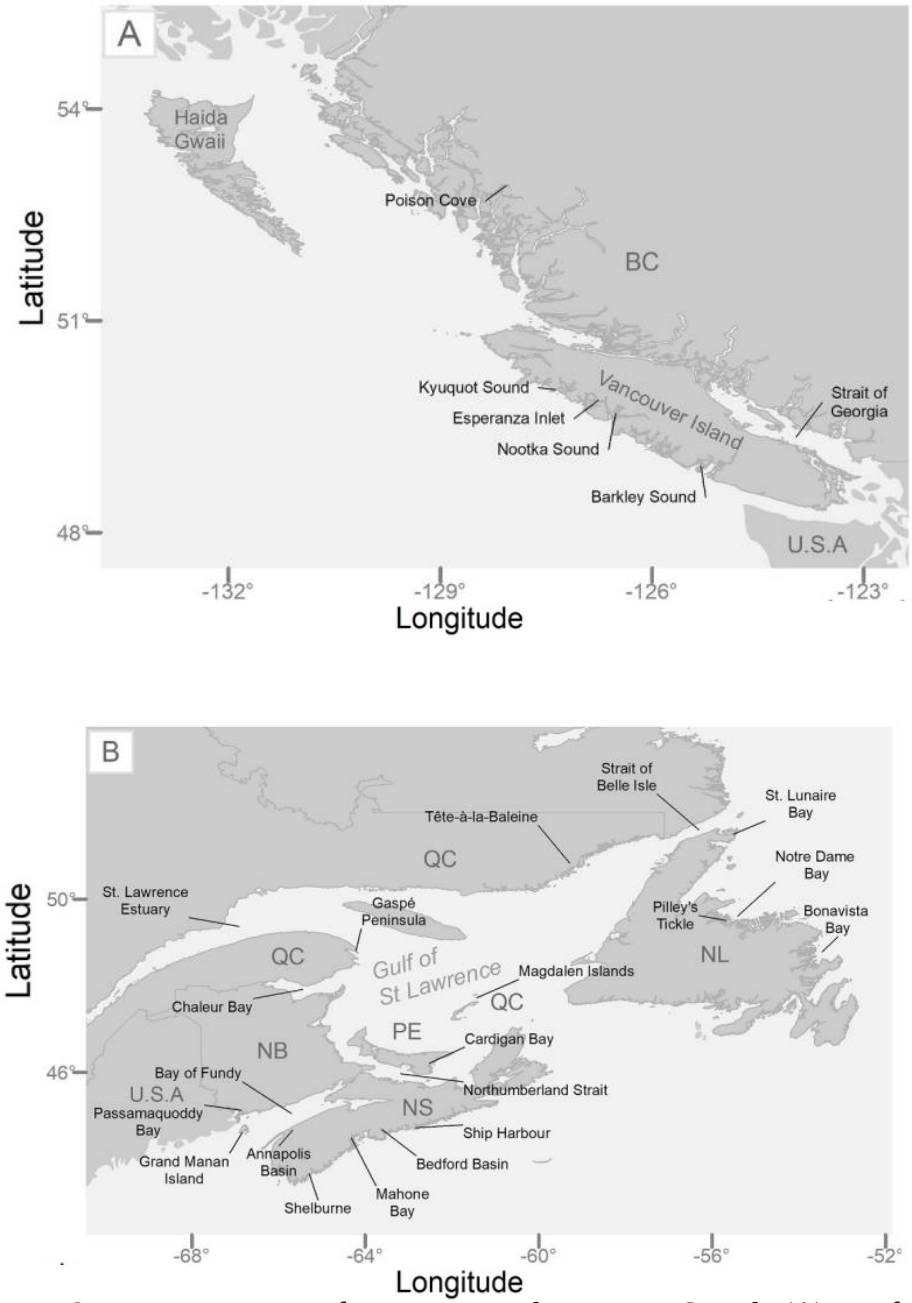


Figure 18. Major Locations of Harmful Algal Events in Canada (A) Pacific Coast and (B) Atlantic Coast (McKenzie et al., 2021).

Neurotoxic and azaspiracid shellfish and ciguatera poisonings occur in other parts of the world, but these poisonings have not been linked to fish or shellfish harvested from Canadian marine waters (McIntyre et al., 2013). The Atlantic coast presents 279 events, and the Pacific coast 221 events. The nature of them, depending on the toxins and the damage they cause, was divided into three types: phycotoxins events associated with amnesic shellfish toxin (AST), diarrhetic shellfish toxin (DST), and paralytic shellfish toxin (PST) (The most common with 315 events).

5.2.a AST Events

The principal toxin responsible for amnesic shellfish poisoning (ASP) is domoic acid (Fig. 19). Historically, domoic acid was identified from the macro red algae *Chondria armata* in Southern Japan. However, following the 1987 outbreak of ASP in Canada, marine diatoms of the genus *Pseudo-nitzschia* spp were also shown to produce domoic acid. The specie is widely distributed worldwide in warm and cold climates (Bates, 1989). Numerous strains of *P. nitzschia* are known to produce domoic acid, including *P. multiseries, P. pseudodelicatissima,* and *P. australis.* Warmer sea temperatures (14–17 ˚C) tend to be associated with higher domoic acid production. However, some strains, such as *P. seriata*, have adapted to growth in cooler waters at four ˚C (Jeffery et al., 2004; Ramsey et al., 1998).

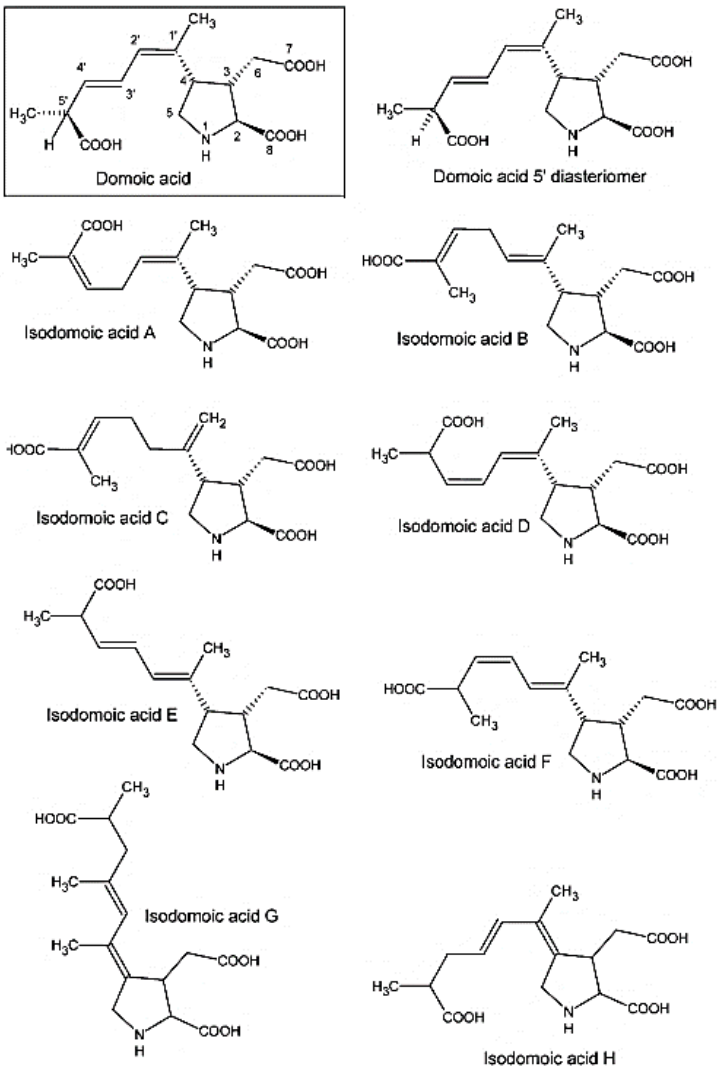


Figure 19. The chemical structures of domoic acid are its 50 diastereomers and its isomers, isodomoic acids A–H (Jeffery et al., 2004).

The toxin becomes an environmental and human health problem because shellfish consume the type of microalgae *Pseudo-nitzschia spp* in abundance when blooms occur. The biotoxin accumulates in their tissues, and when these shellfish are introduced into the human diet, they cause severe amnesic poisoning resulting in permanent short-term memory loss (Trainer et al., 2012; Lefebvre & Robertson, 2010; Kvitek et al., 2008).

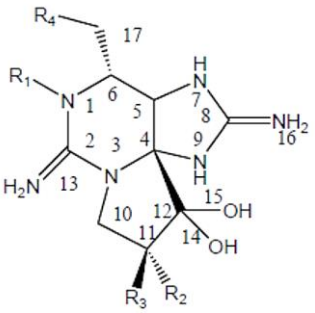
5.2.b DST Events

Diarrhetic Shellfish Poisoning (DSP) is caused by consuming okadaic acid (OA) group toxins, including OA, dinophysistoxin-1, 2 (DTX-1, and DTX-2), and dinophysistoxin-3 (DTX-3) which are produced by dinoflagellate algae *Dinophysis spp.,* and *Prorocentrum spp.* (James et al., 2010; MacKenzie et al., 2002; Rossignoli et al., 2011).

Most DSP outbreaks and toxin detections (primarily DTX-1) have been reported from eastern Canada (Todd, 1997). The cases have been primarily studied in British Columbia (BC) because seafood is a major industry in this province and contributes more than $233 million annually to the economy (BC Ministry of Agriculture, 2013). Infectious shellfish poisonings in BC have been reported to include those caused by Vibrio species, bacterial organisms prevalent in marine waters, and norovirus, an environmentally resistant virus, with humans as the only known reservoir. Most of the incidences attributed to the consumption of bivalve mollusks in BC are due to these two agents (The most common are attributed to *V. parahaemolyticus*) (Patel et al., 2009; Khaira & Galanis, 2007; McIntyre et al., 2013). In addition to human health effects, the lethal and sublethal effects of HABs on shellfish may also be a concern for shellfish farmers in BC. Various species of dinoflagellates (*Protoceratium reticulatum, Noctiluca scintillans, Cochlodinium fulvescens*), as well as raphidophytes (*Heterosigma akashiwo*), silicoflagellates (*Dictyocha fibula and D. speculum*), and diatoms (*Rhizosolenia setigera*, large C*haetoceros* species) can produce harmful irritants or create low oxygen conditions causing shellfish mortality (Landsberg, 2002; Taylor & Trainer, 2002; Keppler et al., 2005; Cassis et al., 2011; McIntyre et al., 2013).

5.2.c PST Events

Paralytic shellfish toxins are potent neurotoxins that cause paralytic shellfish poisoning (PSP) in fish, birds, and mammals, including humans (Raposo et al., 2020). PST is a group of more than 50 neurotoxic alkaloids (Wiese et al., 2010) (Fig. 20).



Division	Name [a]	R1	R2	R3	R4
Carbamate	STX	H	H	H	$OCONH_2$
	NeoSTX	OH	H	H	$OCONH_2$
	GTX1	OH	OSO_3^-	H	$OCONH_2$
	GTX2	H	OSO_3^-	H	$OCONH_2$
	GTX3	H	H	OSO_3^-	$OCONH_2$
	GTX4	OH	H	OSO_3^-	$OCONH_2$
N-sulfocarbamoyl	GTX5 (B1)	H	H	H	$OCONHSO_3^-$
	GTX6 (B2)	OH	H	H	$OCONHSO_3^-$
	C1	H	OSO_3^-	H	$OCONHSO_3^-$
	C2	H	H	OSO_3^-	$OCONHSO_3^-$
	C3	OH	OSO_3^-	H	$OCONHSO_3^-$
	C4	OH	H	OSO_3^-	$OCONHSO_3^-$
Decarbamoyl	dcSTX	H	H	H	OH
	dcNeoSTX	OH	H	H	OH
	dcGTX1	OH	OSO_3^-	H	OH
	dcGTX2	H	OSO_3^-	H	OH
	dcGTX3	H	H	OSO_3^-	OH
	dcGTX4	OH	H	OSO_3^-	OH
Deoxydecarbamoyl	doSTX	H	H	H	H
	doGTX2	H	H	OSO_3^-	H
	doGTX3	H	OSO_3^-	H	H

[a] Abbreviations: STX, saxitoxin; GTX, gonyautoxin.

Figure 20. Chemical structures of common PST and M-toxin.

PST are produced by marine dinoflagellates of the genera *Alexandrium, Gymnodinium,* and *Pyrodinium*. In Canada, it was reported that PST events had occurred annually on the Atlantic (196 events) and the Pacific (119 events) coast since 2000. Fish mortalities, after PST events, were the second most reported event on the two coasts (82 events) (MacKenzie et al., 2002) (Fig. 21).

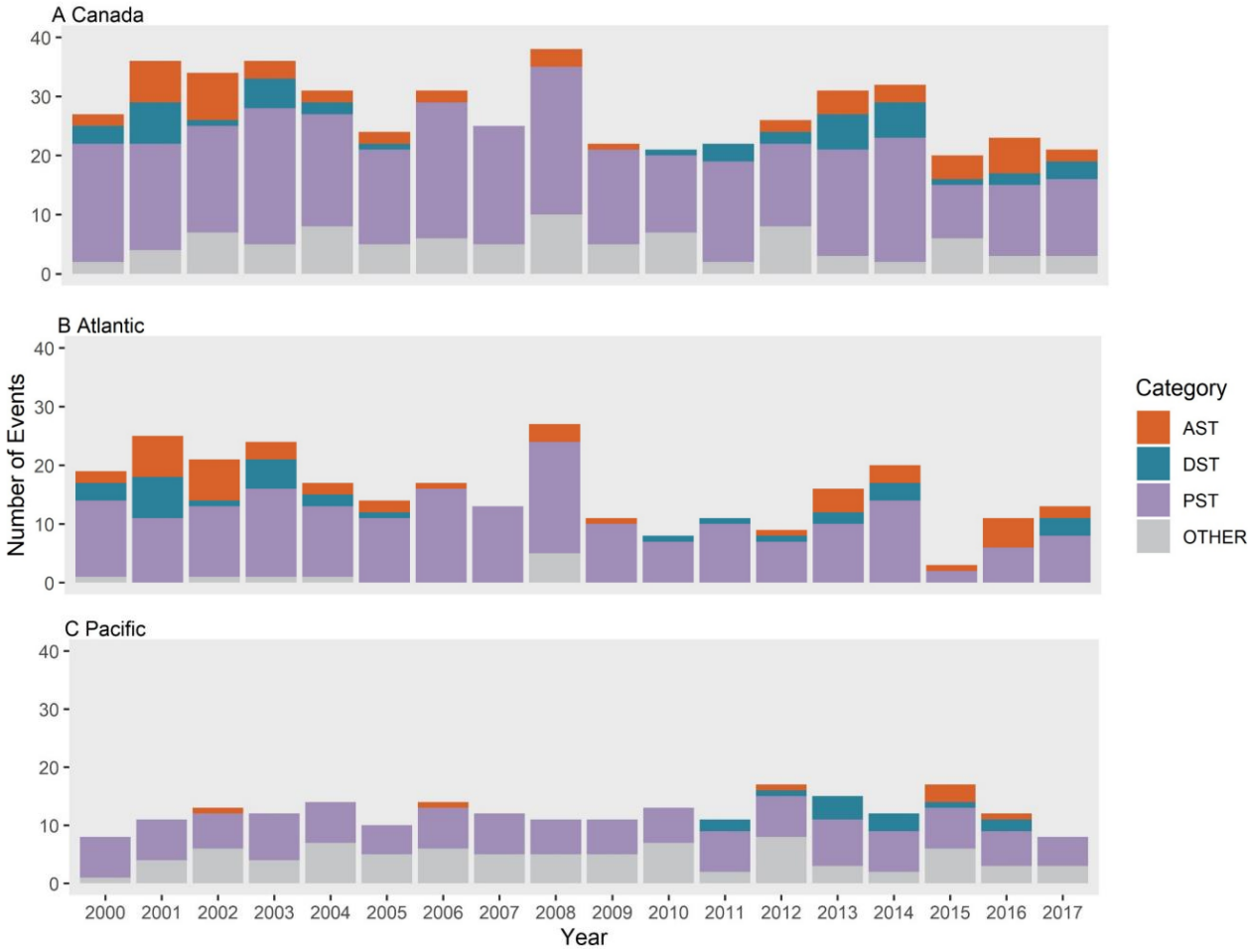


Figure 21. Harmful algal events reported in HAEDAT (2000 to 2017) for AST=amnesic shellfish toxin, DST=diarrhetic shellfish toxin, PST=paralytic shellfish toxin, and OTHER=marine mortalities for (A) Canada, (B) Atlantic Canadian coast, and (C) Pacific Canadian coast (MacKenzie et al., 2002).

Multiple flagellate species have been linked to mass mortalities of farmed Atlantic salmon (Salmo salar) on the Pacific coast since 2001. These mortality events have occurred in most years, from 2001 to 2017, sometimes involving multiple species of flagellates. The Canadian HAEDAT events that resulted from phycotoxins (AST, PST, DST) included those that caused human morbidity or mortality, resulted in the closure of shellfish areas, or were associated with mass deaths of marine species (MacKenzie et al., 2002).

5.3 Mexico

Mexico has 11,000 kilometers of coastline and 1,567 square kilometers of bays, including 58 ports and stations in the Pacific Ocean and 59 in the Caribbean Sea and the Gulf of Mexico. Due to their geographical location and land ranges, the following ports are classified as national ports and serve as economic corridors: Cayo de Arcas, Veracruz, Coatzacoalcos, Tuxpan, and Altamira in the Gulf of Mexico; Punta Venado in the Caribbean Sea; and Ensenada, Isla Cedros, Lázaro, Cárdenas, and Manzanillo in the Pacific Ocean. Like other countries, Mexico has experienced the occurrence of "red tides" and events related to HABs, possibly as a result of the effects of climate change (Secretaría de Comunicaciones y Transportes, 2013; Zepeda-Ortega et al., 2017; Salcedo-Garduño et al., 2018). Previously studies carried out by the Institute of Marine Sciences and Limnology, UNAM, analyzed Mexico's Pacific coastline, harmful algal blooms, and marine biotoxins. The next taken from this study, summarizes the locations of harmful algal bloom (HABs) events registered in Mexico until 2002 (Ochoa et al., 2002) (Fig. 22).

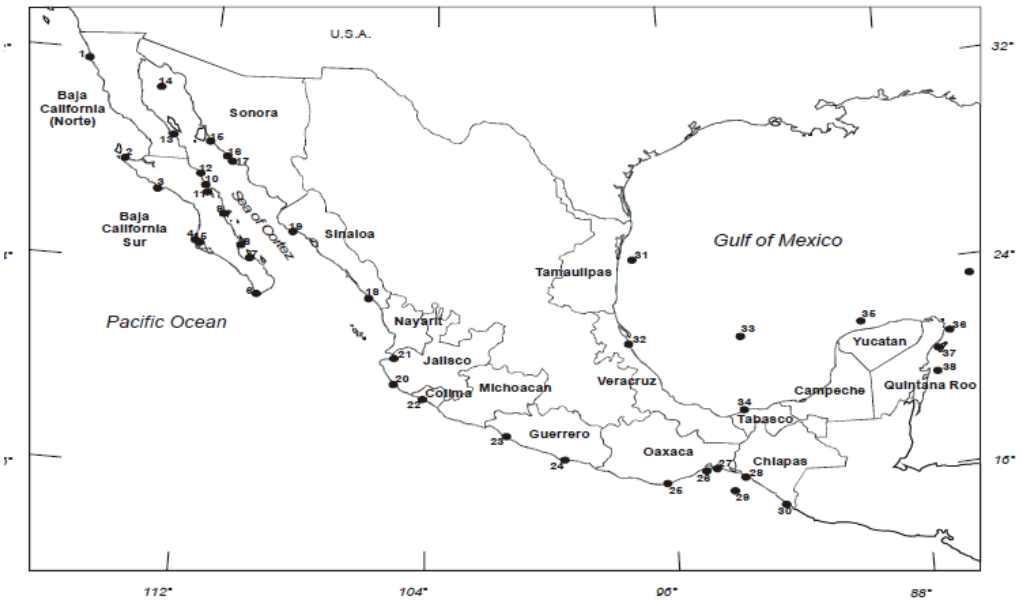


Figure 22. Location of events related to HABs on the Mexican Coast (Ochoa et al., 2002).

The types of marine biotoxins and human diseases associated with harmful algal blooms (HABs) along the Mexican coast are detailed in Table 3, 4 and 5, being dinoflagellates the taxa with the highest incidence.

Biotoxin/ Route of Acquisition	Poisoning/ Intoxication	Microalgae	General Symptoms	Mechanism of Action
Saxitoxin/ Consumption of cultivated or wild molluscs from affected areas.	Paralytic Shellfish Poisoning (PSP), from consumption of molluscs	Dinoflagellates: Previous Name: *Alexandrium catenella, Acatenella tamarense.* Current Name: *Gymnodinium catenatum (*), G. mikimotoii A. minutum (*), A. tamiyavanichi (*), A. andersoni , A. cohorticula, A. minutum, A. tamarense, A. monilatum(*), Pyrodinium bahamense var. compressum (*)* Cyanobacteria. *Cochlodinium, Polykrikoides(*)*	After 30 minutes: tingling; numbness in the face, neck and hands; headache; nausea; vomiting; diarrhea; difficulty breathing; muscle paralysis and death from respiratory failure within 2-24 hours after consumption.	Binds to the sodium channel and inhibits electrical signals that maintain nervous system activity, vegetative and synaptic.
Brevetoxin/ Consumption of cultivated or wild molluscs from affected areas; aerosols of the toxins produced by wave action.	Neurotoxic Shellfish Poisoning from the consumption of molluscs (NSP)	Dinoflagellates: Previous Name: *Ptychodiscus brevis, Gymnodinium breve* Current Name: *Karenia brevis (*,**), K. brevisulcatum, K. selliformis, K. bidigitata, Karenia spp. Pfiesteria piscida (neurotoxic), P. Shumwayae.*	Neurological symptoms after 3-6 hours: chills; headache; muscle weakness; nausea; vomiting; diarrhea; feelings of hot and cold; irritated eyes; double vision; difficulty speaking and swallowing; death from respiratory arrest.	Depolarizing acts on active sodium channels, altering the properties of the membrane of excitable cells to favor the inward flow of sodium ions.
Domoic Acid/ Consumption of molluscs from affected areas	Amnesic Shellfish Poisoning from the consumption of contaminated molluscs (ASP).	Diatoms: *Nitzschia pungens var, Multiseries Pseudonitzschia delicatissima, Pseudonitzschia multiseries, P. Pseudodelicatissima(*).* Previous Name: *N. pseudoseriata Hasle* Current Name: *Pseudonitzschia australis (*), P. seriata (*,**), P. Fraudulenta(*), P. multistriata, P. Pungens(*), P. Calliantha(*), P. Cuspidata(*), Amphora coffaeiformi, Nizschia navis-varingica, Thalassiosira.*	After 3-5 hours, gastrointestinal symptoms such as vomiting, diarrhea and abdominal cramps occur, with mild to severe pain, and dyspnea. Neurological symptoms such as disorientation, nausea, dizziness, low temperature, confusion and temporary memory loss.	Domoic acid binds with glutamate receptors located in the central nervous system. Domoic acid activates these receptors opening ion channels that allow passage into neurons of high concentrations of calcium ions, causing destruction of these cells and the loss of shortterm memory in affected individuals.

Table 3. Marine biotoxins, human diseases, and microalgae of the Mexican coast (Salcedo-Garduño et al., 2018).

Biotoxin/ Route of Acquisition	Poisoning/ Intoxication	Microalgae	General Symptoms	Mechanism of Action
Okadaic Acid/ Consumption of molluscs from affected areas.	Diarrheic Shellfish Poisoning from the consumption of molluscs (DSP).	Dinoflagellates: *Dinophysis acuninata ( ), Dinophysis caudata (*,**), D. norvegica (*), D. fortii (*,**), D. sacculus (*,**), D. miles ( ), D. acuta, D. mitra, D. rotundata, D. rapa, D. tripos, Dinophysis spp., Prorocentrum lima (*,**), P. arenarium, P.belizeamum, P. cassubicum, P. concavum, P. emarginatum, P. hoffmannianum, P. maculosum, P. dentatum(*), P. minimum(*), P. gracile, Procentrum spp., Phalacroma rotundatum*.	After 30 minutes, gastrointestinal symptoms such as diarrhea, nausea, vomiting, abdominal pain and chronic exposure promotes the formation of tumors in the digestive system.	Phosphorylase and phosphatase inhibitor with the latter regulating cellular functions such as mutagenesis.
Ciguatera Scaritoxin Maitotoxin Ciguatoxin/ Transfer through the trophic chain; consumption of tropical and subtropical fishes.	Ciguatera	Dinoflagellates: *Gambierdiscus toxicus* (Maitotoxin) (*,**), *G. australes, G. pacificus, G. polynesiensis, G. yasumotoi, Ostreopsis heptagona, Ostreopsis spp. (*,**), Coolia spp. (,), Prorocentrum lima(*,**)* .	Development of symptoms after 12 to 24 hours: gastroenteric symptoms such as acute gastroenteritis, metallic taste, nausea, diarrhea, stomach pains; neurological symptoms such as numbness and tingling of hands and feet, muscle pain, paresthesia in extremities, tachycardia, bradycardia, hallucinations, and death from respiratory failure.	Depolarizing activates sodium channels, altering membrane properties of excitable cells to favor the inward flow of sodium ions

Table 4. Marine biotoxins, human diseases, and microalgae of the Mexican coast (Salcedo-Garduño et al., 2018).

Biotoxin/ Route of Acquisition	Poisoning/ Intoxication	Microalgae	General Symptoms	Mechanism of Action
Neosaxitoxin Saxitoxin/ Direct contact or water consumption.	Cyanobacteria	*Cyanophyta, Aphanazomenon flosaquae, Anabaena circinalis. Ocillatoria. Microcystis sp. M. aeruginosa. Trichodesmium erythraeum(*,**) and T. Thiebautii.*	Acute toxicity: skin irritation, atypical pneumonia, vomiting abdominal pain and acute gastroenteritis, liver diseases, tumors by chronic exposure.	Binds to the sodium channel and inhibits electrical signals that maintain higher nervous system, vegetative and synaptic activity

Table 5. Marine biotoxins, human diseases, and microalgae of the Mexican coast (Salcedo-Garduño et al., 2018).

Poisonings caused by dinoflagellates have been recorded on the coasts of the Gulf of California, Baja California Sur, Sonora, Sinaloa, Colima, Michoacán, Jalisco, Guerrero, Oaxaca, and Chiapas in the Pacific littoral. In addition to the presence of diatoms that produce the domoic acid toxin in the Gulf of California, Baja California Sur, Sinaloa, Nayarit, and Chiapas and diatoms that produce the domoic acid toxin in the Gulf of California, Baja California Sur, Sinaloa, Nayarit and Chiapas (Ochoa, 2003). Next image is shown the phytoplankton, which produces toxins in HABs. On the coast of Mexico (Fig. 23).

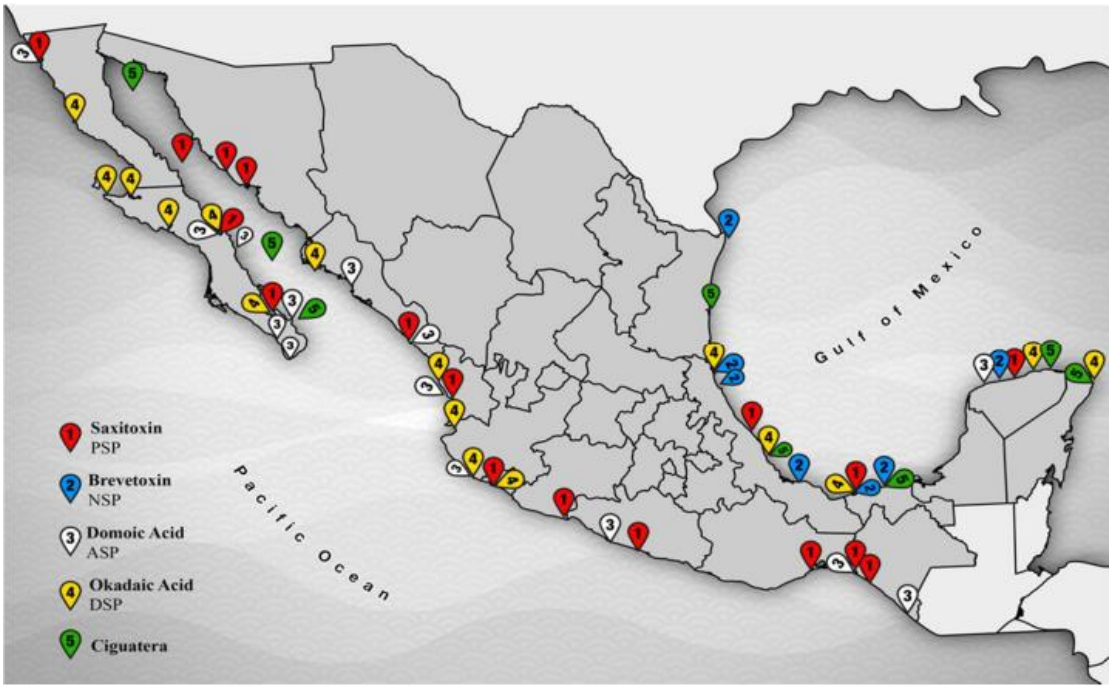


Figure 23. The location of phytoplankton which produces toxins in HABs, is reported on the Mexican Coast (Salcedo-Garduño et al., 2018).

Among the events on the Pacific coast were caused by *Pseudo nitzschia fraudulenta, P. pseudodelicatissima, P. pungens,* and other diatoms that affected the

coastal fauna of the Bay of La Paz stand out (Guluarte-Castro & Bañuelos, 2007; Salcedo-Garduño et al., 2018). The government authorities of Baja California Sur estimated that the fishing families of this bay were affected. As a result, the government distributed $70,000 in economic assistance and food pantries. The region was declared to be in a state of emergency. Federal agencies such as SEDESOL and SAGARPA, among others, provided the affected fishermen with additional resources through programs such as temporary employment (Salcedo-Garduño et al., 2018).

In Oaxaca, 14 HABs from species producing paralyzing toxins were identified between 1989 and 2014. In addition, 139 poisonings and 9 deaths were attributed to the consumption of saxitoxin-contaminated mollusks. These incidents caused more than $500,000 in damages and necessitated a 708 days' sanitary closure to protect public health (Alonso-Rodríguez et al., 2015; Salcedo-Garduño et al., 2018).

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In 2007, the proliferation of the dinoflagellate *Ceratium divaricatum* along the northeast coast of Ensenada, Baja, California, caused anoxic conditions that led to the death of lobsters, starfish, and crabs. Lobsters were the most affected, with dead biomass above 5 tons (Orellana-Cepeda, 2007; Salcedo-Garduño et al., 2018). In the same year, HABs caused by the dinoflagellate *Akashiwo sanguinea* were reported in the region from Punta Abreojos to La Bocana, BCS, killing approximately 100,000 locusts (*Panulirus interruptus*). In addition, 2.5 tons of abalone (*Haliotis spp*.), farmed oysters (*Crassostrea spp*.), as well as various species of snails and fish, perished (Gómez-Tagle, 2007; Gárate-Lizárraga et al., 2007; Gárate et al., 2007; Salcedo-Garduño et al., 2018). This event resulted in a loss of approximately $1,784,616 (Salcedo-Garduño et al., 2018).

5.4 Guatemala

The government could not point out the organism responsible for this incident on the Pacific coast in 1985. Nevertheless, one of the most noteworthy catastrophes was another one that happened in 1987, where 187 persons got poisoned, and 24 died because of the event. Despite human losses, the cause, *Pyrodinium bahamense var compressum*, was identified (Carrillo-Ovalle, 2009; Rosales-Loessener, 1989).

Since the organism was founded, the nation created the Comisión Nacional de Vigilancia de Marea Roja, made up of the Unidad Nacional de Pesca y Acuicultura (UNIPESCA), Instituto Nacional de Sismología, Vulcanología, Meteorología e Hidrología (INSIVUMEH), and the Ministerio de Agricultura Ganadería y Alimentación (MAGA) (Carrillo-Ovalle, 2009; Leiva, 2008).

Following 1989, 1990, 1995, and 2001, new events analogous to the earlier ones were presented to the same organism. Toxins were found in the water over time, reaching 27,000 cells per liter (cells/liter). A red water alert was issued for the entire nation because the tolerance level was around 3000 cells/lit (Fig. 24).

Figure 24. Quetzal port: Zone where the samples were collected.

As shown in the Table 6, the highest level of saxitoxin is found in Las Lisas, a beach in southwestern Guatemala.

Fecha	Procedencia	Molusco	Saxitoxina *UR/100g
22/8/2001	Champerico	Mejillón	280
22/8/2001	Champerico	Ostra	162
25/8/2001	Pto. San José	Ostra	291
25/8/2001	Las Lisas	Concha	254
25/8/2001	Las Lisas	Mejillón	806
25/8/2001	Las Lisas	Almeja	90
25/8/2001	Champerico	Ostra	144
28/8/2001	Las Lisas	Concha	247
28/8/2001	Las Lisas	Mejillón	629
29/8/2001	Tecojate	Almeja	300
29/8/2001	Tecojate	Concha	200
29/8/2001	Las Lisas	Mejillón	508
29/8/2001	Las Lisas	Almeja	ND
29/8/2001	Las Lisas	Concha	117
29/8/2001	Las Lisas	Concha	ND
29/8/2001	Tecojate	Mejillón	287
29/8/2001	Tecojate	Concha	425
30/8/2001	Tecojate	Concha	450
30/8/2001	Las Lisas	Concha	708
30/8/2001	Las Lisas	Mejillón	175
30/8/2001	Tecojate	Mejillón	147
3/9/2001		Mejillón	480
3/9/2001		Almeja	156
3/9/2001		*Crassostrea*	321
12/9/2001		Mejillón	160
2/9/2001		***Anadara***	92

Table 6. Levels of saxitoxin, 2001 (UNIPESCA, 2001 as cited Carrillo-Ovalle, 2009).

This becomes important for the Figure 25 as it shows how the toxins during 2005 gathered in the same region mentioned.

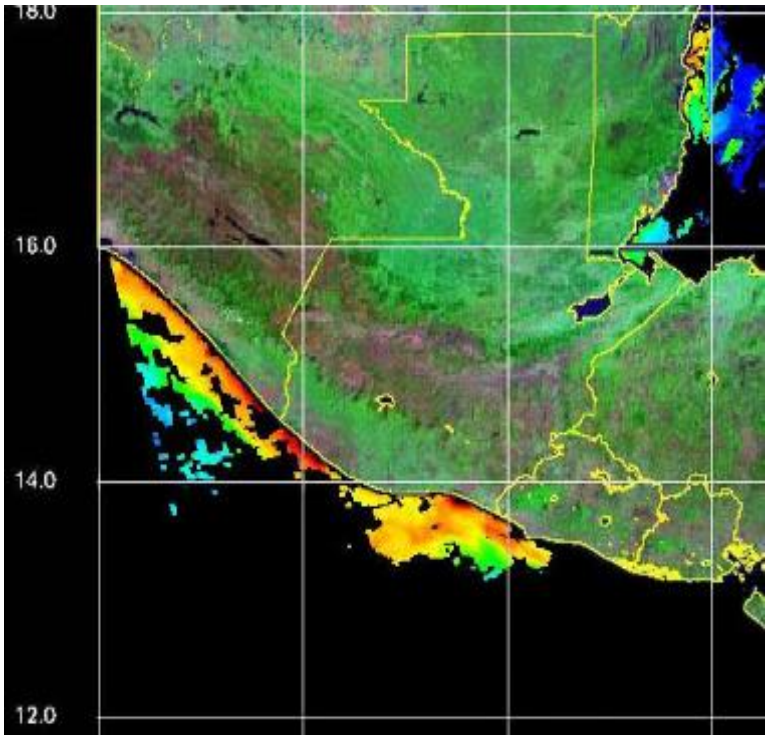

Figure 25. Chlorophyll concentrations during 2005, Pacific coast of Guatemala (Sistema Regional de Visualización y Monitoreo de Mesoamérica, n.d.).

Despite all the research that has been done, in May of 2022, this particular country claimed 4 lives, prompting the Guatemalan government to declare a temporary fishing restriction on the whole Pacific coast (Ministerio de Salud Publica y Asistencia social, 2022).

5.5 Ecuador

Oceanographic features have an impact on the occurrence of HABs. Two biogeographical areas can be identified on South America's Ecuadorian coast of the eastern tropical Pacific: The Gulf of Guayaquil, which the Humboldt Current influences, and the northern coast, which the warm Panama Current influences (Cucalon, 1989; Longhurst, 2006; Borbor-Cordova et al., 2019).

For years, "red tides" have occurred along Ecuador's coast (Borbor-Cordova et al., 2019). Since 1968, 132 incidents have been noted by Ecuadorian researchers, with reports of fish, young shrimp, and shrimp larvae mortality (Torres, 2000; Torres, 2011;

Torres, 2013; Torres, 2015; Torres, 2017; Torres & Tapia, 2002; Borbor-Cordova et al., 2019).

Sadly, at this moment, Ecuador does not have a formal HABs monitoring program to identify the specific phytoplankton species responsible for producing the toxins. However, there is a recent study that integrates the features of the equatorial ocean, synthesized from reports gathered during "red tide" incidents and biological data received from synoptic remote sensing to determine which oceanic circumstances cause the dispersion and occurrence of seasonal HAB in the ocean of the coast of Ecuador. The next figure shows the areas taken to carry out the study, defined by the two biogeographical areas mentioned above, and the provinces and coastal cities involved (Borbor-Cordova et al., 2019) (Fig. 26).

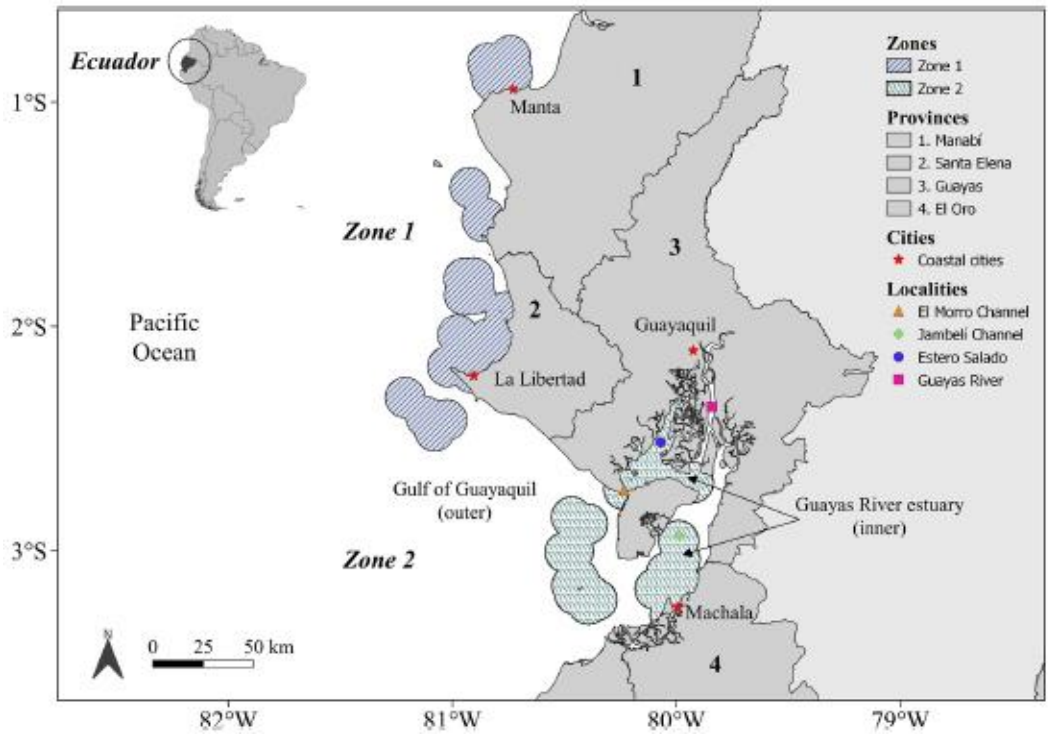

Figure 26. Location of events related to HABs on the Ecuadorian Coast (Borbor-Cordova et al., 2019).

According to the study by Borbor-Cordova et al. (2019), the data utilized for remote sensing observation were the monthly averages of five variables collected in Zones 1 and 2 along the coast of Ecuador from 1997 to 2017 while accounting for the seasons of rain and drought. The oceanographic variables were: Sea surface temperature (SST), Absorption due to phytoplankton at 443 nm (m^-1) (aph), Chlorophyll an

(mg/m^3) and photosynthetically active radiation (Einstein/m^2/day). At the same time, the atmospheric variable was Precipitation (mm). The process needs mean and standard deviation for each variable to normalize it. Several satellites and sensors were employed throughout the investigation to assess and contrast the variables with HABs incidents.

Phytoplankton statistics, on the other hand, were derived from 67 algal bloom occurrences documented from 1997 to 2017 in the literature produced by reports from shrimp hatchery producers, the National Institute of Fisheries, and the Ecuadorian Navy Army Oceanographic Institute (INOCAR). Table 7, 8 and 9 lists the potentially hazardous algal blooms (HABs) seen in Zones 1 and 2 on the coast of Ecuador. The dinoflagellates (DINO), centric diatoms (CDIAT), pennate diatoms (PDIAT), and ciliates were the phytoplankton taxonomic groups (PTG) of the events (CILI). The species were divided into toxic (TOX) and non-toxic (NOC) (Borbor-Cordova et al., 2019).

Species	PTG	Type	ZONE 1		ZONE 2				References for toxicity	References for HABs recurrences in Ecuador
			Wet season		Dry season		Wet season			
			N	Max log cells L^{-1}	*N*	Max log cells L^{-1}	*N*	Max log cells L^{-1}		
Diarrhetic Shellfish Poisoning (DSP)										
Dinophysis caudata (Saville-Kent, 1881)	DINO	TOX	1		1				Ignatiades and Gotsis-Skretas, 2010; Nagai et al., 2011; Anderson et al., 2017	Torres, 2000, 2006, 2011
Dinophysis spp. (Ehrenberg, 1839)	DINO	TOX	1						Yasumoto et al., 1985; Hallegraeff, 1993; Lloyd et al., 2013; Reguera el al., 2014	Torres, 2011
Gonyaulax spp. (Diesing, 1866)	DINO	TOX			1				Wang, 2008	Torres and Palacios, 2007a
Prorocentrum mexicanum (Osoio-Tafall, 1942)	DINO	TOX	1	3.37			1		Ignatiades and Gotsis-Skretas, 2010; Muciño-Marquez et al., 2015	Coello,2010; Torres, unpublished data (u.d)
Prorocentrum micans (Ehrenberg, 1834)	DINO	TOX	1	3.38	2		1		Razali et al., 2015; Lee et al., 2016	Torres, 2000, u.d; Torres anda Tapia, 2002; Torres and Palacios, 2007b
Diarrhetic Shellfish Poisoning (DSP) and Venerupin Shellfish Poisoning										
Prorocentrum cordatum (Ostenfeld) (Dodge, 1975)	DINO	TOX			1		3		Heil et al., 2005; Lundholm, 2011	Torres et al., 2004; Torres and Palacios, 2007b; Torres, 2011
Paralytic Shellfish Poisoning (PSP)										
Alexandrium spp. (Halim, 1960)	DINO	TOX			1				Wang, 2008; Anderson et al., 1990, 2017; Food and Agriculture Organization [FAO], 2004; Ignatiades and Gotsis-Skeretas, 2010	Torres and Palacios, 2007a
Gymnodinium catenatum (Graham, 1943)	DINO	TOX	1		3	6.05	1		Ignatiades and Gotsis-Skretas, 2010	Jiménez and Intriago, 2001; Torres and Palacios, 2007a; Torres, u.d.
Gymnodinium spp. (Stein, 1878)				5.22	6	7.05	6	7.45	Wang, 2008	Torres, 2000, 2006, 2011, u.d; Torres and Palacios, 2007a
Amnesic Shellfish Poisoning (ASP)										
Pseudo-nitzschia spp. (Peragallo and Peragallo, 1900)	DINO	TOX			1	6.68			Rhodes et al., 1998; Trainer et al., 2012; Anderson et al., 2017	Torres, 2006,2011

Table 7. List of potentially harmful algal blooms (HABs) recorded in events along the coast of Ecuador (1997–2017) (Borbor-Cordova et al., 2019).

Species	PTG	Type	ZONE 1		ZONE 2				References for toxicity	References for HABs recurrences in Ecuador
			Wet season		Dry season		Wet season			
			N	Max log cells L^{-1}	*N*	Max log cells L^{-1}	*N*	Max log cells L^{-1}		
Potentially Ichthyotoxic Species										
Gyrodinium spp. (Kofoid and Swezy, 1921)	DINO	NOC	2	4.45	3		2	7.75	Alonso-Rodiguez and Páez-Osuna, 2003; Ignatiades and Gotsis-Skretas, 2010	Torres, 2000, 2011, u.d; Torres et al., 2004; Torres and Palacios, 2007b
Margalefidinium catenatum (Okamaru, 1916; Gómez et al., 2017)	DINO	NOC	2	6.94	1		1		Cortes et al., 2004; Matasuoka et al., 2008; Kudela and Gobler, 2012; Gómez et al., 2017	Torres et al., 2004; Torres, 2011, 2016, u.d.
Potentially Fish-Killing and Bloom-forming Species										
Mesodinium rubrum (Lohmann,1908)	CILI	NOC	9	6.69	4	7.06			Jiménez and Intriago, 2001; Cortés-Lara, 2002; Gárate-Lizárraga et al., 2002; Wang et al., 2008; Torres, 2011	Torres, 2000, 2006, 2011; Jiménez and Intriago, 2001; Coello, 2003; Jiménez and Gualancañay, 2006; Coello and Cajas, 2007
Coscinodiscus spp. (Ehrenberg, 1839)	CDIAT	NOC			1	5.20	1	4.30	Mathew et al., 1988; Torres, 2011	Torres and Palacios, 2007b; Coello et al., 2010
Skeletonema costatum (Greville)(Cleve, 1873)	CDIAT	NOC	1	6.10	1	5.88	4	6.14	Hallegraeff, 1993; Suaréz and Guzmán, 1994; Chen et al., 2007; Anderson et al., 2017	Torres et al., 2004; Torres and Palacios, 2007b; Coello et al., 2010; Torres, 2016
Akashiwo sanguinea (K. Hirasaka)(Daugbjerg et al., 2000)	DINO	NOC	1	7.15					Hallegraeff, 2004; White et al., 2014; Anderson et al., 2017	Coello, 2003; Torres et al., 2004; Torres, 2011
Ceratium tripos (O.F. Muller)(Nitzsch, 1817)	DINO	NOC	1						Suaréz and Guzmán, 1994; Hallegraeff, 2004; Torres at al., 2004; Torres, 2011	Torres, 2000
Cochlodinium spp. (Schutt, 1896)	DINO	NOC			2	6.72	1	6.64	Hallegraeff, 2004; Kudela and Gobler, 2012; Razali et al., 2015	Torres, 2000, 2011; Torres at al., 2004;Torres, 2011
Kryptoperidinium foliaceum (F. Stein) (Lindemann, 1924)	DINO	NOC					1	4.00	Kempton et al., 2002; Saburova et al., 2012; Merino-Virgilio et al., 2014	Jiménez and Gualancañay, 2006; Torres, 2011
Levanderina fissa (Levander) (Moestrup et al., 2014)	DINO	NOC	6	7.00					Alonso-Rodriguez and Paez-Osuna, 2003; Gárate-Lizárraga, 2014	Jiménez and Intriago, 2001; Jiménez and Gualancañay, 2006; Torres, 2011
Noctiluca scintillans (Macartney) (Kofoid and Swezy, 1921)	DINO	NOC	2	3.97	2	6.06	4	641	Thangaraja et al., 2007; Wang et al., 2008; Ibrahem and Al-Shawi, 2015; Razali et al., 2015	Torres, 2000, 2011, 2016, u.d.; Coello, 2003; Torres at al., 2004
Prorocentrum gracile (Schutt, 1895)	DINO	NOC	2						Gul and Saifullah, 2011; Muciño-Márquez et al., 2015; Anderson et al., 2017	Jiménez and Intriago, 2001; Torres at al., 2004
Tripos furca (Ehrenberg) (Gómez, 2013)	DINO	NOC	2		3				Edwards et al., 2006; Ibrahem and Al-Shawi, 2015; Razali et al., 2015; Yurimoto et al., 2015	Torres, 2000, 2006, 2011; Torres and Palacios, 2007a

Table 8. List of potentially harmful algal blooms (HABs) recorded in events along the coast of Ecuador (1997–2017) (Borbor-Cordova et al., 2019).

Species	PTG	Type	ZONE 1		ZONE 2				References for toxicity	References for HABs recurrences in Ecuador
			Wet season		Dry season		Wet season			
			N	Max log cells L^{-1}	*N*	Max log cells L^{-1}	*N*	Max log cells L^{-1}		
Chaetoceros levis (Schutt, 1896)	CDIAT	NOC	1						Razali et al., 2015	Torres, 2000
Thalassiosira spp. (Cleve, 1873; Hasle, 1973)	CDIAT	NOC	1	4.37	2	6.70	1	5.48	Hallegraeff, 2004; Torres, 2011; Anderson et al., 2017	Torres and Palacios, 2007a; Coello et al., 2010; Torres, 2011, u.d.
Navicula spp. (Blainville, 1825)	PDIAT	NOC					1	4.48	Alonso-Rodriguez and Paez-Osuna, 2003; Razali et al., 2015	Coello et al., 2010
Asterionella frauenfeldii (Grunow, 1863)	PDIAT	NOC			1		1		Perumal et al., 1999; Jugnu and Kripa, 2008; Naha et al., 2014	Torres, 2000; Torres at al., 2004
Nitzschia longissima (Brébisson) (Pritchard, 1861)	PDIAT	NOC					1		Merino-Virgilio et al., 2014; Razali et al., 2015; Anderson et al., 2017	Torres at al., 2004
Thalassionema nitzschioides (Grunow) (Mereschkowsky, 1902)	PDIAT	NOC					2	4.78	Perumal et al., 1999; Merino-Virgilio et al., 2014; Begum et al., 2015; Padmakumar et al., 2018	Torres at al., 2004; Coello et al., 2010
Ceratium spp. (Schrank, 1793)	DINO	NOC	1						Thangaraja et al., 2007; Gómez et al., 2008; Padmakumar et al., 2018	Jiménez and Gualancañay, 2006; Torres, 2011
Ceratium trichoceros (Ehrenberg) (Saville-Kent, 1881 ; Kofoid and Swezy, 1921)	DINO	NOC	1			1			Jiménez and Intriago, 2001; Thangaraja et al., 2007; Torres, 2011; Ibrahem and Al-Shawi, 2015	Jiménez and Intriago, 2001
Protoperidinium quarnerense (B. Schröder) (Balech, 1974)	DINO	NOC		1					Torres at al., 2004; Gómez et al., 2008	Torres, 2000
Pyrophacus steinii	DINO	NOC	2	1					Razali et al., 2015; Wall and Cicily, 2015	Torres, 2000, 2011, u.d
Triadinium polyedricum (Schiller) (Wall and Dale, 1971)	DINO	NOC		1					Shin et al., 2016	Torres and Palacios, 2007a
Tripos macroceros (Ehrenberg) (Gómez, 2013)	DINO	NOC	2			1			Thangaraja et al., 2007; Torres, 2011; Ibrahem and Al-Shawi, 2015	Torres, 2000
Tripos dens (Ostenfeld and Johannes Schmidt) (Gómez, 2013)	DINO	NOC	1	3	6.54	1			Homer et al., 1997; Pitcher and Calder, 2000; Alonso-Rodriguez and Páez-Osuna, 2003; Torres, 2011	Torres and Palacios, 2007a; Torres, 2011

The table includes potentially toxic (TOX) and non-toxic (NOC) species. The toxic species or humans are represented through syndromes. The non-toxic species are: (I) Potentially ichthyotoxic species-this group causes physical damage or irritation of the gills, are toxigenic, and cause hypoxia from oxygen depletion. It is also capable of causing the massive death of fish. The causal mechanisms are uncertain and the studies carried out are inconclusive (Andersen, 2012); (ii) Potentially fish-killing species-this group differs from the previous one because the death of the fish is caused by the decrease of oxygen as a consequence of the bloom's great biomass; (iii) Potentially bloom-forming species. The species are classified in four phytoplankton taxonomic groups (PTG): dinoflagellates (DINO), centric diatoms (CDIAT), pennate diatoms, and ciliates (CILI). N, number of occurrences. Log C, maximum abundances in log 10 (cells/L). The occurrences and concentrations are shown by dry and wet seasonality in the study areas (Zone 1 and Zone 2).

Table 9. List of potentially harmful algal blooms (HABs) recorded in events along the coast of Ecuador (1997–2017) (Borbor-Cordova et al., 2019).

The study finds that the presence of hazardous HABs in Zone 1 correlates with low levels of DINO and CDIAT. These biological features appear to be associated with high SST, PRE, and PAR values and low chl-a and aph values (443 nm). In contrast, non-toxic HABs correspond to an abundance of DINO and CILI, low SST and PRE, and a high pH. All of these environmental features define the wet season. During the wet season in Zone 2, TOX events corresponded to high levels of DINO biomass and elevated SST, PAR, and PRE. During the dry season, however, low PRE and PAR and cooler waters were associated with an excess of CDIAT. These characteristics are related to NOC species (Borbor-Cordova et al., 2019).

The results of this study show that larger biomass of HABs was observed in Zone 2 than in Zone 1 when eutrophic and oligotrophic systems were considered, with Dinoflagellates being the taxonomic group most closely related to HABs all along the Ecuadorian coast. Dinoflagellates adapt by colonizing, attaining high biomass levels, and becoming potentially dangerous species in nutrient-rich, high-irradiation estuarine settings like Zone 2. While in Zone 1 oligotrophic, with its stratified and high irradiation conditions, a nutrient stress tolerant assemblage of dinoflagellate species was produced at very low levels of biomass (Smayda & Reynolds, 2003; Suparna, 2005; Smayda & Trainer, 2010; Corcoran et al., 2014; Borbor-Cordova et al., 2019).

The study's final finding is the danger of HABs in the Estero Salado in the wet season, at the entrance of the Guayas Estuary, Manta and La Libertad in the dry season, Gulf of Guayaquil, Jambeli Channel. For instance, Zone 2 had significant concentrations of two species that are thought to be hazardous, *Gymnodinium catenatum* and *Karenia brevis*, particularly during the rainy season. However, during the rainy season, low amounts of *Prorocentrum micans* and *Prorocentrum mexicanum* were found in the oligotrophic Zones 1 and 2, respectively (Borbor-Cordova et al., 2019).

5.5.1 Effects of red tide on human health

The most common health problems associated with red tide are gastrointestinal, respiratory and neurological illnesses. Generally, individuals who become ill from exposure to harmful algal toxins do so by ingesting contaminated

shellfish. However, some harmful algal toxins also end up in the air and infect humans. People get sick from eating clams collected during red tides and, to a lesser extent, from windblown toxins in the form of aerosol. In this particular case of Ciguatoxin poisoning, the illness known as ciguatera is caused by eating contaminated fish. Red tide toxins are generally heat stable, so no matter how well seafood is cooked, people become intoxicated once they ingest it. In addition, they can cause irritation to the eyes and mucous membranes of bathers, which is known as the aerosol effect (Mendoza Avilés, 2016).

5.5.2 Effects of red tide on animal populations

The majority of shellfish filter seawater to gather food. When they eat, they ingest toxic phytoplankton and accumulate toxins in their flesh, which become dangerous, even fatal to fish. Figure 27 shows the monthly record of red tide and fish mortality (1968-2009) in Ecuador.

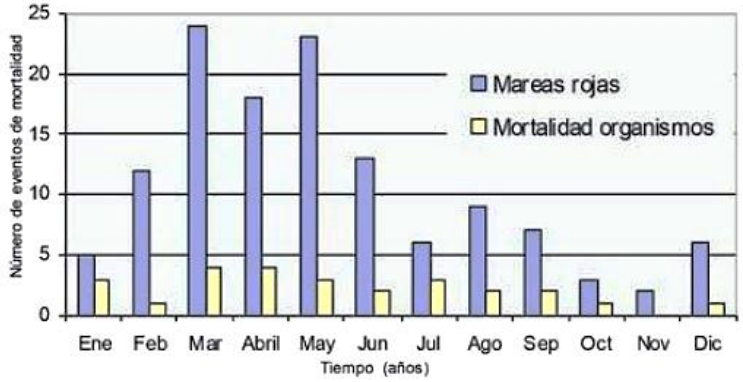

Figure 27. Monthly record of red tide and organism mortality (1968-2009) (Torres, 2013).

Neurotoxins from harmful algal blooms can kill large numbers of fish, washing thousands ashore. Dead fish remain a health risk because of the danger of being eaten by birds or marine mammals that become infected by eating them. Thanks to cytochromes, the massive proliferation of microalgae reaches a concentration of millions of algae cells per liter of seawater, so much so that they can turn seawater from red to yellow. Excessive growth of algae populations is accompanied by the production of toxins, which can lead to the destruction of marine fauna. The deaths of various marine species, such as whales and turtles, have been linked to red tides. The toxins even create foam that makes the feathers of seabirds no longer waterproof, which causes the birds to die.

5.5.3 Immunological Test

5.5.3.1 Methodology

The study area where the bivalve samples were taken was on the Pacific coasts of Machala, Posorja and Santa Elena (Fig. 28).

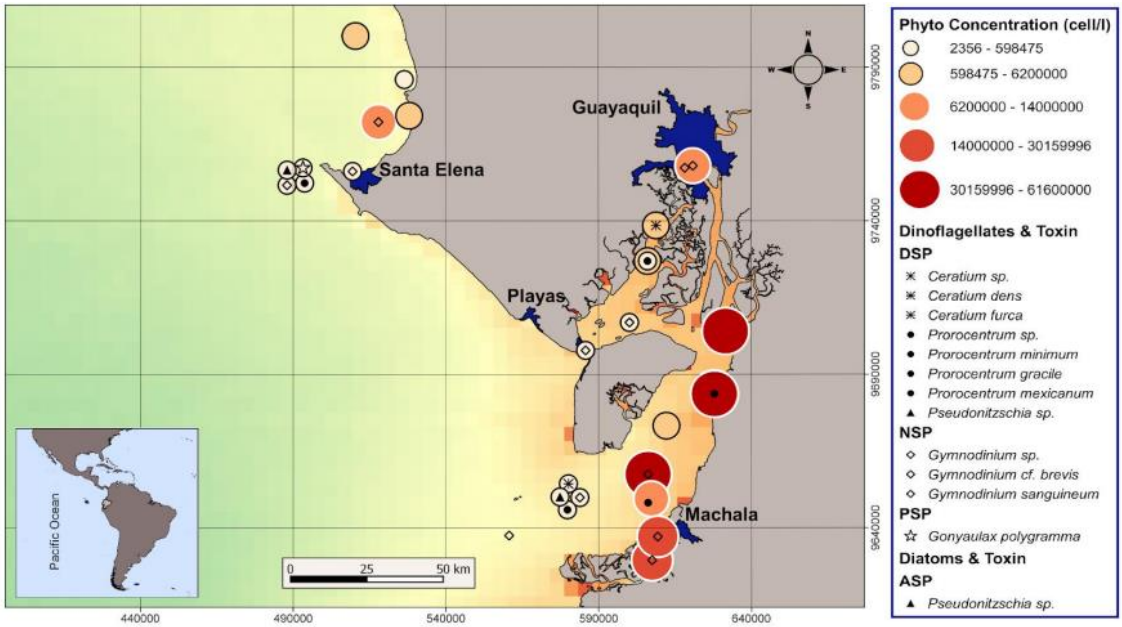

Figure 28. Sampling location in Ecuador (Borbor-Córdova et al., 2018).

5.5.3.1.a Interpretation Test – DSP, PSP and ASP

In order to perform the detection of DSP, PSP and ASP the Interpretation Test was used, which can yield three results, such as (Lira, 2019):

1) If the T-line is darker than the Positive result below, the test is Negative (Fig. 29A).
2) If the T-line is equal to or fainter than the Positive result below, the test is Positive. When in doubt, consider the test Positive. Positive results should be confirmed with analytical testing (Fig. 29B).
3) In the C line is equal to or fainter than the invalid test below, the test is invalid. Repeat the assay with a new test. If the same result occurs, the sample contains an unknown agent that inhibits the test reaction; an alternative testing method should be used (Fig. 29C).

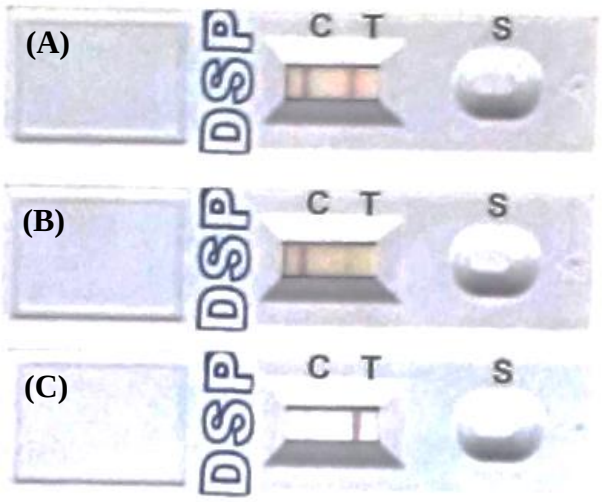

Figure 29. (A) Negative result; (B) Positive result, (C) Invalid result of Interpretation Test (Interpretation Test).

5.5.3.2 Results

According to the results obtained in the interpretation test, it came out positive for Okadaic acid since a result similar to that shown in the interpretation guide is displayed (Lira, 2019) (Fig. 30).

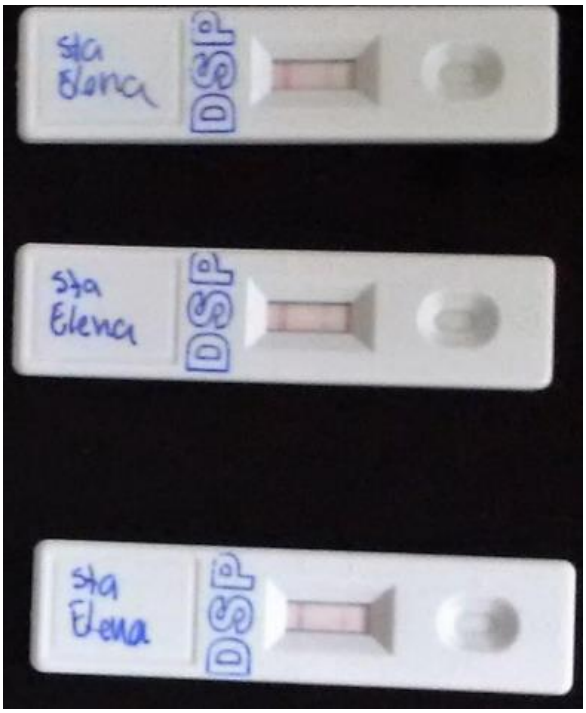

Figure 30. Positive result of Interpretation Test.

According to the results obtained in the interpretation test, it came out negative for Saxitoxin since a result similar to that shown in the interpretation guide is displayed (Lira, 2019) (Fig. 31).

Figure 31. Negative result of Interpretation Test.

According to the results obtained in the interpretation test, it came out negative for Domoic acid since a result similar to that shown in the interpretation guide is displayed (Lira, 2019) (Fig. 32).

Figure 32. Negative result of Interpretation Test.

Chapter 6: Conclusions

Harmful algal bloom, also known as red tide, is a spread of aquatic microalgae, visible to the naked eye because the ocean turns a different color than usual. The expression red tide is usually used to refer to large concentrations of phytoplankton in certain coastal areas of the ocean that become blotchy. This are mainly caused by the rapid growth of dinoflagellates, a type of phytoplankton. However, seawater must contain abundant food to promote the explosive growth of dinoflagellates otherwise they will never grow. The main problem from red tide is the generation of toxins which are harmful for human and animals as well.

In Canada there are several studies are evaluating the last three decades of harmful algal events. Also here there are presence of phycotoxins events associated with amnesic shellfish toxin (AST), diarrhetic shellfish toxin (DST), and paralytic shellfish toxin (PST).

In United States of America marine birds and mammals are susceptible to a neurotoxic produced by some HABS and the main poisoning that are affecting the population are Paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP), amnesic shellfish poisoning (ASP), and azaspiracid shellfish poisoning (AZP) are all names given to human poisoning syndromes caused by eating shellfish. However, there are some laboratories that are studying and monitoring the effects of the mentioned toxins.

In Mexico, there are some diseases documented from consumption of molluscs. The toxins here are Saxitoxin, Brevetoxin, Domoic Acid, Okadaic Acid, Ciguatoxin.

In Guatemala some research also establish toxins contend found in the water over time. And, there are Institutes working such as Comisión Nacional de Vigilancia de Marea Roja. Moreover, related with marine species the highest level of saxitoxin is found in Las Lisas.

Since other country's use the data and reports of some Institutions and commissions Ecuador does not have a formal monitoring program to identify the specific phytoplankton species responsible for producing the toxins. However, there is a potentially harmful algal blooms (HABs) recorded in events along the coast of Ecuador

1997–2017 that show content of dinoflagellates (DINO), centric diatoms (CDIAT), pennate diatoms (PDIAT), and ciliates were the phytoplankton taxonomic groups (PTG) of the events (CILI), there is and advice to stablish a technique to understand the incidence of these species. It is also important to note that the samples taken from bivalves in Posorja, Machala and Santa Elena showed positive results for Okadaic acid and negative results for Domoic acid and Saxitoxin. For future research work it is necessary to consider those samples that showed positive results and develop HPLC and Mass Spectroscopy for a better identification of the toxin.

Finally, after this research, it is possible to see how red tides have not only harm humans by eating contaminated food. They also affect all sea life as well as the sea water making it dangerous for humans to swim or even collecting shellfish especially since symptoms can appear an hour later since contact.

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