Journal of Food Chemistry & Nanotechnology

https://doi.org/10.17756/jfcn.2020-095

Research Article

Open Access

Dinoflagellates Important Marine Producers of Natural Bio-Compounds with High Biotechnological and Pharmacological Potential

Lydia Ferrara

Università degli Studi di Napoli Federico II Napoli, NA, Italy

*Correspondence to:
Lydia Ferrara
Università degli Studi di Napoli Federico II
Napoli, NA, Italy
E-mail: lyferrar@unina.it

Received: May 11, 2020
Accepted: July 13, 2020
Published: July 15, 2020

Citation: Ferrara L. 2020. *Dinoflagellates* Important Marine Producers of Natural Bio-Compounds with High Biotechnological and Pharmacological Potential. *J Food Chem Nanotechnol* 6(3): 138-149.

Published by United Scientific Group

Abstract

Microalgae are unicellular photosynthetic organisms that can produce organic carbon from CO_2 using sunlight energy and for their ability to fix atmospheric carbon in organic compounds are considered primary producers in the marine food chain, plankton. The various aquatic species that feed on plankton can represent, due to the incidence of numerous factors, the basis for the proliferation of pathogenic microorganisms and the production of biotoxins, known as phycotoxins, which have attracted the attention of researchers not only for their complex chemical structure and for various pharmacological activities (cytotoxic, anti cancer, antifungicide, antibiotic, etc...), but also for their ability to modify and activate important metabolic pathways. These biomolecules, thanks to the remarkable reproductive speed, formed the substrate to study and understand complex cellular functions, with important effects on human health. *Dinoflagellates* are microscopic, unicellular, flagellated algae, which represent one of the most important groups of marine phytoplankton and freshwater and in addition to the Red Tide phenomenon are responsible for the production of highly toxic biotoxins. Important human poisonings occur as a result of the ingestion of bivalve shellfish due to their ability to filter water and, consequently, accumulate pollutants in their body. Some microalgae are able to produce ichthyotoxins that, by acting on gills, can cause prolonged death of fish, and which, if consumed by humans, can lead to serious health risks. *Dinoflagellates* have attracted the attention of researchers because of the possibility of beneficial use of their metabolites: glycolipids containing polyunsaturated fatty acids are considered molecules responsible for allelopathic effects and therefore these algae are exploited by agronomists in the cultivation of terrestrial plants. *Tetradotoxin,* although a very toxic molecule, has proven to be important for knowledge of nerve transmission; exerts anesthetic action to block sodium channels; relieves cranial symptoms in cases of heroin withdrawal. *Goniautotoxins* have been shown to be a safe and effective therapeutic tool as a painkiller as they can be taken for long periods without showing unwanted side effects. *Amphidinolids* show in vitro a strong cytotoxicity to L1210 murine lymphoma and human epidermal carcinoma cells. The components of these algae also have antibacterial antimicrobial, antioxidant, protect against UV radiation, and for the richness of functional foods, including docosahexaenoic acid can also be used for children's products.

Keywords

Dinoflagellates, Marine toxins, Diarroic shellfish poisoning, Pharmacological activity

Introduction

Fish products are a food of considerable nutritional power, but they can

also be particularly harmful to health, to the presence of pathogenic microorganisms as a result of pollution of the aquatic ecosystem. In fact, they are able to metabolize and accumulate in the tissues various molecules equipped with toxicity for both aquatic fauna and for humans, known as "marine biotoxins", to which they follow particular disorders that are sometimes even lethal [1].

The substrate for biotoxins synthesis is plankton and the "phycotoxins", i.e. algal biotoxins, are the most important and have attracted the attention of researchers both for the presence of molecules with new chemical structures, and because they have highlighted numerous pharmacological activities [2]. The consumption of bivalve molluscs is considered one of the main culprit of transmission to humans not only of bacterial and viral diseases, but above all of intoxications from algal biotoxins, for the widespread habit of consuming them under-cooked or even raw [3, 4].

Traditional cooking methods (by boiling, steaming, or baking) have little impact on paralyzing toxins because they pass from the tissue in which they are contained to the coking juices. Industrial processes, if there are novery high amounts of toxins, are more effective: both cooking in alkaline pH solution preceded by a detoxification process and thermal treatment followed by canning reduce the presence of most toxins below the safety limits. The boxing process is the most suitable, with no alteration of the sensory characteristics of shellfish.

The problem of the presence of algal toxins in molluscs is becoming very important from a sanitation point of view, as a result of both the increased development of marine algae species recognized as harmful, and for algal flowering phenomena, presumably linked to the eutrophization of coastal areas. Recent researche has revealed a relationship between the presence of pollutants in the water, in particular nitrogen and phosphorus, and the concentration of toxic substances in algae: at low concentrations of these pollutants algal metabolism is aimed at the production of primary components such as proteins and polysaccharides, while a high degree of pollution corresponds to an increase in toxins. The phenomenon of algal blooms has been known since ancient times and is manifested many areas of the world, linked to the very high proliferation of algal cells, with a change in the colour of the water from red to yellow to brown, depending on the species, as a result of an increase in temperature, brightness and pollution [5, 6].

Algal biotoxins

Biotoxins are substances produced by a living organism capable of inducing, even at low concentrations, harmful biological manifestations on certain living species. Toxic substances are to be considered secondary metabolic products typical of the different species and, according to some authors, they would be produced as a mechanism of defense against any predators as well as being involved in the phenomenon of prolonged algal bloom of the species. The active ingredients have generally been identified based on the macroscopic symptoms of poisoning they provide to humans, or are listed together with the name of the toxin or the species that produces them.

The algae responsible for the intoxication phenomena are red microalgae, the *Dinoflagellates* (Figure 1), belonging to the group of the *Dinoficeae*. Often they are phosphorescent algae, provided with a skeleton or shell of a substance similar to cellulose, elegantly sculpted, which extends into long extensions, the flagella, generally two, located in furrows and directed to each other. Their chemical composition is characterized by substances of different nature: amino acids, purinic derivatives, polyethers, polyhydroxylated compounds that have both hydrophilic and lipophilic characteristics [7]. The difference in solubility corresponds to a different expression of toxicity; in fact, water-soluble toxins are responsible for respiratory paralysis (Paralytic Shellfish Poisoning) (PSP) and forms of temporary amnesia (Amnesic Shellfish Poisoning) (ASP), while fat-soluble toxins are primarily responsible for severe and prolonged gastrointestinal disorders (Diarrhetic Shellfish Poisoning) (DSP).

DSP is the most common intoxication, symptoms occur after about thirty minutes after ingestion of contaminated shellfish and the action is commensurate with the amount of mollusk ingested. The syndrome is mainly diarrhetic in nature, caused by polyetheric toxins containing *Okadaic acid*, ($C_{44}H_{68}O_{13}$) (Figure 1) produced by *Dinoflagellates* belonging to the *Dinoficeae* group, genus *Dinophysis* and *Prorocentrum*, which are normally present in the seas around the world [8, 9].

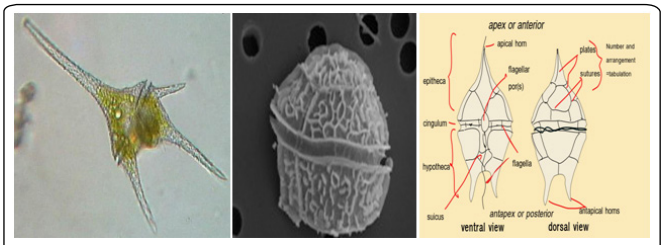

Figure 1: *Dinoflagellates* photosynthetic protist and fossil *Dinoflagellate* anatomy.

With regards the mechanism of action of *Okadaic acid*, it has been shown that it causes long- lasting contractions in the vascular smooth musculature; this happens without the intervention of neurotransmitters, in fact adrenaline and acetylcholine do not affect the action of that acid. *Okadaic acid* is held responsible for promoting the tumor on rat skin following local applications and its presence in drinking water induces the formation of tumours in the stomach of rats. Accurate pharmacologica studies have proposed an action mechanism for *Okadaic acid* and its derivatives, based on inactivation of the function of the tumor suppressor gene function, through hyperphosphorylation, especially at the level of serine and threonine residues, due to inhibition of certain phosphatases. It is likely to result in a build-up of phosphorylated proteins, which are then involved in the promotion of the tumour [10, 11].

DSP toxins are stable to heat, although prolonged boiling can decrease the concentration of *Okadaic acid* within shellfish. Most of the DSP syndromes have occurred in Japan where shellfish cultivation is widespread and along the eastern and western coasts of North America linked to the "Red Tides" produced by *Dinoflagellates*.

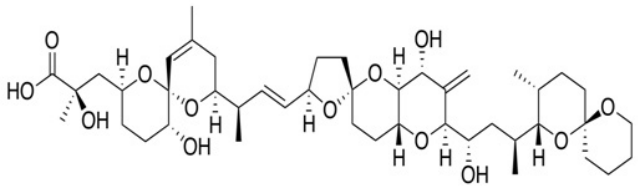

Figure 2: Okadaic Acid: a polyketide, polyether derivative of a C_{38} fatty acid.

New poisoning syndromes resulting from *Dinoflagellate* toxins have recently been characterized due to the presence in addition to the *Okadaic acid* of other substances, *Azaspiracid* ($C_{47}H_{71}NO_{12}$), *Yessotoxin* ($C_{55}H_{82}O_{21}S_2$) and *Palitoxin* ($C_{129}H_{223}N_3O_{54}$) (Figure 3-5), compounds that have different toxicological effects and mechanisms of action. Such toxins can be functionally classified as neurotoxins and hepatotoxins, based on their clinical symptoms. Their neurotoxicity is mediated by different and highly specific interactions with the ion channels involved in neurotransmission.

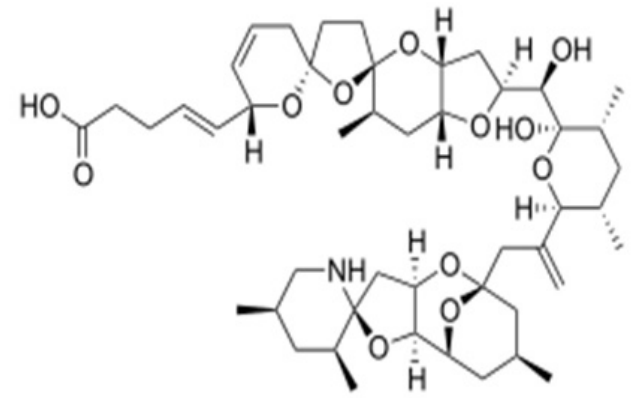

Figure 3: Azaspiracid: a polycyclic ether marine algal toxins produced by *Azadinium spinosum*.

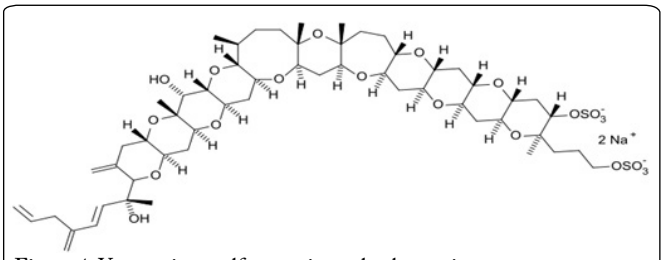

Figure 4: Yessotoxin: a solfate marine polyether toxin.

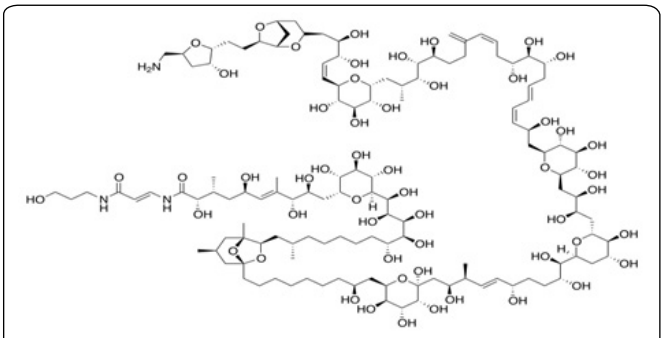

Figure 5: Palitoxin: a polyhydroxylated and unsaturated compound by *Ostreopsis* dinoflagellates.

In fact, in addition to *Okadaic acid*, responsible for DSP syndrome is *Prorocentrum minimum* (Figure 6) an algae that contains *Venerupin,* a substance of which the structure is not yet known, but harmful for the liver, which has caused shellfish poisoning resulting in gastrointestinal diseases in the humans. This species, in addition to being responsible for many deaths among humans, is responsible for the remarkable killings of shellfish in Japan, in the Gulf of Mexico, in Florida [12-14]. This algae, in addition to promoting very large red tides due to its high productivity, is also very resistant to changes in temperature and salinity. It has recently been found in the Mediterranean Sea and in particular along the coasts of Adriatic Sea.

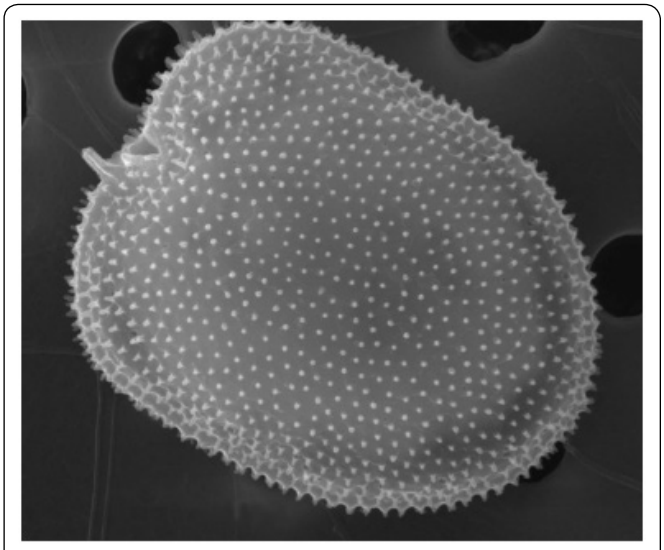

Figure 6: *Prorocentrum minimum*.

Venerupin is a molecule that exhibits hepatotoxic activity and is responsible for VSP (*Venerupin* Shellfish Poisoning) syndrome. Disorders of this toxin are both gastrointestinal and neurological, but not paralyzing, caused by the ingestion of oysters and clams. Poisoning is characterized by a long incubation 24-48 hours followed by a sudden onset of symptoms: nausea, vomiting, diarrhea, headache, loss of appetite and agitation. In severe cases, liver dysfunction, delirium, liver coma, death can occur [15, 16].

Azaspiracid poisoning (AZA), a group of marine algal toxins first reported by the Netherlands, was caused by the presence of *Dinoflagellate Azadinium spinosum*, which can accumulate in crustaceans and thus cause disease in humans. *Azaspiracid* is a polyether poliotoxin that inhibits ion channels of potassium with tension. Shellfish contaminated with AZA as a result of ingestion can cause severe acute symptoms including nausea, vomiting, diarrhea and stomach cramps [17].

AZAs can contaminate various organisms including: scallops oyster mussels, clams, sponges and crabs and through these vector organisms, enter the human food chain, thus posing a potential risk to public health. Oysters are currently the only ones able to accumulate toxin at levels comparable to mussels, the species that accumulates more and the only one that has so far generated intoxication. Toxins can remain

in shellfish for more than eight months because, although hepatopancreas is the first site where they accumulate, they migrate to other tissues in the body where detoxification occurs more slowly [18].

The structure of AZA was first uneaked in 1998 by Satake's group and collaborators, following their isolation from Irish mussels (*Mytilus edulis*). The structure included: a cyclic aminic group (aza), a three-ring spiranic group (spiro) and a terminal carboxylic residue (acid), hence the name "azaspiracid" [19, 20].

Studies of acute oral toxicity were performed using partially purified toxin extracts administered in mice through gastric probe. Mice treated with a dose six times higher than the lethal dose showed no symptoms of poisoning or lethality within 24 hours of treatment, but autopsies of mice sacrificed after 4 hours after treatment showed changes at the gastrointestinal level, with fluid buildup in the ileo and microvilli necrosis: this clinical picture is similar to chronic inflammatory bowel disease, like Crohn's disease. It was noted that the toxin was absorbed dose-dependent and the highest concentrations were detected after 24 hours in the kidneys, spleen and lungs, followed by those in the liver and heart. After one week AZA levels had dropped significantly in all organs except the kidneys [21].

Several studies have shown the action of AZAs also on voltage-dependent ion channels: they in fact alter the flow of intracellular calcium [22-25]; protonic homeostasis [26], causing cell membrane hyperpolymerization [27].

The study of toxins produced by *Dinoflagellates* has shown that the most representative class of substances is polyethers. *Protoceratium reticulatum* and *Lingulodinium polyedrum* (Figure 7 and 8) are two species of *Dinoflagellates* responsible for the production of *Yessotoxins*, that are substances that show toxicity to both the heart and the liver and pancreas, digestive organs.

Brevitoxin B, the first toxic compound of which structure was defined exactly by crystallographic analysis, has been

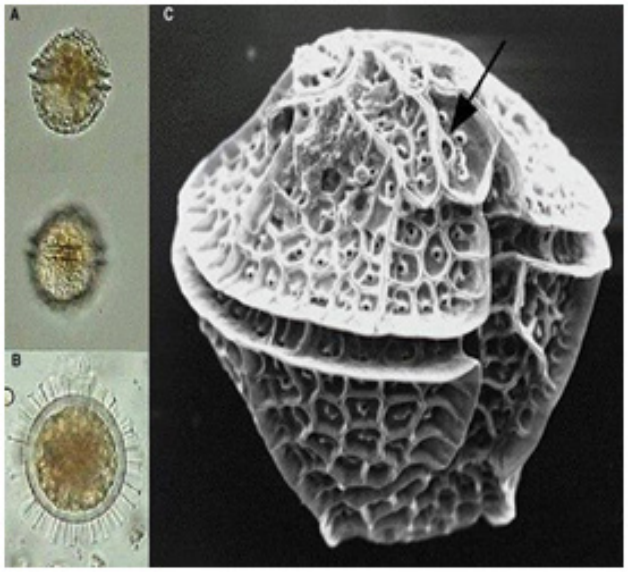

Figure 7: *Protoceratium reticulatumand* cysts.

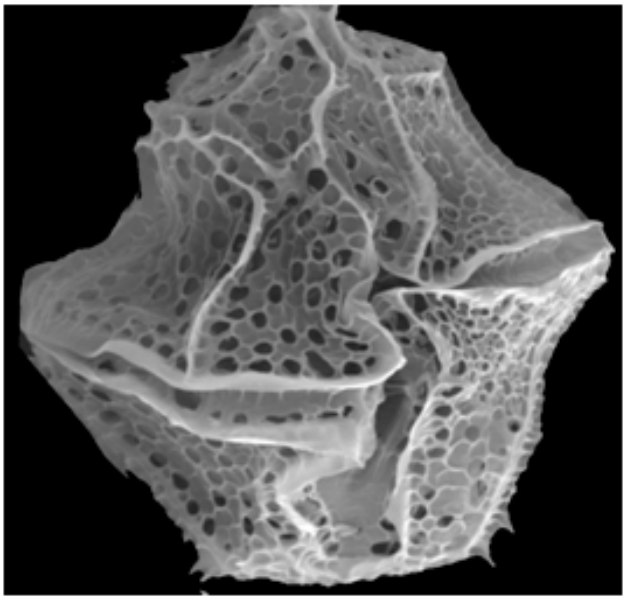

Figure 8: *Lingulodinium polyedrum.*

isolated from the *Dinoflagellate Gymnodinium breve* (Figure 9). Its toxicity is due to the stimulation of the nerve fibers that act on sodium channels by increasing the entry of the Na ion by depolarizing the neuronal membrane and inhibition of skeletal muscle activity; if taken for aerosol, due to the presence in the air of droplets of sea water polluted by algae, it produces irritation of airways with rhinorrhea, conjunctivitis and cough [28]. Oysters and mussels are the most infected for the filtering action of seawater and the accumulation of the toxin in the hepatopancreas. The toxin is also resistant to the temperature of 120 °C and to pH values between 2 and 10; the lethal dose for fish is between 0.2-0.5 mg/kg.

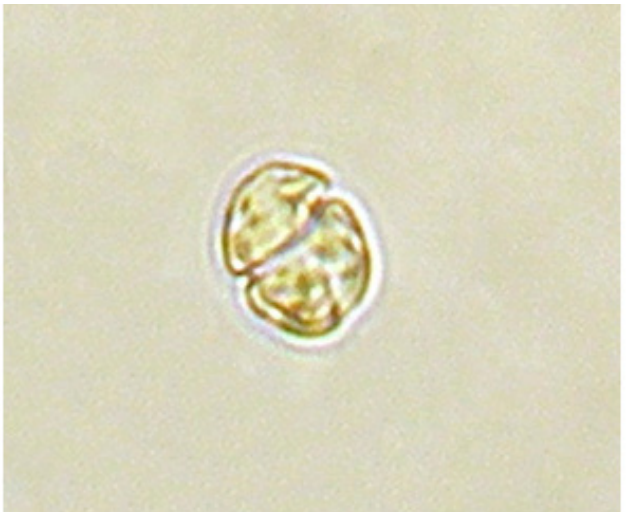

Figure 9: *Gymnodinium breve.*

Pfiesteria piscicida (Steidinger & Burkholder) (Figure 10) is a species of unicellular algae belonging to the *Dinoflagellates*, and is responsible for the phenomenon of Red Tides and the death of many fishes and shellfish. It has only been described since 1990 as it can occur in very complex life forms that include cysts, amoeboid forms and toxic zoospores [29, 30].

Ingestion of fish infected with *Pfiesteria piscicida* can cause nausea, vomiting, abdominal pain, diarrhea, neurological symptoms, psychiatric alteration, eye irritation, skin lesions [31, 32]. Intoxication occurs both through direct contact with fish and polluted water and by aerosol containing toxic particles.

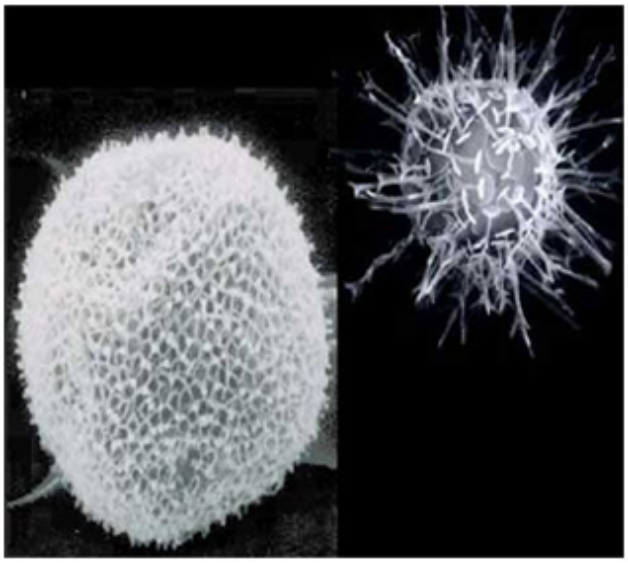

Figure 10: *Pfiesteria pescicida* and Cyst.

Toxicity depends on various factors related to both the polluted environment, salinity, temperature, light, nutrients, and the stage of life (free, amoeboid, spores) and the presence of prey. Currently there are no specific tests to determine the presence of toxins except biological tests on fish using, in particular, tilapia [33].

Pharmacological activity of biomolecules

The seas and oceans have always been considered very important for the research of new drugs from marine organisms: toxins, in fact, are structurally made up of complex molecules with multiple functional groups, each of which is equipped with biological activity and can be applied in the pharmaceutical sector. Many studies have been undertaken to identify the presence of new molecules with anticancer, antibiotic, analgesic, antispasmodic, hypotensive, antiviral activity.

Dinoflagellates are unicellular microalgae found in plankton that contain molecules with pharmacological activity, although their notoriety is linked to the presence of biotoxins that make seafood unsafe for health [34]. In recent years, interest has increased in new pharmacologically active biocompounds for use in the biotechnology and microalgae are primary producers that, due to their high productivity, provide at low cost various substances very useful for human health, being able to be used both as medicines and for applications in the biomedical and toxicology sector [35].

Tetradotoxin ($C_{11}H_{17}N_3O_8$) (Figure 11), for example, because of its high toxicity, has never been utilized as a drug, but as pharmacological reagent. It is extracted from the fish of the *Tetradontidae* family known as "puff fish", since it is not possible to extract from from the only *Dinoflagellate* that produces it, *Alexandrium tamarense*. It causes paralysis of peripheral nerve endings due to inhibition of sodium permeability to nerve membranes. This paralysis is reversible, and Tetradotoxin has proved to be an important indicator for the study of the transmission of nerve arousal [36]. *Tetradotoxin* is really a powerful and selective drug, with an analgesic/anaesthetic effect associated with its sodium channel blocking properties. An increase in the activity of live sodium channels is reported in many forms of carcinoma and is an indicator of metastasis [37]. The use of *Tetradotoxin* to block sodium canal activity not only reduces the presence of metastases, but highlights the pathway to finding new drugs that act like toxin, but are less dangerous [38, 39]. *Tetradotoxin* has also been used with minor side effects to relieve cranial symptoms in cases of heroin withdrawal [40].

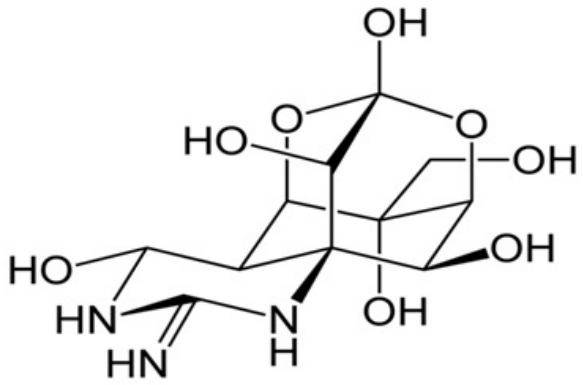

Figure 11: Tetradotoxin: an extremely toxic compound from pufferfish.

In many marine *Dinoflagellates*, such as *Alexandrium catenella* and *Alexandrium tamarense* (Figure 12 and 13) there are phytotoxins that accumulate in molluscs and the intake of these causes paralyzing symptoms and even death for humans. The mechanism of action of these phytotoxins was studied, highlighting that their toxicity is due to a blockage of neuronal transmission influencing the permeability of sodium in nerve cells. Researchers have highlighted the group of *Goniautotoxins* ($C_{10}H_{17}N_7O_8S$) (Figure 14) among the paralyzing toxins and have demonstrated their action as scarring and painkillers. They have shown that local applications of small amounts of paralyzing toxins produce a reversible paralysis of the striatum muscle,which turns out to be dependent dose. Patients with chronic tension-type headache were infiltrated locally with 50 mg of *Goniautotoxins* at the site where the pain was present and after a few minutes they showed a clear attenuation of pain [41]. Patients did not need to use other drugs and no adverse side effects were reported , and no second infiltration was carried out over a long period of about eight weeks, so the use of *Goniautotoxins* proved to be a safe and effective therapeutic tool as a painkiller.

Patients treated with an infiltration of *Goniautotoxins* during a surgery of arthroplasty in the knee showed a greater decrease in pain than those treated with conventional pain protocol, purchasing early the complete extension of the knee. No adverse effects were reported during the three-day hospital stay, during which patients did not experience any pain from the prolonged action of the *Goniautotoxins* [42].

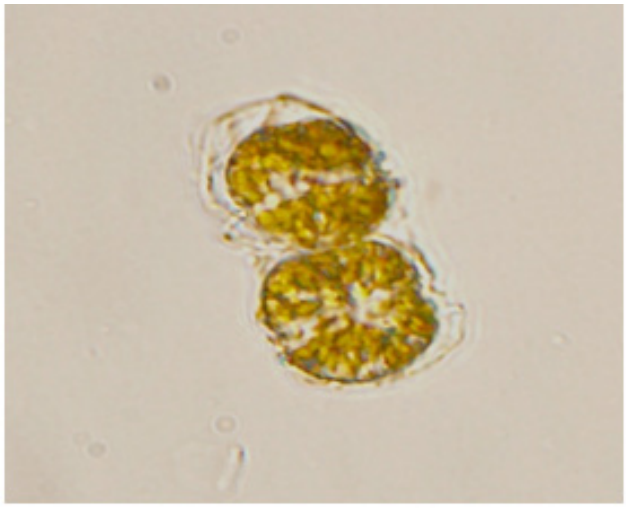

Figure 12: *Alexandrium catenella.*

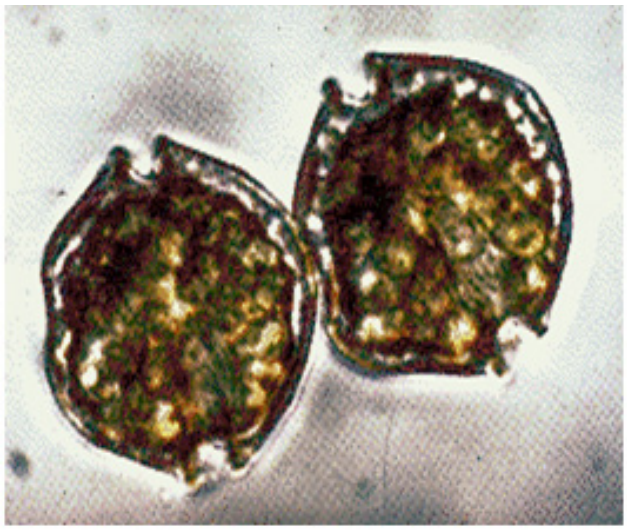

Figure 13: *Alexandrium tamarense.*

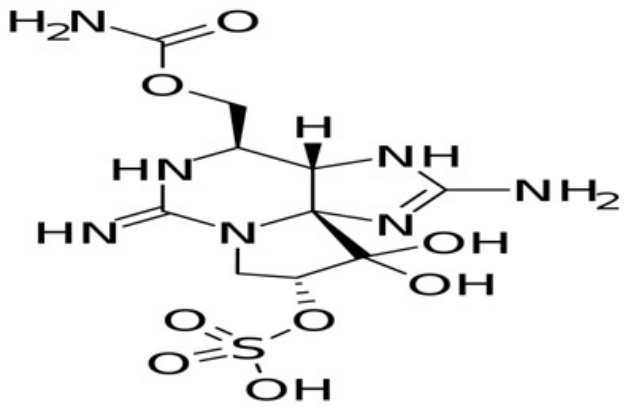

Figure 14: *Gonyautoxin*: a large group of neurotoxins by *Alexandrium sp.*

Gastrointestinal pathology is a very complex form, the onset of which is little known. Visceral pain is a characteristic symptom of functional disorders such as irritable bowel syndrome and inflammatory bowel disease that afflict many individuals around the world. The use of drug therapies often does not get good results for the lack of effectiveness and to cause many unwanted adverse effects. Scientists have recognized some receptor sites of the perception of painful sensations on which new biologically active natural compounds could more effectively act. Marine toxins represent, in fact, high affinity and selectivity to different molecular mediators of visceral pain, acting in particular on ion channels and receptors involved in pain generation [43]. Their use is very useful for studying the properties of ionic channels and receptors involved in pain perception, improving knowledge of their pathophysiological properties. A major disadvantage is that the toxins have low oral bioavailability, so injecting is required, which is generally unwelcome to the patient, high production costs and low conservation stability.

Dinoflagellates also produce many cytotoxic and/or long-chain polychetid macrolide: *Amphidinolids* (Figure 15) and *Cholopsinols* that are products of the genus *Amphidinolids*. The *Amphidinolids* show strong cytotoxicity towards cells L1210 murine lymphoma and *in vitro* epidermal human carcinoma; in particular, a N-type macrolide ampidinolide has been isolated from *Amphidinolids operculatum var. November Gibbosum* (Figure 16). The metabolic extract of a variety of *Dinoflagellate* cells such as *Amphidinolids carterae* (Figure 17), highlighted hemolytic, antifungal and cytotoxic properties, particularly towards *Candida albicans* (MIC = 64 μg/mL) [44-46].

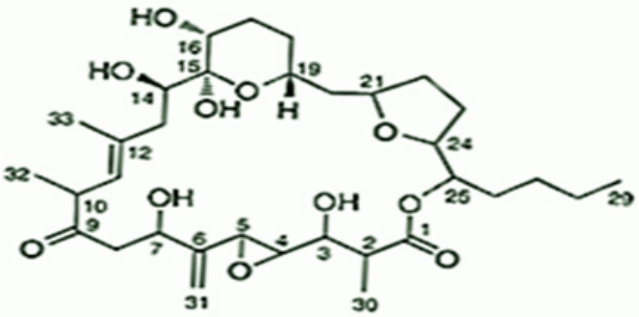

Figure 15: *Amphidinolide*: a macrolides class isolated from the genus *Amphidinium.*

The genus *Karenia* consists of unicellular, photosynthetic, planktonic organisms found in marine environments known mainly for their dense blooms of toxic algae and red tides that cause considerable ecological and economic damage causing serious animal mortality. *Karenia brevis* (Figure 18) is known to cause respiratory distress and poisoning in humans by neurotoxic crustaceans to build up toxins in tissues [47]. *Karenia brevis* (Figure 18) is found all over the world in oceanic and coastal waters and when algal blooms are formed and the availability of nutrients decreases, the genus *Karenia* begins to die releasing their neurotoxins that are destructive to the nervous system. Toxins characterized as *Brevetoxins* ($C_{50}H_{70}O_{14}$) (Figure 19) are lipsoluble and act by activating the tension-sensitive sodium channels and causing them to stay open for long periods of time with uncontrolled depolarization of the neural membrane and persistent neuronal arousal [48-50].

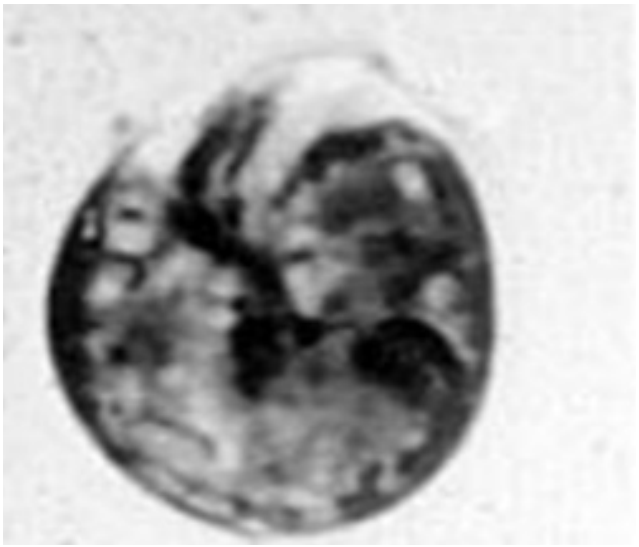

Figure 16: *Amphidinium opercolatum.*

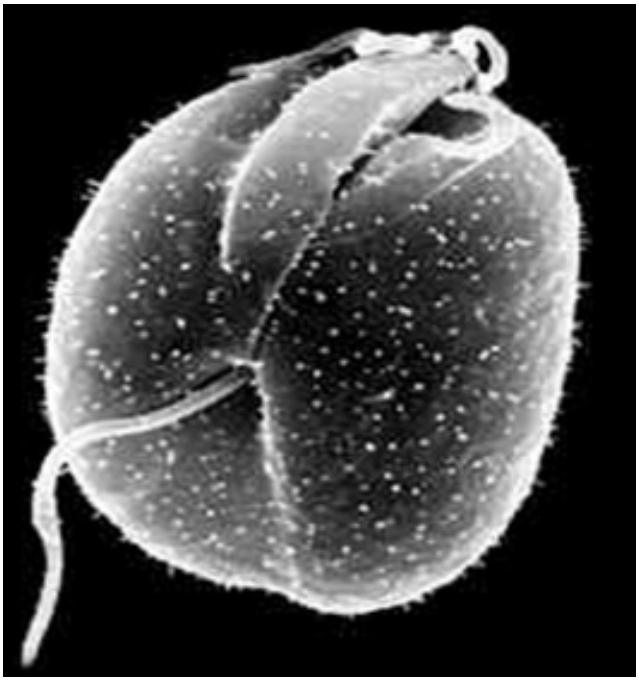

Figure 17: *Amphidinium carterae.*

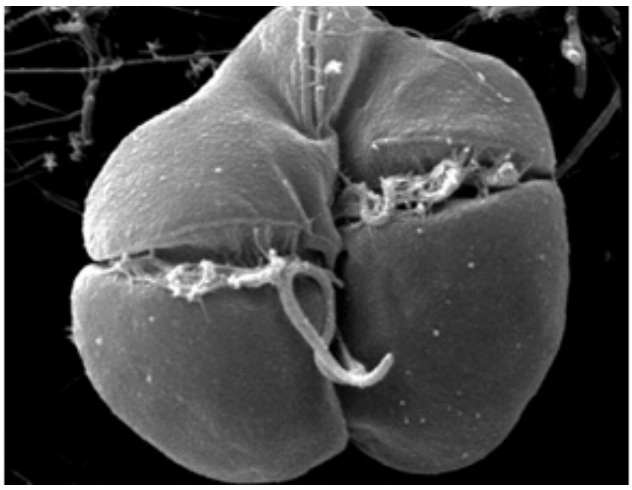

Figure 18: *Karenia brevis.*

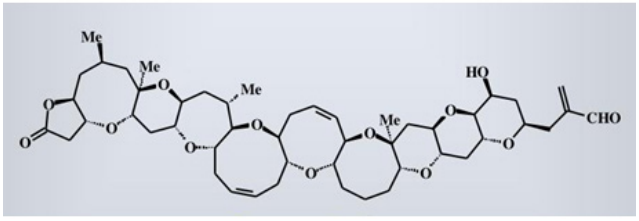

Figure 19: *Brevetoxins*: cyclic polyether compounds produced by *Karenia brevis*

No deaths have been recorded in association with *Brevetoxins*, but serious effects have been noted, such as nausea, vomiting and a variety of neurological symptoms, confused language, skin irritation directly exposed to water, irritation ocular. Exposure to *Brevetoxins* occurs by ingestion or inhalation: *Karenia brevis* cells are weak, so the action of the waves can break the cells, releasing the *Brevetoxins* as aerosols.

Exposure to *Brevetoxins* is all the more harmful the greater the contact time and the death of marine mammals is due to the ingestion of organisms that have accumulated high concentrations of *Brevetoxins* in their tissues. Humans are at risk, mainly through respiratory exposure which can result in a severe inflammatory response of bronchial mucous [51, 52]. Respiratory symptoms highlighted for exposure to marine aerosol containing *Brevetoxins* are coughing, involuntary sneezing, tearing, rhinorrhoea, burning sensation in the throat and nose, and breathing difficulties [53-56]. Various forms of *Brevetoxins* are known to have cytotoxic activities with DNA damage; they affect cell proliferation in a dose-dependent way, are genotoxic and cause cell death through an apoptotic mechanism.

Some experimental work has also shown that aerosol causes inflammation of the smooth bronchial musculature and broncho constriction even to animals that have been exposed [57, 58].

*Dinoflagellate*s of the genus *Amphidinolids carterae* contain a carotenoid, *Peridinin*, which forms a complex with chlorophyll that is responsible for the brown coloration of algal blooms. In fact *Peridinin* absorbs light at wavelengths between 470 -550 nm, of blue-green color and is able to transfer energy to chlorophyll molecole by giving it fluorescence [59]. *Peridinin*, like other carotenoid structure pigments act as sunscreen for both corals and algae with which they live in symbiosis.

The obtained fluorophore is very stable and finds different applications in immunological tests and flow cytometry for cell counting, determining cellular characteristics and their function, detection of microorganisms, diagnosis of pathologies such as blood tumors, etc.

Peridinin being a carotenoid is equipped with antioxidant and anticancer activity as has been demonstrated in a study on the proliferation and survival of lines of T cells infected with the HTLV-1 leukemia virus. Results showed an inhibition of cell proliferation dependent on the dosage of *Peridinin* used. Sugawara et al., highlighted colon cancer cell apoptosis for treatment with *Peridinin* isolated from *Dinoflagellates*, *Heterocapsa triquetra* (Figure 20) [60, 61]. Photoactivated

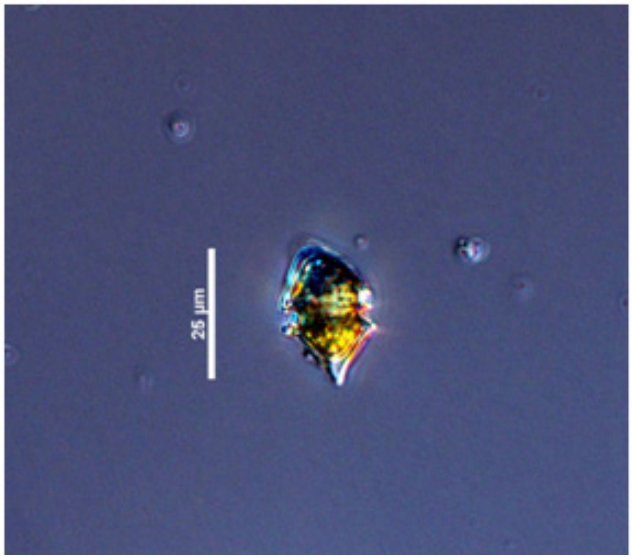

Figure 20: *Heterocapsa triquetra.*

porphyrines, pigments with a molecular structure similar to chlorophyll and hemoglobin, found in *Dinoflagellates* also showed antimicrobial activity [62, 63]. *Heterocapsa circularisquama* (Figure 21) is a *Dinoflagellate* toxic to bivalves but not to fish: it contains hemolytic porphyrin, which shows light-dependent cytotoxicity towards cancer cells. His antibacterial activity was also highlighted preferentially towards light-dependent gram+ [64].

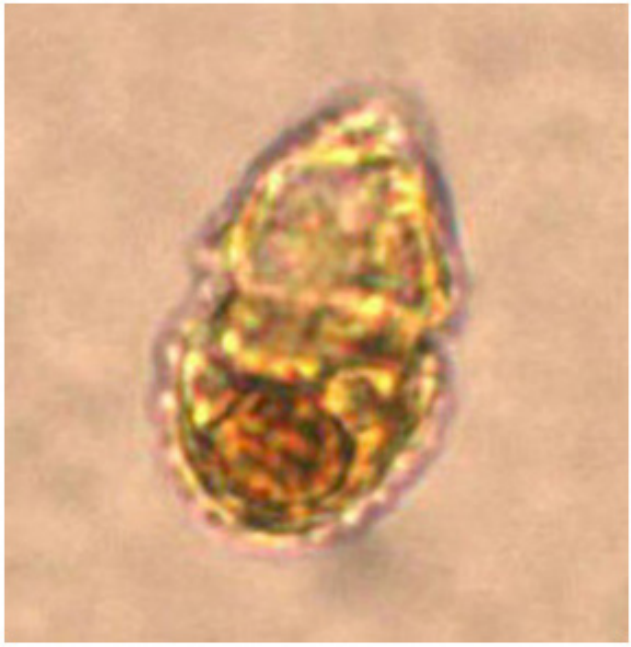

Figure 21: *Heterocapsa circularisquama.*

Crypthecodinium chonii (Figure 22) is the only non-toxic *Dinoflagellate* that is used industrially for the production of docosaesaenoic acid (DHA), high value omega-3 poly-unsaturated fatty acid that possesses various physiological and nutritional functions, used for the enrichment of baby products. The acid is contained entirely within cells and is distributed in both phospholipids and conservation lipids and therefore the extraction method is very long and complex. Particular attention should be used, then, to prevent oxidation phenomena that would not guarantee the purity of the final product [65]. During the production of docosahexaenoic acid this algae also produces polysaccharides, of which are known antioxidant, anti-inflammatory, antiadhesive, anti-coagulant, anti-cancer, anti-viral and immunomodulating properties [66-68]. Numerous studies on various cancer cell lines show that marine polysaccharides have high cytotoxicity and apoptogenic activities that can be considered a future alternative for the production of natural antitumor drugs compared to synthetic drugs.

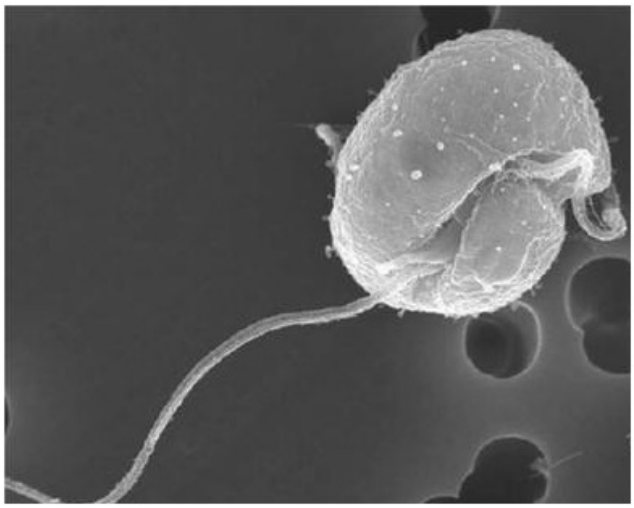

Figure 22: *Cripthecodinium chonii.*

Conclusions

Toxic algal blooms are a serious problem for all aquatic environments. They are not a new phenomenon, but they currently occur very frequently, and have taken on a strong expansion in Asian countries and America. The main cause is environmental degradation, but climatic change, the misuse of fertilisers and pesticides, industrial discharges, overcrowding, and engineering work also contribute [69, 70].

Dinoflagellates, responsible for algal blooms known as "Red Tides", are unicellular microalgae that produce biotoxins that make seafood toxic, as well as being responsible for a high death of fish. The increase in algal blooms in recent decades requires greater surveillance of seawaters and the need to take appropriate action to study this phenomenon, in an attempt to avoid serious repercussions on the environment, the economy and, above all, on the health of men. There are no specific therapies against algal biotoxins because, being ionophores, they affect the transport of ions (sodium and potassium pumps) at the cellular level. Currently, the only intervention, if the intoxication is reported in a timely manner , is to resort to the elimination of toxic residues from the digestive system by gastric lavender or with activated charcoal dust. In more severe cases, when neurological symptoms are present and respiratory paralysis is feared, it is necessary to resort to intubation of the patient to to subject him to mechanical ventilation [71-73].

In addition, detoxification methods are provided for health, especially in relation to mussels, before they are put on the market. The most commonly used method involves the transfer of toxic shellfish into waters free of toxic plankton, to allow self-purification, but it is a method that involves a long time; the transfer of shellfish is very tiring and expensive. Electric shocks or the use of chlorine reduce the duration of contamination, but one runs the risk of altering the sensory properties of the product, decreasing its appeal.The use of ozone has recently been proposed, which has been shown to be effective in preventing the accumulation of toxins by shellfih, without any alteration of them, but has shown no an efficient action towards invertebrates species that accumulate cysts of microorganisms, or that bind toxins to their tissues for long periods of time. There is still no effective, rapid and universal method of detoxification for all shellfish and as the costs of such treatments are still high, monitoring areas exposed to algal blooms, mussels, is still high [74-76].

Dinoflagellates are often studied because they are related to harmful algal blooms but are also capable of producing bioactive compounds for the treatment of human pathologies. In addition to proteins, fatty acids, vitamins and pigments they contain bioactive compounds such as carotenoids, polysaccharides, vitamins, lipids and powerful neurotoxins that can be applied as drugs by showing activities, analgesic, anticancer, anti-cholesterol, cytotoxic, anti-infective, immunosuppressants or as nutraceuticals. As primary producers, these marine microalgae are also rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), polyunsaturated fatty acids known as omega-3 and are responsible for many human health benefits, particularly in reducing heart disease such as arrhythmia, stroke and hypertension, as well as acting on depression, rheumatoid arthritis and asthma [77-79] Industrially they are added to infant milk formula or other foods, they are used as food additives for the presence of dyes, such as feed, even in aquaculture, pharmaceutical compounds, cosmetics and potentially as a source of biofuels [80-82].

Recent research has highlighted in *Amphidinolids carterae* the presence of enzymes of biotechnology interest such as polyketides synthasi (PKS) which are both responsible for the synthesis of toxins and other polyketides with interesting ecological and biotechnological functions such as antiprifier, allelopathic, anti-cancer, antifungal activity and/or beneficial effects for the treatment of Alzheimer's disease [83].

L-asparaginase is a polyketide enzyme synthase that catalyzes the hydrolysis of L-asparagine into L-aspartic acid and is used to treat acute lymphoblastic leukemia, acute myeloid leukemia, non-Hodgkin's lymphoma; malignant cells having a reduced ability to produce asparagine synthase, they use the asparagine present in the blood. By limiting the supply of asparagine, the growth of cancer cells is inibite [84]. This enzyme also has applications in the food industry to reduce acrylamide, a carcinogenic substance, in many foods: adding L-asparaginase to chips, biscuits, crispy bread is effectively reduced the formation of acrylamide [85].

The study of toxins, in addition to helping to reduce cases of food poisoning, can facilitate the research and development of new drugs by studying the routes of action and receptors to which they claim. To ensure the safety of shellfish it is not sufficient to subject them to analysis to identify the nature and extent of contamination, but it is essential to monitor aquatic environments to identify the toxic algal species present and the risks to human health. Should not be neglected, then the analysis of Red Tide aerosols for the identification of toxins and microorganisms present in it, to assess the risks related to time and exposure dose for human health.

Conflict of Interest

The author declares she has no conflicts of interest.

References

1. Vilariño N, Louzao MC, Vieytes MR, 2010. Botana LM. Biological methods for marine toxin detection. *Anal Bioanal Chem* 397(5): 1673-1681. https://doi.org/10.1007/s00216-010-3782-9
2. Farabegoli F, Blanco L, Rodríguez LP, Vieites JM, Cabado AG. 2018. Phycotoxins in marine shellfish: origin, occurrence and effects on humans. *Mar Drugs* 16(6): 188. https://doi.org/10.3390/md16060188
3. Blanco J. 2018. Accumulation of *Dinophysis* toxins in bivalve molluscs. *Toxins (Basel)* 10(11): 453. https://doi.org/10.3390/toxins10110453
4. Tangen K. 1983. Shellfish poisoning and the ocurrence of potentially toxic dinoflagellates in Norwegian waters. *Sarsia* 68(1):1-7. https://doi.org/10.1080/00364827.1983.10420550
5. Hallegraeff GM. 2010. Ocean climate change, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge. *J Phycol* 46: 220-235. https://doi.org/10.1111/j.1529-8817.2010.00815.x
6. Hallegraeff GM. 2014. Harmful algae and their toxins: progress, paradoxes and paradigm shifts, toxins and biologically active compounds from microalgae. In: Rossini GP (ed) Toxins and biologically active compounds from microalgae, CRC Press, Boca Raton, Florida, USA, pp 3-20.
7. Gallardo-Rodríguez JJ, Sánchez-Mirón A, García-Camacho F, López-rosales L, Chisti Y, et al. 2012. Bioactives from microalgal *dinoflagellates*. *Biotechnol Adv* 30(6): 1673-1684. https://doi.org/10.1016/j.biotechadv.2012.07.005
8. Tachibana K, Scheuer PJ, Tsukitani Y, Kikuchi H, Van Engen D, et al. Okadaic acid, a cytotoxic polyether from two marine sponges of the genus *Halichondria*. *J Am Chem Soc* 103(9): 2469-2471. https://doi.org/10.1021/ja00399a082
9. Valdiglesias V, Prego-Faraldo MV, Pásaro E, Mendez J, Laffon B 2013. Okadaic acid: more than a diarrheic toxin. *Mar Drugs* 11(11): 4328-4349. https://doi.org/10.3390/md11114328
10. Suganuma M, Okabe S, Marino MW, Sakai A, Sueoka E, et al. 1999. Essential role of tumor necrosis factor a(TNF^{-a}) in tumor promotion as revealed by TNF^{-a}-deficient mice. *Cancer Res* 59(18): 4516-4518.
11. Fujiki H, Sueoka E, Watanabe T, Suganuma M. 2018. The concept of the okadaic acid class of tumor promoters is revived in endogenous protein inhibitors of protein phosphatase 2A, SET and CIP2A, in human cancers. *J Cancer Res Clin Oncol* 1444(12): 1-8. https://doi.org/10.1007/s00432-018-2765-7
12. Tangen K. 1980. Brown water in the OSlofjord, Norway,in september 1979 caused by the toxic *Prorocentrum minimum* and other *dinoflagellates* Blyttia. 38: 145-158.
13. Marasovic I, Pucher–Petkovic T. 1985. Effects of eutrophication on the coastal phytoplankton community. *Rapp Comm Int M Int Mer Medit* 29: 137-139.
14. Heilet CA, Gilbert PM, Fan C. 2005. *Prorocentrum minimum* (Pavillard) Schiller-a review of a hamful algal bloom species of growing worldwide

importance. *Harmful Algae* 4(3): 449-470. https://doi.org/10.1016/j.hal.2004.08.003

15. Marasovic I, Pucher-Petkovic T, Petrova-Karadjova V. 1990 *Prorocentrum minimum* (Dinophyceae) in the Adriatic and Black Sea. *J Mar Biol Ass* 70(2): 473-476. https://doi.org/10.1017/S0025315400035542
16. Tubaro A, Bornancin A, Hungerford J, Sosa S. 2008. Pharmacology and toxicology of diarrheic shellfish toxins. Seafood and Freshwater toxins. Taylor & Francis Group, CRC Press, Boca Raton, New York, USA, pp 231-239.
17. Twiner M, Rehmann N, Hess P, Doucette GJ. 2008. Azaspiracid shellfish poisoning: a review on the chemistry, ecology, and toxicology with an emphasis on human health impacts. *Mar Drugs* 6(2): 39-72. https://doi.org/10.3390/md20080004
18. Jauffrais T, Marcaillou C, Herrenknecht C, Truquet P, Séchet V, et al. 2012. Azaspiracid accumulation, detoxification and biotransformation in blue mussels (*Mytilus edulis*) experimentally fed *Azadinium spinosum*. *Toxicon* 60(4): 582-595. https://doi.org/10.1016/j.toxicon.2012.04.351
19. Satake M, Ofuji K, Naoki H, James KJ, Furey A. 1998. Azaspiracid, a new marine toxin having unique spiro ring assemblies, isolated from Irish mussels, *Mytilus edulis*. *J Am Chem Soc* 120: 9967-9968. https://doi.org/10.1021/ja981413r
20. Furey A, O'Doherty S, O'Callaghan K, Lehane M, James KJ. 2010. Azaspiracid poisoning (AZP) toxins in shellfish: toxicological and health considerations. *Toxicon* 56(2): 173-190. https://doi.org/10.1016/j.toxicon.2009.09.009
21. Aasen JA, Espenes A, Hess P, Aune T. 2010. Sub-lethal dosing of Azaspiracid-1 in female NMRI mice. *Toxicon* 56: 1419-1425. https://doi.org/10.1016/j.toxicon.2010.08.007
22. Cao Z, LePage KT, Frederick MO, Nicolaou KC, Murray TF. 2010. Involvement of caspase activation in Azaspiracid-induced neurotoxicity in neocortical neurons. *Tox Sci* 114(2): 323-334. https://doi.org/10.1093/toxsci/kfp312
23. Roman Y, Alfonso A, Louzao MC, de la Rosa LA, Leira F, et al. 2002. Azaspiracid-1, a potent, nonapoptotic new phycotoxin with several cell targets. *Cell Signal* 14(8): 703-716. https://doi.org/10.1016/s0898-6568(02)00015-3
24. Roman Y, Alfonso A, Vieytes MR, Ofuji K, Satake M, et al. 2004. Effects of azaspiracids 2 and 3 on intracellular cAMP, [Ca^{2+}], and pH. *Chem Res Toxicol* 17(10): 1338-1349. https://doi.org/10.1021/tx0341862
25. Alfonso A, Roman Y, Vieytes MR, Ofuji K, Satake M. 2005. Azaspiracid-4 inhibits Ca^{2+} entry by stored operated channels in human T lymphocytes. *Biochem Pharmacol* 69(11): 1627-1636. https://doi.org/10.1016/j.bcp.2005.03.022
26. Alfonso A, Vieytes MR, Ofuji K, Satake M, Nicolau KC, et al. 2006. Azaspiracids modulate intracellular pH levels in human lymphocytes. *Biochem Biophys Res Comm* 346(3): 1091-1099. https://doi.org/10.1016/j.bbrc.2006.06.019
27. Vale C, Nicolau KC, Frederick MO, Vieytes MR, Rotana LM. 2010. Cell volume decrease as a link between Azaspiracid-induced cytotoxicity and c-Jun-N-Terminal kinase activation in cultured neurons. *Tox Sci* 113(1): 158-168. https://doi.org/10.1093/toxsci/kfp246
28. Cheng YS, Zhou Y, Irvin CM, Pierce RH, Naar J, et al 2005. Characterization of marine aerosol for assessment of human exposure to brevetoxins. *Environ Health Perspect* 113(5): 638-643. https://doi.org/10.1093/toxsci/kfp246
29. Noga EJ, Khoo L, Stevens JB, Fan Z, Burkholer JM. 1996. Novel toxic dinoflagellates causes epidemic disease in estuarine fish. *Mar Pollut Bull* 32(2): 219-224. https://doi.org/10.1016/0025-326X(95)00114-3
30. Peglar MT, Nerad TA, Anderson OR, Gillevet PM. 2004. Identification of amoebae implicated in the life cycle of *Pfiesteria* and *Pfiesteria*-like dinoflagellates. *J Eukaryot Microbiol* 51(5): 542-552. https://doi.org/10.1111/j.1550-7408.2004.tb00290.x
31. Litaker RW, Vandersea MW, Kibler SR, Madden VJ, Noga EJ, et al. 2002. Life cycle of the heterotrophic dinoflagellate *Pfiesteria piscicida* (Dinophyceae). *J Phyc* 38(3): 442-463. https://doi.org/10.1046/j.1529-8817.2002.01242.x
32. Lovko VJ, Vogelbein WK, Shields JD, Haas LW. 2003. A new larval fish biossay for the pathogenicity of *Pfiesteria spp* (Dinophyceae). *J Phycol* 39(3): 600-609. https://doi.org/10.1046/j.1529-8817.2003.02106.x
33. Moeller PDR, Morton SL, Mitchell BA, Siverten SK, Fairey ER, et al. 2001. Current progress in isolation and and characterizaton of toxins isolated from *Pfiesteria piscicida*. *Environ Health Perspect* (Suppl 5): 739-743.s https://doi.org/10.1289/ehp.01109s5739
34. Taylor FJR. 1987. *Diniflagellates*: an introduction in the biology of *Dinoflagellates*. Taylor FJR (ed) Blackwell Scientific Publications: Oxford,UK, pp 1-13.
35. Mimouni V, Ulmann L, Pasquet V, Mathieu M, Picot L, et al. 2012. The potential of microalgae for the production of bioactive molecules of pharmaceutical interest. *Curr Pharm Biotechnol* 13(15): 2733-2750. https://doi.org/10.2174/138920112804724828
36. Hagen NA, Laponte B, Ong-Lam M, Dubuc B, Walde D, et al. 2011. A multicentre open-label safety and efficacy study of tetradotoxin for cancer pain. *Curr Oncol* 18(3): 109-116. https://doi.org/10.3747/co.v18i3.732
37. Zhongxiu L, Jian L, Jitao L, Zhijun T, Hai R, et al. 2014. Toxic Dinoflagellate *Alexandrium* tamarese induces oxidative stress and apoptosis in hepatopancreas of shrimp (*Fenneropenaeus chinensis*). *J Ocean Univ China* 13(6): 1005-1011. https://doi.org/10.1007/s11802-014-2397-8
38. Djamgoz MBA, Coombes RC, Schwab A. 2014. Ion trasport and cancer: from initiation to metastasis. *Philos Trans R Soc Lond B Biol Sci* 369(1638): 20130092. https://doi.org/10.1098/rstb.2013.0092
39. Lastraioli E, Iorio J, Arcangeli A. 2015. Ion canne expression as promising cancer biomarker. *Biochim Biphys Acta* 1848(10 Pt B): 2685-2702. https://doi.org/10.1016/j.bbamem.2014.12.016
40. Song H, Li J, Lu CL, Kang L, Xie L, et al. 2011. Tetrodotoxin alleviates acute heroin withdrawal syndrome: a multicentre,randomized, double-blind,placebo-controlled study. *Clin Exp Pharmacol Physiol* 38(8): 510-514. https://doi.org/10.1111/j.1440-1681.2011.05539.x
41. Lattes K, Venegas P, Lagos N, Lagos M, Pedraza L, et al. 2009. Local infiltration of Gonyautoxin is safe and effective in treatment of chronic tension-type headache. *Neurol Res* 31(3): 228-233. https://doi.org/10.1179/174313209X380829
42. Hinzpeter J, Barrientos C, Camorano A, Martinez A, Palet M, et al. 2016. Gonyautoxins :first evidence in pain management in total knee arthroplasty. *Toxicon* 119: 180-185. https://doi.org/10.1016/j.toxicon.2016.06.010
43. Baj A, Bistoletti M, Bosi A, Moro E, Giaroni C, et al. 2019. Marine toxins and nociception: potential therapeutic use in the treatment of visceral pain associated with gastrointestinal disorders. *Toxins (Basel)* 11(8): 449. https://doi.org/10.3390/toxins11080449
44. Kobayashi J, Shimbo K, Kubota T, Tsuda M. 2003. Bioactive macrolides and polyketides from marine dinoflagellates. *Pure Appl Chem* 75(3): 337-342. https://doi.org/10.1021/np0605844
45. Chakraborty TK, Das S. 2001. Chemistry of potent anti-cancer compounds, amphidinolides. *Curr Med Chem Anti-cancer Agents* 1(2): 131-149. https://doi.org/10.2174/1568011013354660
46. Nuzzo G, Cutignano A, Sardo A, Fontana A. 2014. Antifungal amphidinol 18 and its 7-sulfate derivative from the marine dinoflagellate *Amphidinium carterae*. *J Nat Prod* 77(6): 1524-1527. https://doi.org/10.1021/np500275x
47. Brand LE, Cmpbell L, Bresnan E. 2012. Karenia: the biology and ecology of a toxic genus. *Harmful Algae* 14: 156-178. https://doi.org/10.1016/j.hal.2011.10.020
48. Naar J, Bourdelais A, Tomas C, Kubanek J, Whitney PL, et al. 2002. A

competitive ELISA to detect brevetoxins from *Karenia brevis* (Formely *Gymnodinium breve*) in seawater shellfish and mammalian body fluid. *Envir Health Perspect* 110(2): 179-185. https://doi.org/10.1289/ehp.02110179

49. Magana HA, Contreras C, Villareal TA. 2003. A historical assessment of *Karenia brevis* in the westrn Gulf of Mexico. *Harmful Algae* 2(3):163-171. https://doi.org/10.1016/S1568-9883(03)00026-X
50. Porter H, Di J, Beet A, Kirkpatrick B, Reich A, et al. 2014. The human health effects of Florida Red Tide (FRT) blooms: an expanded analysis. *Envir Inter* 68: 144-153. https://doi.org/10.1016/j.envint.2014.03.016
51. Shimoda T, Krzanowski JJr, Nelson R, Martin DF, Polson J, et al. 1988. *In vitro* red tide effects on human bronchial smooth muscle. *J Allergy Clin Immunol* 81(6):1187-1191. https://doi.org/10.1016/0091-6749(88)90889-5
52. Sas KM, Baatz JE. 2010. Brevetoxin-2 induces an inflammatory response in an alveolar macrophage cell line. *Int J Hyg Environ Health* 213: 352-358. https://doi.org/10.1016/j.ijheh.2010.06.007
53. Backer LC, Fleming LE, Rowan A, Cheng YS, Benson JM, et al. 2003. Recreational exposure to aerosolized brevetoxins during Florida red tide events. *Harmful Algae* 2(1): 19-28. https://doi.org/10.1016/S1568-9883(03)00005-2
54. Backer LC, Kirkpatrick B, Fleming LE, Cheng YS, Pierce R, et al. 2005. Occupational exposure to aerosolized brevetoxins during Florida red tide events: effects on a healthy worker population. *Environ Health Perspect* 113(5): 644-649. https://doi.org/10.1289/ehp.7502
55. Woodcock AH. 1948. Note on concerning human respiratory irritation associated with high concentrations of plankton and mass mortality of marine organisms. *Sears Found J Marine Res* 7: 56-62.
56. Pierce RH, Henry MS, Blum PC, Lyons J, Cheng YS, et al. 2003. Brevetoxin concentrations in marine aerosol: human exposure levels during a Karenia brevis harmful algal bloom. *Bull Environ Contam* 70(1): 161-165. https://doi.org/10.1007/s00128-002-0170-y
57. Abraham WM, Bourdelais AJ, Sabater JR, Ahmed A, Lee TA, et al. 2005. Airway pesponses to aerosolized brevetoxins in an animal model of asthma. *Am J Respir Crit Care Med* 171(1): 26-34. https://doi.org/10.1164/rccm.200406-735OC
58. Asai S, Krzanowski JJ, Anderson WH, Martin DF, Polson JB, et al. 1982. Effects of the toxin of red tide, *Ptychodiscus brevis* on canine tracheal smooth muscle: a possible new asthma-triggering mechanism. *J Allergy Clin Immunol* 69(5): 418-428. https://doi.org/10.1016/0091-6749(82)90116-6
59. Hofmann E, Wrench PM, Sharples FP, Hiller RG, Wlte W, et al. 1996. Structural basis of light harvesting by carotenoids peridinin-chlorophyll-protein from *Amphidinium carterae Science* 272 (5269): 1788-1791. https://doi.org/10.1126/science.272.5269.1788
60. Ishikawa C, Jomori T, Tanaka J, Senba M, Mori N. 2016. Peridinin, a carotenoid, inhibits proliferation and survival of HTLV-1-infected T-cell lines. *Int J Oncol* 49(4): 1713-1721. https://doi.org/10.3892/ijo.2016.3648
61. Sugawara T, Yamashita K, Sakai S, Asai A, Nagao A, et al. 2007. Induction of apoptosis in DLD-1 human colon cancer cells by peridinin isolated from the dinoflagellate, *Heterocapsa triquetra. Biosci Biotechnol Biochem* 71(4): 1069-1072. https://doi.org/10.1271/bbb.60597
62. Malik Z, Hanania J, Nitzan Y. 1990. Bactericidal effects of photoactivated porphyrins--an alternative approach to antimicrobial drugs. *J Photochem Photobiol B* 5(3-4): 281-293. https://doi.org/10.1016/1011-1344(90)85044-w
63. Shannon E, Abu-Ghannam N. 2016. Antibacterial derivatives of marine algae: an overview of pharmacological mechanisms and applications. *Mar Drugs* 14(4): 81. https://doi.org/10.3390/md14040081
64. Wencheng L, Cho K, Yamasaki Y, Takeshita S, Hwang K, et al. 2018. Photo-induced antibacterial activity of a porphyrin derivative isolated from the harmful dinoflagellates *Heterocarpa circularisquama Aquat Toxicol* 201: 119-128. https://doi.org/10.1016/j.aquatox.2018.06.004
65. Mendes A, Reis A, Vasconcelos R, Guerra P, Lopes Da Silva T. 2009. *Crypthecodinium cohnii* with emphasis on DHA production: a review. *J Appl Phycol* 21: 199-214. https://doi.org/10.1007/s10811-008-9351-3
66. Laurienzo P. 2010. Marine polysaccharides in pharmaceutical applications: an overview. *Mar Drugs* 8(9): 2435-2465. https://doi.org/10.3390/md8092435
67. Gardeva E, Toshkova R, Yossifova L, Minkova K, Gigova L. 2012. Cytotoxic and apoptogenic potential of red microalgal polysaccharides. *Biotechnol Biotechnol Equip* 26(4): 3167-3172. https://doi.org/10.5504/BBEQ.2012.0035
68. de Jesus Raposo MF, de Morais RM, de Morais AM. 2013. Health applications of bioactive compounds from marine microalgae. *Life Sci* 93(15): 479-486. https://doi.org/10.1016/j.lfs.2013.08.002
69. Gauthier L, Tison-Rosebery J, Morin S, Mazzella N. 2019. Metabolome response to anthropogenic contamination on microalgae: a review. *Metabolomics* 16(1): 8. https://doi.org/10.1007/s11306-019-1628-9
70. Anderson DM, Glibert PM, Burkholder JM. 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries* 25: 704-726. https://doi.org/10.1007/BF02804901
71. Dominguez HJ, Paz B, Daranas AH, Norte M, Franco JM, et al. 2010. Dinoflagellate polyether within the yessotoxin, pectenotoxin and okadaic acid toxin groups: characterization, analysis and human health implications. *Toxicon* 56(2): 191-217. https://doi.org/10.1016/j.toxicon.2009.11.005
72. Ferreiro SF, Carrera C, Vilariño N, Louzao MC, Santamarina G, et al. Acute cardiotoxicity evaluation of the marine biotoxins OA, DTX-1 and YTX. *Toxins (Basel)* 7(4): 1030-1047. https://doi.org/10.3390/toxins7041030
73. Davidson K, Baker C, Higgins C, Higman W, Swan S, et al. 2015. Potential threats posed by new or emerging marine biotoxins in UK waters and examination of detection methodologies used for their control: Ciclici imines. *Mar Drugs* 13(12): 7087-7112. https://doi.org/10.3390/md13127057
74. Otero P, Alfonso A, Alfonso C, Aráoz R, Molgó J, et al. 2011. First direct fluorescence polarization assay for the detection and quantification of spirolides in mussel samples. *Anal Chim Acta* 701(2): 200-208. https://doi.org/10.1016/j.aca.2011.05.034
75. Fux E, McMillan D, Bire R, Hess P. 2007. Development of an ultra-performance liquid chromatography-mass spectrometry method for the detection of lipophilic marine toxins. *J Chromatogr A* 1157(1-2): 273-280. https://doi.org/10.1016/j.chroma.2007.05.016
76. Adarme-Vega TC, Lim DKY, Timmins M, Vernen F, Li Y, et al. 2012. Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production. *Microb Cell Fact* 11: 96. https://doi.org/10.1186/1475-2859-11-96
77. Gong Y, Wan X, Jiang M, Hu C, Hu H, et al. 2014. Metabolic engineering of microorganisms to produce omega-3 very long-chain polyunsaturated fatty acids. *Prog Lipid Res* 56: 19-35. https://doi.org/10.1016/j.plipres.2014.07.001
78. Nauroth JM, Liu YC, Van Elswyk M, Bell R, Hall EB, et al. 2010. Docosahexaenoic acid (DHA) and docosapentaenoic acid (DPAn-6) algal oils reduce inflammatory mediators in human peripheral mononuclear cells *in vitro* and paw edema *in vivo*. *Lipids* 45(5): 375-384. https://doi.org/10.1007/s11745-010-3406-3
79. Sprague M, Betancor MB, Tocher DR. 2017. Microbial and genetically engineered oils as replacements for fish oil in aquaculture feeds. *Biotechnol Lett* 39(11): 1599-1609. https://doi.org/10.1007/s10529-017-2402-6
80. Ganuza E, Benítez-Santana T, Atalah E, Vega-Orellana O, Ganga R, et al. 2008. *Cryptocodinium cohnii* and Schizochytrium sp. as potential substitutes to fisheries-derived oils from sea bream (*Sparus aurata*) microdiets. *Aquaculture* 277(1-2): 109-116. https://doi.org/10.1016/j.aquaculture.2008.02.005
81. Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussgnug JH,

et al. 2008. Second generation biofuels: high-efficiency microalgae for biodiesel production. *Bioenerg Res* 1: 20-43. https://doi.org/10.1007/s12155-008-9008-8

82. Kamat PK, Rai S, Nath C. 2013. Okadaic acid induced neurotoxicity: an emerging tool to study Alzheimer's disease pathology. *Neurotoxicology* 37: 163-172. https://doi.org/10.1016/j.neuro.2013.05.002

83. Pieters R, Hunger SP, Boos J, Rizzari C, Silverman L, et al. 2011. L-asparaginase treatment in acute lymphoblastic leukemia: a focus on *Erwinia asparaginase*. *Cancer* 117(2): 238-249. https://doi.org/10.1002/cncr.25489

84. Xu F, Oruna-Concha MJ, Elmore JS. 2016. The use of asparaginase to reduce acrylamide levels in cooked food. *Food Chem* 210: 163-171. https://doi.org/10.1016/j.foodchem.2016.04.105

85. Benson J, Hahn F, March T, McDonald J, Sopori M, et al. 2004. Inhalation toxicity of brevetoxin 3 in rats exposed for 5 days. *J Toxicol Environ Health A* 67(18): 1443-1456. https://doi.org/10.1080/15287390490483809