TOXICITY OF SEA ALGAL TOXINS TO HUMANS AND ANIMALS

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Abstract: Marine algal toxins are responsible of more than 60000 intoxication/year, with an overall mortality of about 1.5%. Human intoxications are due to consumption of seafood and respiratory exposure to aerosolized toxins. Algal toxins are also responsible for extensive die-offs of fish and shellfish, as well as mortality in seabirds, marine mammals and other animals depending on marine food web. Lots of information are available concerning acute intoxications, while little is known about environmental health effects of chronic exposure to low levels of algal toxins. Toxins are produced by two algal groups, dinoflagellates and diatoms, representing about 2% of known phytoplankton species (60–80 species out of 3400–4000) and can reach humans directly (via consumption of shellfish) or through food web transfer to higher trophic levels (zooplankton and herbivorous fish). Most toxins are neurotoxins and all are temperature stable, so cooking does not ameliorate toxicity in contaminated seafoods; five seafood poisoning syndromes exists: paralytic shellfish poisoning, neurotoxic shellfish poisoning, ciguatera fish poisoning, diarrhetic shellfish poisoning, and amnesic shellfish poisoning.

Paralytic Shellfish Poisoning (**PSP**) is caused by the consumption of molluscan shellfish contaminated with a suite of heterocyclic guanidines collectively called saxitoxins (STXs), causing almost 2,000 cases of human poisonings per year, with a 15% mortality rate. In addition to human intoxications, PSP has been implicated in deaths of birds and humpback whales. STX elicits its effects by inhibiting sodium channel conductance and there-by causing blockade of neuronal activity, mainly at the peripheral

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V. Evangelista et al. (eds.), *Algal Toxins: Nature*, *Occurrence*, *Effect and Detection.*

nervous system level, where its binding results in rapid onset of symptoms (less than 1 hr) that are classic for PSP: tingling and numbness of the perioral area and extremities, loss of motor control, drowsiness, incoherence, and in the case of high doses, respiratory paralysis.

Neurotoxic Shellfish Poisoning (NSP) generally results from consumption of molluscan shellfish contaminated with brevetoxins (PbTx), a suite of nine structurally related ladderlike polycyclic ether toxins. Brevetoxins bind with high affinity sodium channel altering the voltage sensitivity of the channel, resulting in inappropriate opening of the channel under conditions in which it is normally closed, and inhibiting channel inactivation, resulting in persistent activation or prolonged channel opening. Symptoms of NSP include nausea, tingling and numbness of the perioral area, loss of motor control, and severe muscular ache. NSP has not been documented as a fatal intoxication in humans. *Gymnodinium breve* red tides are also frequently associated with massive fish kills. The extreme sensivity of fish may result from lysis of cells passing through the gills, with direct transfer of toxin across the gill epithelium. *G. breve* was also responsible of a manatees die-off in Florida concurrent with a persistent red tide. The demonstration of brevetoxin immunoreactivity in lymphoid tissue of the manatees raises the possibility of immunosuppression as a second mode by which brevetoxin exposure may affect human health, particularly in individuals with chronic exposure to aerosolized toxin during prolonged red tide incidents.

Ciguatera Fish Poisoning (CFP) is another seafood intoxication caused by ladderlike polyether toxins, primarily attributed to the dinoflagellate, *Gambierdiscus toxicus*, which produces a precursors to ciguatoxin which is biotransformed to ciguatoxins and bioaccumulated in the highest trophic levels. Large carnivorous fishes associated with coral reefs are a frequent source of ciguatera. Baracuda, snapper, grouper, and jacks are particularly notorious for their potential to carry high toxin loads; however, smaller herbivorous fishes may also be ciguatoxic, particularly when viscera are consumed. CFP is estimated to affect over 50,000 people annually and is no longer a disease limited to the tropics because of travel to the tropics and shipping of tropical fish species to markets elsewhere in the world; outbreaks are sporadic and unpredictable at others. The symptoms of ciguatera vary somewhat geographically as well as between individuals and incidents and may also vary temporally within an area, but they generally include

early onset (2–6 hr) gastrointestinal disturbance–nausea, vomiting, and diarrhea–and may be followed by a variety of later onset (18 hr) neurologic sequelae such as numbness of the perioral area and extremities, reversal of temperature sensation, muscle and joint aches, headache, itching, tachycardia, hypertension, blurred vision, and paralysis. Ciguatera on rare occasions can be fatal. A chronic phase may follow acute intoxication and can persist for weeks, months, or even years.

Diarrhetic Shellfish Poisoning (**DSP**) is a comparatively milder seafood intoxication that consists of rapid onset (3 hr) gastrointestinal symptoms such as vomiting and diarrhea that generally resolve within 2–3 days. The diarrhetic shellfish toxins (DTX) are a class of acidic polyether toxins consisting of at least eight congeners including the parent compound, okadaic acid. Okadaic acid, DTX-1, and DTX-2 are the primary congeners involved in shellfish poisoning, with the other congeners believed to be either precursors or shellfish metabolites of the active toxins. The DTXs are inhibitors of ser/thr protein phosphatases. Ser/thr protein phosphatases are critical components of signaling cascades in eukaryotic cells that regulate a diverse array of cellular processes involved in metabolism, ion balance, neurotransmission, and cell cycle regulation. Diarrhea associated with DSP is most likely due to the hyperphosphorylation of proteins, including ion channels, in the intestinal epithelia, resulting in impaired water balance and loss of fluids. Okadaic acidlike polyether toxins have been identified as tumor promotors, thus raising the question of what effect low levels of chronic exposure to DSP toxins may have on humans as well as wildlife such as marine turtles.

Amnesic Shellfish Poisoning (**ASP**) is the only shellfish intoxication caused by a diatom (*Pseudo-nitzschia spp.*). The first recorded occurrence of ASP was in Prince Edward Island, Canada in 1987 when approximately 100 people became ill and several died after consuming contaminated mussels. The toxic agent involved in the outbreak was identified as domoic acid. Domoic acid is a water-soluble tricarboxylic amino acid that acts as an analog of the neurotransmitter glutamate and is a potent glutamate receptor agonist. The symptoms of ASP include gastrointestinal effects (e.g. nausea, vomiting, diarrhea) and neurologic effects such as dizziness, disorientation, lethargy, seizures, and permanent loss of short-term memory. Persistent activation of the kainate glutamate receptor results in greatly elevated intracellular Ca^{2+} through cooperative interactions with *N*-methyl-d-aspartate

and non-*N*-methyl-d-aspartate glutamate receptor subtypes followed by activation of voltage dependent calcium channels. Neurotoxicity due to domoic acid results from toxic levels of intracellular calcium, which leads to neuronal cell death and lesions in areas of the brain where glutaminergic pathways are heavily concentrated. The CA1 and CA3 regions of the hippocampus, an area responsible for learning and memory processing, are particularly susceptible. However, memory deficits occur at doses below those causing structural damage. Domoic acid has been identified as the causative agent in the mass mortality of pelicans and cormorants in Monterey Bay, California, in 1991 and in the extensive die-off of California sea lions in the same region in 1998. In both instances the vector for toxin transfer was anchovy.

Pfiesteria Piscicida, a fish-killing dinoflagellate first identified in aquaculture tanks in North Carolina, has been linked to fish kills in the mid-Atlantic region of the United States and is characterized by the presence of open, ulcerative lesions. *Pfiesteria* has been termed an "ambush predator" because it is believed to release a toxin that narcotizes or kills fish and then phagocytizes the sloughed tissue from its prey. *Pfiesteria* has been linked to a human intoxication syndrome, with symptoms that include fatigue, headache, respiratory irritation, skin lesions or burning sensations on contact, disorientation, and memory loss. The toxins responsible for fish lethality or neurologic symptoms have not yet been identified. There is currently no evidence that toxicity is transferred through food.

In conclusion we can state that marine algal toxins impact human health through seafood consumption and respiratory routes. The apparent increase in their occurrence over the past three decades has raised alarm and lead to the establishment of algal and toxin monitoring programs which will assist in providing time series needed to assess interannual and long-term variability in algal and toxin occurrence.

Keywords: Algae, toxins, syndromes, treatment

1. General Characteristics of Producing Organisms

Marine algal toxins are produced by phytoplankton, phytobenthos and bacteria, and are also called phycotoxins.

Phycotoxins are secondary metabolites produced by dinoflagellates and diatoms, which present pharmacologically active compounds which can be harmful to aquatic flora and fauna. Their role is both important for normal physiology of the cell and for the defense against external environmental insults, namely predators (Amzil et al., 2001; Quod et al., 2001).

Marine toxins are not dangerous *per se*, but they became an hazard when dinoflagellates and diatoms proliferate, under particular environmental conditions, i.e. eutrophication, and toxins can accumulate along different steps of trophic chains, particularly mollusks and fish. In these case, the so called HARMFUL ALGAL BLOOMS (HABs) occur, causing a great increase in cells and toxins concentrations (Smayda, 1997; van Dolah, 2000).

Phycotoxins have an great and important toxicological role as they produce a huge number of human illness linked to seafood consumption and contaminated aerosol inhalation. They are also responsible for massive die-off of fish, shellfish and marine vertebrates (van Dolah, 2000).

Generally speaking they are responsible for acute intoxications, which are well known from the toxicological, chemical and etiological point of view, while little is known concerning chronic exposure to low levels of toxins (Landsberg, 1996; Burkholder, 1998; Edmunds et al., 1999; Landsberg et al., 1999).

More than 3000 dinoflagellates and diatoms species are known at present, but only 2% of then (about 60–80 species) have proved to be toxic or harmful. This little group of species, anyway, is responsible for about 60000 human intoxication/year, 1.5% of them fatal. Fatalities are generally linked to ingestion of saxitoxins, tetrodotoxin and, in rare cases, ciguatera and domoic acid (Landsberg, 1996; Burkholder, 1998; Edmunds et al., 1999; Landsberg et al., 1999).

Incidence of HABs has increased in recent years, both in frequency and in geographical distribution (Fig. 1). Causes of this expansion are various, and include on one side the increased awareness concerning the issue and the establishment of monitoring, surveillance and research programs on toxins. This lead to a faster and more detailed identification of blooms and toxic episodes (Anderson, 1989; Smayda, 1990; Hallegraeff, 1993).

On the other side, human activities can directly and indirectly contribute to this expansion. Ballast waters transport or shellfish transplantation can directly act by easing the transfer of toxic, non indigenous species from side to the other of the world. Local and regional environmental changes, i.e. eutrophication and pollution, and/or climate variations at the local or global scale can indirectly act by inducing algae proliferation, thus increasing toxins concentrations (van Dolah, 2000)

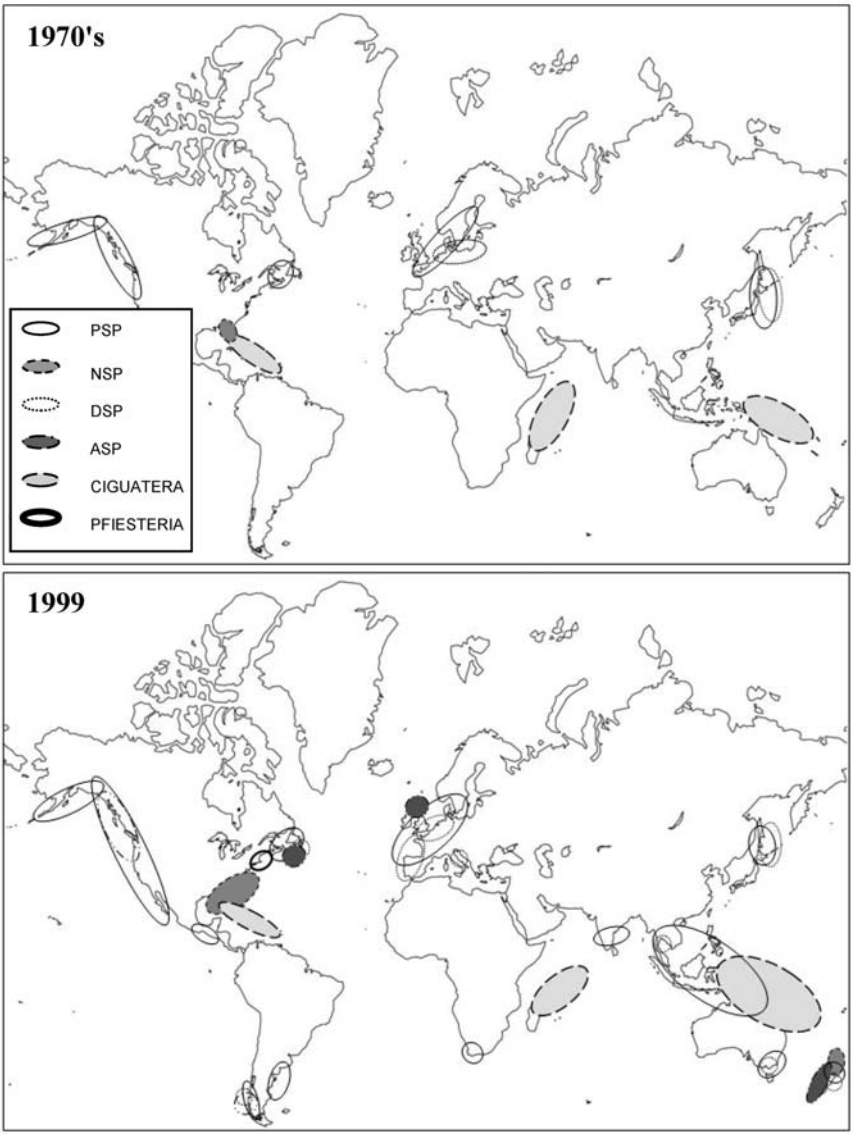


Figure 1. Time changes in HABs distribution (from van Dolah (2000), modified).

Algal blooms can be classified following various criteria: 1) the kind of bloom formed; 2) the chemical structure of the toxin; 3) the solubility in solvents; 4) the syndrome they induce.

Starting from the **kind of bloom** formed, 4 groups have been identified, which are more or less dangerous to humans and /or animals (Andersen, 1996):

– Blooms of species which produce basically harmless water discolorations, with the result that the recreational value of the area decreases due to low visibility of the water and eventually, under exceptionally weather conditions in sheltered bays, the blooms can grow so dense that they cause escape reactions and indiscriminate fish kills and kills of benthic invertebrates due to oxygen depletion. Species forming this kind of bloom are *Noctiluca scintillans, Ceratium* spp, *Prorocentrummicans, Heterocapsa triquetra*, *Skeletonema costatum*, *Trichodesmiumerythraeum*, *Eutreptiella* spp., *Phaeocystis pouchetii*, *Emiliania Huxley*, *Mesodinium rubrum*.

– Blooms of species which produce potent toxins which accumulate in food chains and cause a variety of gastrointestinal and neurological illnesses m humans and other higher animals such as. *Alexandrium tamarense, Alexandrium funndyense, Gymnodinium catenatum, Pyrodinium bahamense* var. *compressum*, *Dinophysis fortii, Dinophysis acuminata, Dinophysis acuta, Dinophysis norvegica*, *Pseudo-nitzschia multiseries, Pseudo-nitzschia pseudodelicatissima, Pseudo-nitzschia australis*, *Gambierdiscus toxicus*, *Gymnodinium breve*, *Anabaena flos-aquae, Nodularia spumigena* can produce these blooms.

– Blooms of species which, in most cases are non-toxic to humans but harmful to fish and invertebrates (especially in intensive aquiculture systems) e.g. by intoxication, damaging or clogging of the gills or other means. Examples of producing species: *Alexandrium tamarense, Chaetoceros convolutus, Gyrodinium aureolum, Chrysochromulina polylepis, Prymnesium parvum, Heterosigma akashiwo, Chattonella antiqua, Aureococcus anophagefferens, Phiesteria piscimortuis, Nodularia spumigena.*

– Blooms of species which produces toxins which are toxic to humans and which are transported by air in aerosols from the bloom area to the coast. *Gymnodinium breve, Pfiesteria piscicida*.

There is no general rule to define harmful concentrations of cells in an algal bloom, the concentration in a HAB is species specific.

Some algae cause harm at low concentrations, with no discoloration in the water, e.g. *Alexandrium tumarense* where PSP toxins are detected in

shellfish at concentrations below 10^3 cells/L, whereas other algae cause harmful effects when they occur in higher in higher concentrations, with discoloration of the water as a result, a "red tide". For example *Gyrodinium aureolum* kills fish and benthic animals at concentrations higher than 10^7 cells/L (Andersen, 1996).

Five main classes of toxins have been identified starting from their **chemical structure**:

1. Amino acid-like compounds (domoic acid and derivatives)
2. Purine derivatives (saxitoxins and derivatives)
3. Cyclic imines (spirolides, gymnodines and pinnatoxin A)
4. Linear and macrocyclic non-azotated polyethers (okadaic acid, pectenetoxins, azaspiracid, primnesines)
5. Trasfused polyehters (brevetoxins, yessotoxins, ciguatoxins).

All the toxins can be classified starting from their **solubility** in water and organic solvents:

1. Hydrophilic compounds (saxitoxins, domoic acid, tetrodotoxin)
2. Lipophilic compounds (okadaic acid, brevetoxins, ciguatoxins)

Finally, most known classification is that starting from the **syndrome** they induce. Starting from this principle 5 different syndromes can be identified:

1. **Diarrhetic Shellfish Poisoning** (DSP) is caused by a group of toxins, represented by okadaic acid, and is characterized by gastrointestinal symptoms (nausea, diarrhea, vomiting, abdominal pain) which following chronic exposure can evolve in digestive system tumors.
2. **Paralytic Shellfish Poisoning** (PSP) is caused by saxitoxins and is characterized by gastrointestinal and neurological symptoms, with nausea, vomiting, diarrhea, tingling or numbness around lips, gradual and more and more severe paralysis, respiratory difficulty, death through respiratory paralysis. It can cause death in humans.
3. **Amnesic Shellfish Poisoning** (ASP) toxin is domoic acid, and main sign of this syndrome is loss of short term memory, accompanied by gastrointestinal and neurological symptoms.

4. **Neurotoxic Shellfish Poisoning** (NSP) toxin is brevetoxin, and typical signs of toxicity are tingling and numbness of perioral area, loss of motor control and severe muscular ache. It is also responsible for some irritative episodes following exposure through contaminated aerosol.
5. One last syndrome is named ciguatera and is due to **ciguatoxin**. Together with tetrodotoxin, this is the only toxin transmitted by fish and not by shellfish. Typical symptoms are diarrhea, abdominal pain, nausea, vomiting, and lots of neurological signs. It can rarely cause death in humans.

When describing a syndrome, different toxins can be included as etiological agent, and that's the criteria followed in present chapter; it should be noted anyway that in some cases these toxins have only chemical similarity to the main toxin, causing the syndrome, and nay have a different action on humans and animals. So in the group of diarrheic toxins yessotoxins is included, even if its main action is at the neurological level.

Many other toxins have been studied and new ones are discovered in recent years, ut they are not included in a precise syndrome: tetrodotoxin, palitoxin, Pfiesteria toxins among the others. These toxins will be described in next sections.

Lots of studies have been conducted to define ideal conditions for algal growth and toxin production, but no clear scenario has been identified (Quod et al., 2001).

One of main question concerning phycotoxins is if they are produced by algae themselves or by symbiotic bacteria. In some species the production of toxins seems to be independent of bacteria presence, i.e. in *Prorocentrum lima*, producing okadaic acid. Studies conducted on saxitoxins production lead to no conclusive result, as these toxins have been found in autotrophous dinoflagellates, fresh water cyanobacteria, macrophytes and some bacteria. Finally it has been proved that tetrodotoxin is produced by symbiotic bacteria, which can be found in various aquatic and terrestrial organisms (Oshima et al., 1984; Scheuer, 1996; Shimitzu, 1996; Dantzer and Levin, 1997; Gallacher and Smith, 1999; Ritchie et al., 2000; Quod et al., 2001).

2. Role of Phycotoxins in Marine Environment

Lots of toxins producing algae contain very potent active principles, showing specific biological activity, which are thought to have a physiological or a defensive role. Indeed a certain correlation was observed between the

production of diarrhetic toxin and photosynthetic activity. Finally, a positive correlation was found between chlorophyll and diarrhetic toxins. An additional factor in favor of this hypothesis is the fact that almost all species are strict of facultative photosynthetic organisms. Okadaic acid has been found to be located into chloroplasts of some dinoflagellates (Zhou and Fritz, 1994; Morton and Tindall, 1995; Wright and Cembella, 1998; Barbier et al., 1999; Quod et al., 2001).

All these data lead to the hypothesis that at least some phycotoxins can modulate photosynthesis.

Other toxins are thought to have a physiological role: saxitoxins seem to be important n chromosome organization, due to their localization close to the nucleus and paralyzing toxins are thought to act also ad pheromones (Anderson and Cheng, 1988; Wyatt and Jenkinson, 1997; Cembella, 1998).

Phycotoxins have proved to have some antibacterial and antifungal activity; these activities are thought to allow dinoflagellates to inhibit growth of competitors like bacteria and fungi, as well as other algal species development. This inhibitory action was observed in various species of *Prorocentrum*, *Amphidinium* and in *Gambierdiscus toxicus* (Nagai et al., 1990; Lewis and Holmes, 1993; Nagai et al., 1993).

A defensive role of phycotoxins against herbivores has also been considered, acting through the alteration of ionic channel functioning , as shown with some studies demonstrating a reduction in grazing activity from macro- and micro- zooplankton.

This inhibiting activity, called allelopathy, has been studied and observed in vitro against other dinoflagellates or microalgae, even if no real evidence exist witnessing an allopathic action in the wild (Elbrächter, 1976; Kayser, 1979; de Jong and Admiraal, 1984; Yasumoto et al., 1987; Gentien and Arzul, 1990; Scheuer, 1990; Rausch de Traubenberg and Morlaix, 1995; Windust et al., 1996; Paul, 1997; Windust et al., 1997; Wright and Cembella, 1998; Sugg and Van Dolah, 1999).

The impact of toxins on and their bioaccumulation along food chains depends on the characteristics of trophic chains themselves and on environmental conditions (Fig. 2).

Thus temperate and tropical ecosystems differ greatly. Indeed, tropical waters are generally olygotrophic and blooms which develop are usually reduced as, affecting very complex ecosystems. These blooms can form a mosaic in close areas: so some zones are toxic, and a close one is not. In these ecosystem fish species, feeding on algae, are responsible for toxicity (Bourdeau et al., 2001) (Fig. 3).

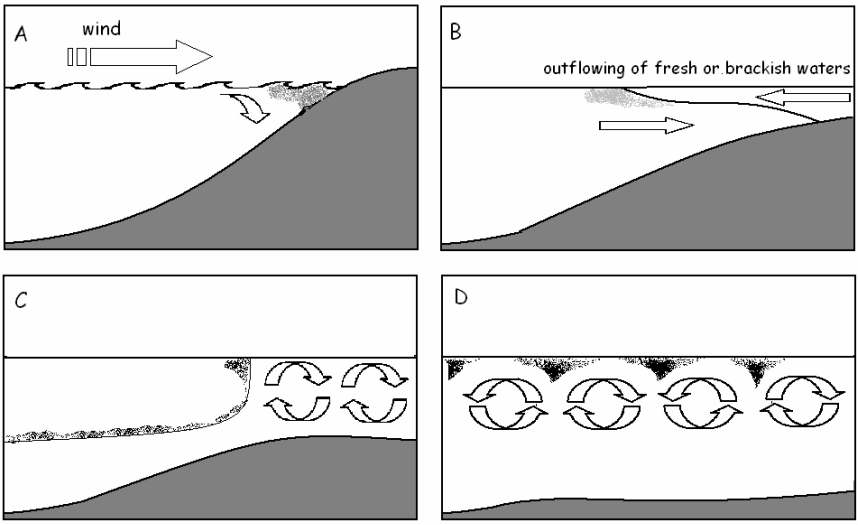


Figure 2. Blooms formation in coastal areas.

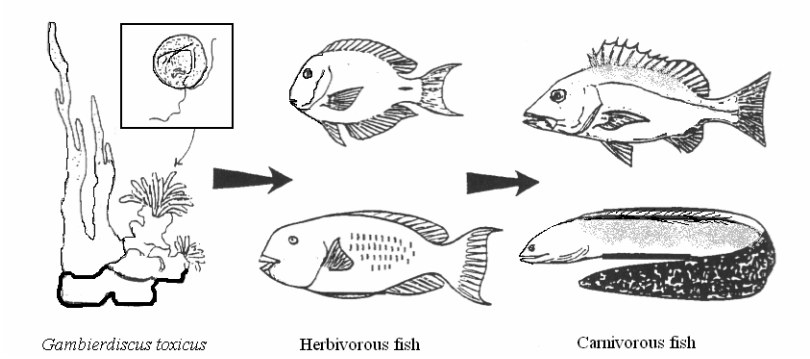


Figure 3. Transfer of phycotoxins along tropical food chains.

In temperate waters, which are more eutrophic, basic step of trophic chain are filtering organisms, like mussels. Only in some case fish, at different levels of trophic chain, can be directly interested by toxicity and accumulation of toxins, for direct contact with the poison or by ingestion of producing algae. These fish species are generally planktophagous species, living in packs (Bourdeau et al., 2001).

In these areas, phycotoxins can affect all levels of food chains.

It is recognized that harmful algae and their toxins can influence ecosystems from both the top-down (i.e. affecting predators and influencing grazing) and from the bottom-up (i.e. affecting plankton and benthic communities). Acute or chronic exposure to HABs and their toxins, either directly or through the food web, puts these populations at increased risk (White, 1980; White, 1981; Ives, 1985; Geraci et al., 1989; Gosselin et al., 1989).

Acute or chronic exposure to HABs and their toxins, either directly or through the food web, place certain populations at increased risk (Fig. 4). Microalgal toxins and their chronic effects need to be recognized as major threats to animal health, sustained fisheries, endangered species, and ecosystems. Long-term effects of biotoxins on the health of aquatic animals include increased susceptibility to disease, immunosuppression, abnormal development, and the induction of tumors. Animals at all trophic levels that are exposed to biotoxins in the long term through their diet may die or display impaired feeding and immune function, avoidance behavior, physiological dysfunction, reduced growth and reproduction, or pathological effects.

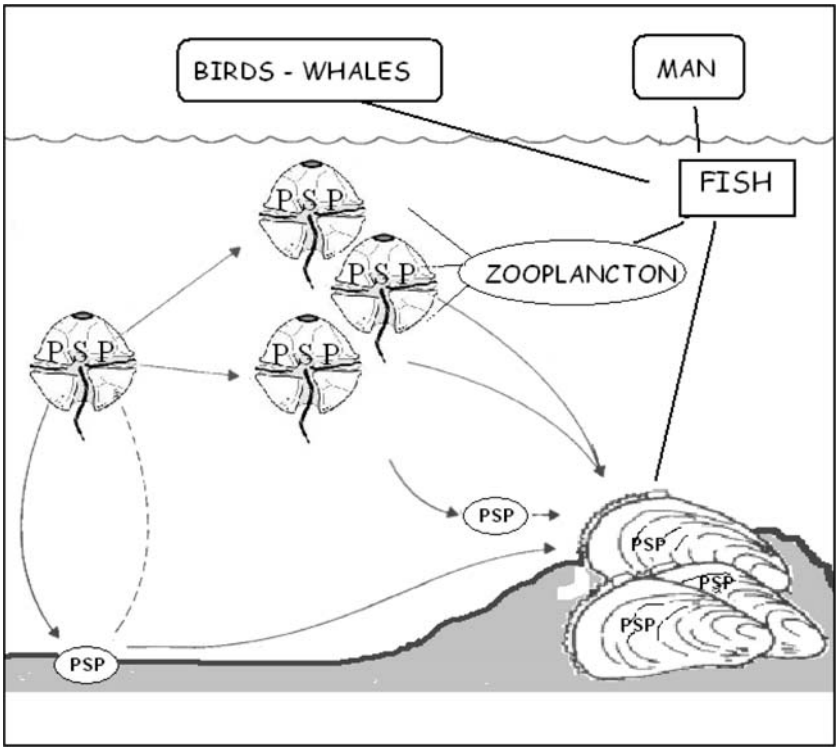


Figure 4. Accumulation and transfer of phycotoxins along food chain. The example of PSP.

2.1. ZOOPLANKTON

In many occasions zooplankton can feed on toxic dinoflagellates without any adverse effect.

When some effect occurs, it is usually a sub-lethal one, like a reduction in feed consumption. This alteration can be seen as the appearance of an avoiding behavior, so that some species avoid grazing on toxic dinoflagellates, while others feed on them without any problem.

Other effects observed are regurgitation of food, tachycardia, uncontrolled motor activity or reduced motility. Some authors think that long term exposure to low levels of toxins can induce reduction of growth rate and motor inhibition, which make copepods more sensible to predation, also easing accumulation along food chain (White, 1981; Hayashi et al., 1982; Boyer et al., 1985; Watras et al., 1985; Huntley et al., 1986; Gill and Harris, 1987; Ives, 1987; Sykes and Huntley, 1987; Uye and Takamatsu, 1990; Anderson and White, 1992).

2.2. FISH SPECIES

Phycotoxins can have a direct effect on fish species, causing larval and adult massive death. Anyway they can also have some important effect linked to long term accumulation of the toxins, turning them poisonous for consumers, being them humans or animals.

A real accumulation of toxins hardly occurs, as the toxicity of phycotoxins to fish is quite high, so in many cases fish die before they can accumulate discrete amounts of toxins. When accumulation occurs, liver and digestive tract are main target of accumulation. In the case of paralyzing toxins, altered swimming, equilibrium loss and complete immobility have been observed; if fish survive, recovery is complete (White, 1980; White, 1984; Carreto et al., 1993).

Being toxins stored in liver and digestive tract, consumption of whole fish, as happens in Borneo and Philippines, can produce deadly episodes, as registered in past years (Maclean, 1979; Maclean, 1989).

Many fish species have proved to accumulate toxins in their body: mackerels, *Sardinella* sp., *Mugil* and *Sillago*; one of the most known species which are able to accumulate toxins are puffer fish, which can stock tetrodotoxin in their viscera. Some doubt exist regarding brevetoxins capacity of accumulate; poisoning episodes in marine mammals seems to confirm the transfer of brevetoxins via plankton-eating fish (Beales, 1976; Estudillo and Gonzales, 1984; Bourdeau et al., 2001).

All specie able to accumulate toxins seems to have some adaptation to the poison; it the case of puffer fish and tetrodotoxin. Indeed, puffer fish have developed resistance to the toxin by a mutation of proteic sequence of sodium channel, which is the target of the toxin (Nakamura et al., 1984).

2.3. SEABIRDS

Lots of reports exist regarding seabirds die-off following contaminated fish eating.

Cormorants, terns, pelicans are among species more frequently affected by paralyzing toxins and domoic acid. Interestingly, different sensitivity was observed among species, cormorants being more sensible than others.

In a toxic episode concerning pelicans and cormorants, anchovy, which were responsible for the transfer of domoic acid to birds, showed no toxic symptoms, showing how in many cases blooms con be underestimated, if not correctly monitored (Coulson et al., 1968; Armstrong et al., 1978; Anderson and White, 1992; Fritz et al., 1992; Anderson, 1994b).

2.4. MARINE MAMMALS

Various episodes, reported since ‘80s, witness for toxicity of phycotoxins to marine mammals and their transfer along food chains. Whales die-off was observed in USA following an *Alexandrium tamarense* bloom, transfer agent being mackerel who fed on the dinoflagellates and who accumulated saxitoxins in liver and kidney. Interestingly, no toxin was found in muscle of contaminated fish. Some concern exist regarding saxitoxins as real causative agent of the die-off, as the levels found were below the toxic threshold defined for humans. Anyway, it has been thought that chronic exposure to lower doses could lead to accumulation of toxic levels (Geraci et al., 1989; Anderson, 1994a; Anderson. 1994b).

Brevetoxins have been considered responsible for a die-off of dolphin in USA during 1987–88. Two possible way of absorption have been considered: 1) intoxication following toxic aerosol inhalation; 2) continuous absorption of low levels of toxin accumulated by natural preys of dolphins (menhadens and mackerels). Absorbed doses were considered as non toxic per se, but they probably caused a defedation of animals, which then experienced bacterial or viral secondary infections (Gerlach, 1989; Anderson and White, 1992; Van Dolah et al., 2003).

The toxin was also responsible for some important die-off of manatees following *Gymnodium breve* blooms. Again, contaminated aerosol inhalation or toxin ingestion have been considered as death cause. In a case reported in 1982, tunicates were considered as transfer organisms, while in a second case, dating 1996, little or no tunicates were found in gastric content of manatees (Freitas et al., 1996; Bossart et al., 1998; Landsberg and Steidinger, 1998).

Domoic acid was responsible of sea lions intoxication in California in 1998, anchovies being the transfer organisms. Anchovies contained the highest amounts of toxin in their internal organs, while sea lions had highest levels in faeces (Lefebvre et al., 1999; Scholin et al., 2000).

Not all marine mammals experience accidental or passive intoxication by phycotoxins. Indeed, it has been observed that sea otter seems to be able to distinguish between contaminated and non contaminated parts of preys, as they discard flesh and siphon of contaminated animals. The observation that sea otters are absent in areas containing saxitoxins at level higher than toxic threshold seems to confirm this hypothesis (Kvitek et al., 1991; Patyten, 1999).

3. Diarrhetic Shellfish Poisoning

Diarrhetic toxins include various toxins: dynophisistoxins, whose principal compound is **okadaic acid** (OA), which is responsible for the syndrome, **pectenotoxins** (PTX) and **yessotoxins** (YTX).

This group is an example of a set of molecules grouped together because of their physico-chemical characteristics, even if biological effects are completely different. Due to these toxicological differences, it is now considered the possibility of modifying their classification, using toxicological criteria (Amzil et al., 2001).

DSP is a relatively recent discovered syndrome, but it is considered that it should exist since long time: gastroenteric symptoms indeed could have lead to the attribution of the syndrome to bacterial or viral infections, leading to ad underestimation of its incidence.

Poisoning follows ingestion of mussels containing *Dynophisis* spp. and *Prorocentrum* spp.; it has been observed that very low *Dynophisis* concentrations (50 cells/L) could lead to mussels contamination and toxicity (Kat, 1983; Marcaillou-Le Baut et al., 2001) (Fig. 5).

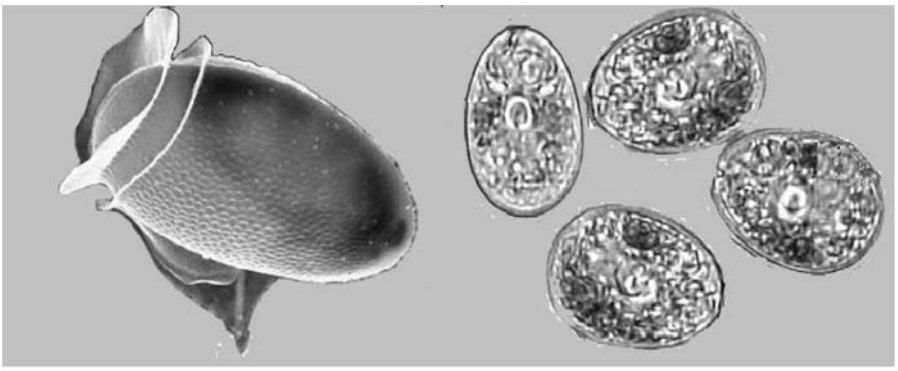


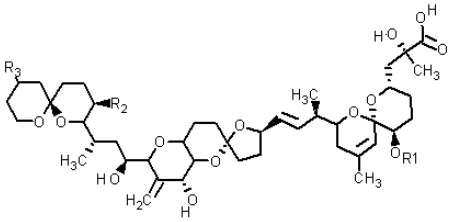
Figure 5. Diarrhetic toxins producing organisms (A *Dynophisis* spp. and B *Prorocentrum* spp).

3.1. CHEMICAL STRUCTURE

3.1.1. *Okadaic acid*

Okadaic acid is a liposoluble cyclic polyether with a carboxylic function (Fig. 6). Various derivatives exists of OA, which originate by modification of OA structure: DTX1 is a methyl derivatives, and DTX2 is an isomer of OA (Draisci et al., 1996; Quilliam, 1998; Van Egmond et al., 2004).

Some more toxins have been identified, originating from the acylation of the okadaic acid molecule, leading to the formation of DTX3, named, acyl-ester. These acyl-esters probably are metabolic derivatives, as they are only found in mussels' digestive gland (Hu et al., 1995c; Windust et al., 1997; Barbier et al., 1999).



Okadaic acid (OA)	R1=H	R2=Me	R3=H
dinophysistoxin 1 (DTX 1)	R1=H	R2=Me	R3=Me
dinophysistoxin 2 (DTX 2)	R1=H	R2=H	R3=Me
dinophysistoxin 3 (DTX 3)	R1=acyl	R2=Me	R3=Me

Figure 6. Okadaic acid and its derivatives molecular structure.

3.1.2. *Yessotoxins*

These toxins are sulphated polyether compounds; two main molecules have been identified; yessotoxin and 45-hydroxy-yessotoxin.

YTX is main toxin in Adriatic Sea mussels, other homologues were identified: homo-YTX, 45-hydroxyhomo-YTX, carboxy-YTX and adriatoxin (Lee et al., 1989; Ciminiello et al., 1997; Satake et al., 1997a; Satake et al., 1997b; Ciminiello et al., 1998; Tubaro et al., 1998; Yasumoto and Satake, 1998; Ciminiello et al., 2000) (Fig. 7).

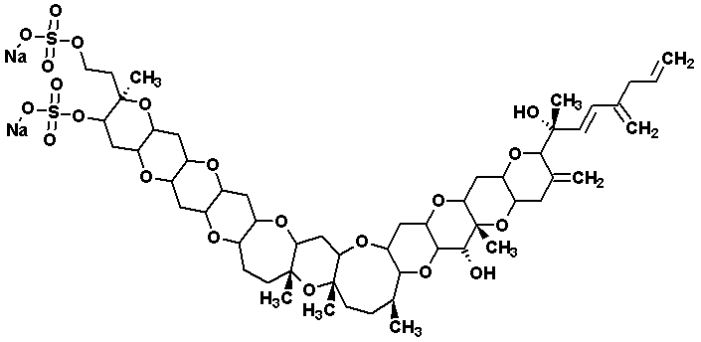


Figure 7. Yessotoxins chemical structure.

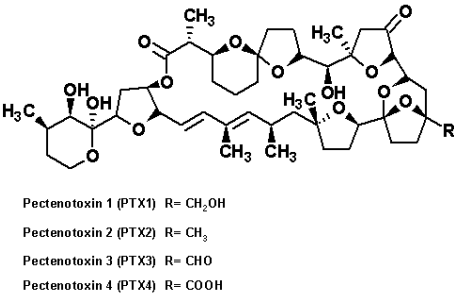


Figure 8. Chemical structure of pectenotoxins.

3.1.3. *Pectenotoxin*

Pectenotoxins (PTXs) are a group of cyclic polyether macrolide sharing the same basic structure. Actually, eight different PTXs (PTX1 to 7 and PTX10) and two new derivatives of PTX2 (PTX2 seco-acid and 7-epi-PTX2 seco-acid) have been described and characterized mainly in shellfish. PTX2 is suspected to be the precursor toxin of the whole PTXs through biotransformation processes which take place in the digestive glands of bivalves

(Lee et al., 1989; Yasumoto et al., 1989; Draisci et al., 1996; Suzuki et al., 1998; Suzuki et al., 2001) (Fig. 8).

3.2. MECHANISM OF ACTION

3.2.1. *Okadaic acid*

Okadaic acid is an inhibitor of protein phosphatases (PP), which induce dephosphorylation of proteins by protein kinases (PK). The accumulation of phosphorilated proteins lead to tumor promotion and contraction of smooth muscles. This last effect is responsible for diarrhea and abdominal pain which are among principal symptoms (Puiseaux-Dao et al., 2001).

It has been observed that only some PP are inhibitied, namely serine/ threonin PPs 1, 2A, 4, 5 and 6, while it seems that the different conformation of PP 2B and 7 makes them particularly resistant: the binding to these PP is probably partially obstructed by the catalytic part of the.

For the non-competitive interaction of OA with PP chemical structure maintenance is mandatory, as loss of carboxyl group, esterification or reduction to okadaol make the toxin no more toxic enzyme (Bialojan and Takai, 1988; Walter and Mumby, 1993; Honkanen et al., 1994; Takai et al., 1995; Dawson and Holmes, 1999).

Binding to catalytic unit is reversible, but break of the bound is very slow.

Hyperphosphorilation induced by okadaic acid occurs in any kind of cell and targets not only serine and threonin, but also tyrosine. Proteins affected are those of cytoskeleton, those involved in signal transduction, transcription and in gene expression (Afshari, 1994; Sawa et al., 1999; Puiseaux-Dao et al., 2001).

Okadaic acid can also alter cell morphology, induce apoptosis and cell death and modify cell physiology: alteration of ions current across membrane, of glucose balance, of resorption of glucocorticoids receptors, increase in T3 secretion (Shibata et al., 1982; Hescheler et al., 1988; Haystead et al., 1989; Ozaki and Haraki, 1989; Mironov and Lux, 1991; Chiavaroli et al., 1992; Neumann et al., 1993; Wang et al., 1993; Arufe et al., 1999; Galigniana et al., 1999).

Diarrhea, one of the main symptoms of DSP, is due to hyperphosphorilation of intestinal epithelia, with the loss of intestinal structure and of *villi*; this expose superior part of intestinal crypt cells and produce an important loss of water (Edebo et al., 1988; Lange et al., 1990; Yuasa et al., 1994; Tripuraneni et al., 1997; Puiseaux-Dao et al., 2001).

3.2.2. *Yessotoxins*

Mechanism of action of these toxins is not known. Toxicological studies have shown that YTXs do not induce sodium channel activation, while and increase in intracellular calcium following intra-cytoplasmatic reserves depletion and extra-cellular calcium use. This effect, coupled with cardiac lesions linked to degeneration of capillary endothelial cells, mitochondria and swelling of cardiac cells, make these toxins more similar to maitotoxins than to domoic acid (Aune, 1989; Alfonso et al., 2000; Puiseaux-Dao et al., 2001).

3.2.3. *Pectenotoxin*

PTXs do not inhibit protein phosphatases nor induce diarrhea in mammals. Most toxicological data available on PTXs (both *in vivo* and *in vitro*) have been obtained with PTX1, showing liver damage following intraperitoneal injection in mice and morphological changes in freshly prepared hepatocytes. Highest lethality for PTX2 with respect to all other PTXs further supports the hypothesis of PTX2 as the parental compound of PTX group. Thus, successive oxidation of substituent in C18 in the digestive glands of bivalves would diminish the toxicity of PTXs. PTX2 has been proven to induce lethality of brine shrimp (*Artemia salina*), as well as cytotoxic activity against several human cell lines, although significant differences were observed in the relative LC_{50} values obtained for each of them (Terao et al., 1986; Sasaki et al., 1998; Suzuki et al., 1998; Hori et al., 1999; Eaglesham et al., 2000).

Apoptosis induction by PTXs has also been proved. Primary cultures of rat and salmon hepatocytes exposed to PTX1 in the micromolar range showed rapid apoptotic changes, but no further studies concerning apoptotic activity of PTXs have been carried out in human cells. No additional data are available on acute and chronic effects of PTXs, and the exact mechanism of action of these toxins is currently unknown.

3.3. SYMPTOMS AND TREATMENT IN HUMANS

3.3.1. *Okadaic acid*

Symptoms of DSP appear within 4 hours after ingestion of contaminated mussels and include vomiting, diarrhea, abdominal pain. More rarely neurological appears.

Recovery is complete within 3 days and there seems to be no long term effect and no deadly episodes (Marcaillou-Le Baut et al., 2001).

3.3.2. *Yessotoxins*

Yessotoxins have not been associated with human poisoning, but only in animals (Marcaillou-Le Baut et al., 2001).

3.3.3. *Pectenotoxins*

Pectenotoxins poisoning have been reported in human since 1997. Symptoms registered are nausea, vomiting and diarrhea. Treatment of toxicosis is similar to that of DSP (Marcaillou-Le Baut et al., 2001).

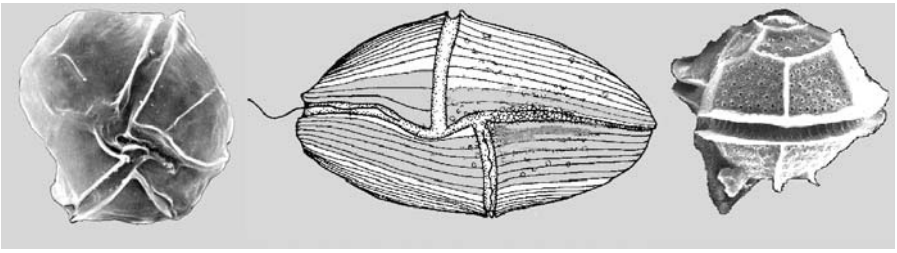
4. Paralytic Shellfish Poisoning (PSP)

This syndrome is caused by **saxitoxins** (STX), a group of toxins including about 20 different molecules.

STX was one of the first marine toxins recognized as responsible for human intoxications, the first report dating up to 1798, even if PSP symptoms were attributed to saxitoxins only after 1920.

Saxitoxins are responsible for about 2000 human cases/year, with a mortality rate ranging from 15 to 50% (van Dolah, 2000; Marcaillou-Le Baut et al., 2001).

The name of the toxin comes from the mollusk in which it was firstly identified, *Saxidomus giganteus*. It is produced by both temperate and tropical dinoflagellates of the genera *Alexandrium*, *Gymnodium* and *Pyrodinium* (Fig. 9).



A B C

Figure 9. Saxitoxins producing organisms A. *Alexandrium* sp., B. *Gymnodium* sp C. *Pyrodinium sp*.

This is one of the few toxins which are produced by both marine and fresh water (cyanobacteria) organisms, even if no report of intoxication exists for fresh water sources.

The major transvector for the toxins are bivalve mollusks, even if also crabs and snails feeding on coral reef seaweeds seem to be able to accumulate them (Fig. 4).

4.1. CHEMICAL STRUCTURE

Saxitoxins are tricyclic, substituted alkali, hydro-soluble, thermo-stable and resistant to acidic environment (Fig. 10). Alkaline pH or oxidizing compounds can inactivate the toxins, which are all derivatives of two basic molecules, saxitoxin and neosaxitoxin, which undergo sulphatation at different sites of their molecules; the chemical characteristics of these molecules are resumed in Table 1 (Amzil et al., 2001).

At physiological pH, to functional groups, the 1,2,3- and the 7,8,9-guanidinic group, present a positive charge, which give the molecules their water solubility characteristics.

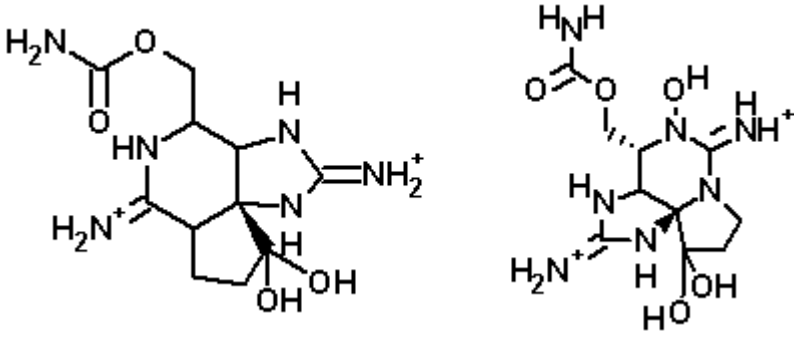


Figure 10. Saxitoxins and neosaxitoxins molecular structure.

Saxitoxins are grouped in 3 classes, based on their toxicity:

- Carbamates derivatives: saxitoxin, neosaxitoxin, GTX1-GTX4;
- N-sulphocarbamoyl derivatives: B1, B2, C1-C4;
- Decarbamoyl derivatives (dc-derivatives)

Decarbamoyl derivatives have an intermediate toxicity between carbamate (highly toxic) and N-sulphocarbamoyl compounds.

TABLE 1. Structure of saxitoxins (from Amzil et al. (2001), modified).

R1	R2	R3	R4	Carbamates toxins	N-sulphocarbamoyl toxins	Decarbamoyl toxins
H	H	H		STX	B1	dcSTX
H	H	OSO3		GTX2	C1	dcGTX2
H	OSO_3	H		GTX3	C2	dcGTX3
OH	H	H		NEO	B2(GTX6)	dcNEO
OH	H	OSO3		GTX1	C3	dcGTX1
OH	OSO_3	H		GTX4	C4	dcGTX4

4.2. MECHANISM OF ACTION

Being polar molecules, STX can not cross blood-brain barrier and thus they affect peripheral nervous system by targeting voltage-dependent sodium channels, which regulate action potential propagation along neurons (Fig. 11). Indeed, these trans-membrane proteins, responsive to membrane potential, control ions movement across the membrane of nervous cells (Hines et al., 1993; Gessner et al., 1997; Andrinolo et al., 1999).

Sodium channel is composed by 3 sub-unit, α, β-1 and β-2, α sub-unit presenting most of the functional properties.

Sub-unit α is composed by 4 repeated domains, numbered I to IV, eah containing 6 membrane-spanning regions, labeled S1 to S6. S4 is a highly conserved region acting as voltage sensor. When transmembrane voltage stimulates S4, it moves towards the extracellular side of the membrane, opening the channel. Another important part of the channel is the amino acidic sequence connecting domains III and IV, which is responsible for channel inactivation (closure) after prolonged activation (West et al., 1992; Catterall, 2000; Goldin et al., 2000; Yu and Catterall, 2004).

Saxitoxins binds to site 1 of the channel, an amino acid sequence negatively charged placed in the external part of the membrane connecting S5 and S6. Specific amino acids have been identified as responsible of the binding of the toxin to the channel. This binding cause a complete block of the sodium channel preventing the ions from passing into the neurons. The block of inward flow of sodium impedes the release of neurotransmitters at the synaptic level, and this causes paralysis of muscle cells (Evans, 1972; Catterall et al., 1986; Kao, 1986; Puiseaux-Dao et al., 2001).

A first hypothesis considered the toxin acting like a stopper on the pore. More recent studies anyway have clarified that there in no direct action on the pore; the toxin binds to an external site to the channel, close but not

inside the pore (Fig. 12). The interaction of only part of the toxin molecule and/or an alteration of the channel structure induced by the toxin are considered more probable (Puiseaux-Dao et al., 2001).

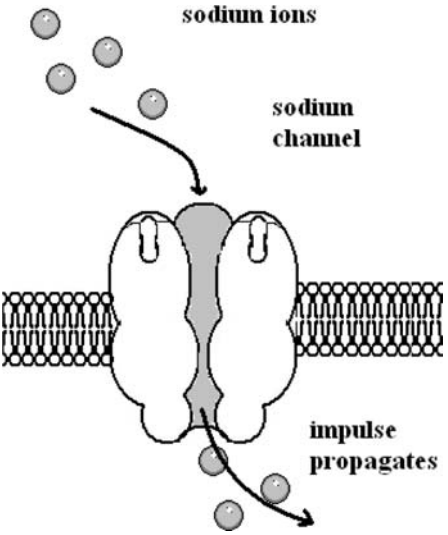


Figure 11. Sodium channel functioning.

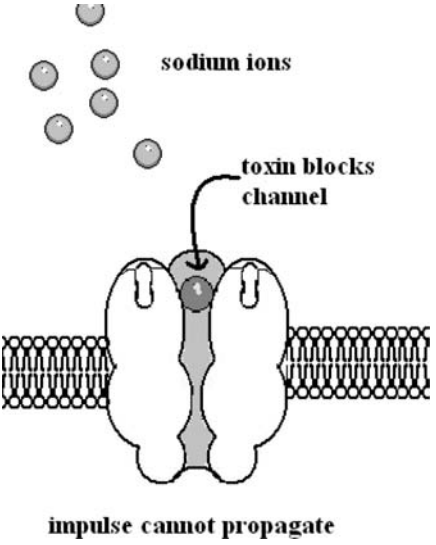


Figure 12. Saxitoxins mechanism of action of sodium channel.

4.3. SYMPTOMS AND TREATMENT IN HUMANS

PSP, s the oldest known intoxication and one of the most dangerous for humans, with a high rate of mortality.

Native populations of Canada perfectly knew the existence of the toxin and prohibited consumption of mussels coming from contaminated areas, which were considered as a food taboo (Marcaillou-Le Baut et al., 2001).

It is a worldwide distributed poisoning, with cases reported for North and South America, Europe, Africa and Asia. It is commonly thought that it is indeed more probable that the toxin or the dinoflagellates have not been detected or searched for.

Symptoms observed during PSP poisoning are characteristic, easy to recognized and impossible to be confused with allergy and viral or bacterial pathologies.

First symptoms appear 5 to 30 minutes after ingestion of mussels and develop following a precise sequence in few hours. The severity of signs depends on the dose ingested and on individual sensitivity.

Usually, recovery is complete in few days, even if in more severe intoxication death can occur following respiratory paralysis. Symptoms have been classified following the severity of intoxication, and are resumed in Table 2.

Even if it is a well known toxin, no antidote has been found for its treatment.

Most efficient treatment is a symptomatic one, including gastric lavage and active charcoal or alkaline dinks administration, which favor the inactivation of the toxins and their elimination with urine. Indeed, their clearance via kidney is rapid, close to 24 hours (Hines et al., 1993; Gessner et al., 1997; Andrinolo et al., 1999).

Forced ventilation is useful in more severe intoxications, when respiratory paralysis occurs, as it can counteract paralysis.

4.4. TOXICOSES IN ANIMALS

Saxitoxins have been implicated in a mass mortality episode of humpback whales which occurred during late 1987-beginning of 1988 in Massachusetts. During an *A. tamarense* bloom, whales were forced to feed on mackerels, as their natural preys, sand lance, was largely absent from the affected area. Mackerels fed on *A. tamarense* and were found to contain a mean concentration of 80 μg/100 g tissue, which were deadly toxic for whales. Indeed, short after feeding on fish, humpback whales were found

dead, without any sign of emaciation (blubber was abundant) or starvation (stomachs contained digested fish). Estimated dos absorbed by whales was 3.2 μg/kg b.w., well below toxic threshold defined for humans. Geraci et al. (1989) consider two possible mechanisms as responsible for apparent higher sensitivity of cetaceans to saxitoxins: 1) approximately 30% of the whales body weight is blubber, into which the water-soluble STXs would not partition, thus being more highly concentrated in metabolically sensitive tissues; 2) the diving physiology of whales concentrates blood to the heart and brain and away from those organs required for detoxification, further concentrating neurotoxins in sensitive tissues (Ridgway and McCormick, 1971; Geraci et al., 1989; Haya et al., 1989; Levin, 1992; Haulena and Heath, 2001; Van Dolah et al., 2003).

TABLE 2. PSP signs classification.

Severity of intoxication	Symptoms
Mild	Mouth paresthesia which can expand to the whole face and neck, to fingers and ears. Nausea, headache, vomiting
Severe	Paresthesia expand to arms and legs. General sensation of numbness, muscular weakness and floating sensation. Altered speech, dysarthria, severe ataxia, motory incoordination. Some respiratory problem appears.
Extreme	Peripheric paralysis, including respiratory paralysis which can lead to death if not rapidly trated.

Another important episode, which had a great importance also from the conservation point of view, is the one affecting a monk seal (*Monachus monachus*) population in Mauritania during 1997. Close to 70% of the total population of monk seals died following an *A. minutum*, *G. catenatum* and *D. acuta* bloom. Affected seals showed lethargy, motor incoordination, paralysis, symptoms who could be ascribed to STX intoxication. Lungs of dead animals showed severe respiratory distress and congestion, and viscera of the animals contained up to 12 μg/100 g liver and 3 μg/100 g brain of decarbamoyl saxitoxins. Again, observed levels were below the toxic threshold for humans, but a higher sensitivity should be considered also in this species (Osterhaus et al., 1997; Hernandez et al., 1998; Osterhaus et al., 1998; Forcada et al., 1999).

Long term effect of STX were also considered, as toxin presence con alter the distribution of predator species, as hypothesized for sea otters (*Enhydra lutris*).

Alaskan populations of sea otters, consuming 20–30% of their body weight as bivalves, avoid eating butter clams during *Alexandrium* blooms. Butter clams can highly accumulate STX in the siphon and can retain it for more than 1 year, probably as a defensive system against predation.

Sea otters seems capable of distinguish between toxic and non-toxic clams and areas, and feed only on healthy mussels in safe areas. This hypothesis was confirmed by a feeding study on caged sea otters. If animals were fed with contaminated clams, they reduced the rate of consumption and selectively discarded most of toxic tissues, i.e. siphons and kidney, thus reducing the risk of intoxication (Kvitek and Beitler, 1991; Kvitek et al., (1991).

Seabirds were also affected by saxitoxins: Nisbet (1983) reported of a massive die-off of common terns in Massachusetts which occurred in 1978. Transfer species was found to be sand-launce, the terns' principal food. Interestingly, it was observed that almost all tens that died were pre-laying females, while all other animals recovered after vomiting. No breeding alteration was observed, while an apparent age-dependent sensitivity was observed, as highest mortality was for 3 years-old females.

5. Neurotoxic Shellfish Poisoning

Neurotoxic Shellfish Poisoning is a little common intoxication, which has not been documented as a fatal intoxication in humans, and results from consumption of molluscan shellfish contaminated with **brevetoxins**. Interestingly, intoxications have been observed not only as a consequence of mussels ingestion, but also after inhalation of contaminated aerosol. Its toxicological importance is more related to massive fish death.

The producing organism is *Gymnodium breve*, which differs from other dinoflagellates because it is an unarmored dinoflagellates; the lack of an external shell make this microalga easily lysed in turbulent waters (Fig. 13). The lysis allows the toxin to be released IN water, making aerosol and droplets potentially toxics (Amzil et al., 2001).

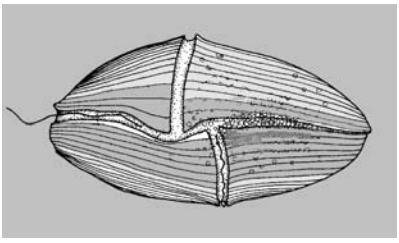


Figure 13. Gymnodium breve.

5.1. CHEMICAL STRUCTURE

Brevetoxins (PbTX) are liposoluble, ladder-like polycyclic ether toxins, classified into two types based on their backbone structure: PbTXB and PbTXA, counting up to 12 different molecules (McFarren et al., 1965; Lin et al., 1981) (Fig. 14).

The two families are composed by molecules counting 10 or 11 cycles. Some of these compounds are thought to be produced in mussels by metabolic processes (Table 3).

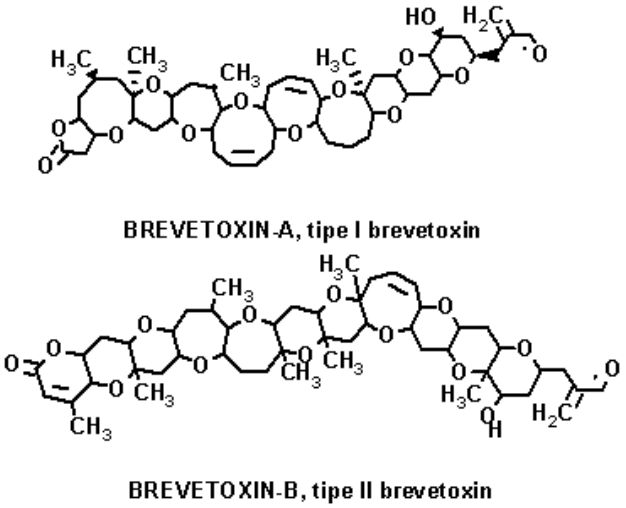


Figure 14. Molecular structure of PbTXA and B.

TABLE 3. Different molecular structure of type A and type B brevetoxins derivative.

Type A	R	Type B	R
Brevetoxins-1	$CH_2C(=CH_2)CHO$	Brevetoxins-2	$CH_2C(=CH_2)CHO$
Brevetoxins-7	$CH_2C(=CH_2)CH_2OH$	Brevetoxins-3	$CH_2C(=CH_2)CH_2OH$
Brevetoxins-10	$CH_2C(CH_3)CH_2OH$	Brevetoxins-9	$CH_2C(CH_3)CH_2OH$
		Brevetoxin-8	CH_2COCH_2Cl

5.2. MECHANISM OF ACTION

PbTX acts by binding with high affinity to voltage-dependent sodium channel. Brevetoxins bind to site 5 of the channel (for sodium channel description see PSP paragraph), altering bio-physic properties of the channel itself (Bidard et al., 1984; Poli et al., 1986; Lombet et al., 1987; Baden, 1989; Dechraoui et al., 1999; Puiseaux-Dao et al., 2001).

They induce and increase in membrane sodium permeability and a shift of activation potential toward more negative values.

It has been supposed that this action results from the intercalation of brevetoxins backbone (whose length is similar to that of membrane lipidic double layer) between transmembrane domains of nervous and muscular cells, provoking spontaneous and/or repeated discharges which appears at very low action potential levels. Discharges are generally followed by a block of excitation, due to important and prolonged membrane depolarization (Huang et al., 1984; Atchison et al., 1986; Sheridan and Adler, 1989; Gawley et al., 1995; Jeglitsch et al., 1998).

Hyperexcitation lead to an initial increase in neurotransmitters secretion, followed by a decrease and an inhibition due to overstimulation, Ca^{2+} being not essential for the release: it has been observed that Na^{+} alone can activate neurotransmitters liberation (Molgo et al., 1990; Molgo et al., 1991; Molgo et al., 1993; Meunier et al., 1997).

Finally, an increase in cell volume was observed. Two different mechanism have been considered:

6. In mielinic nervous fibers sodium ions entrance alters osmotic balance, causing water entrance into the cell and swallow of cell itself. A 50% hyperosmotic external solution can counteract this increase in volume.
7. In motor neurons terminal synaptic vesicles fuse with terminal axon body, allowing vesicular membrane incorporation after neurotransmitter release induced by the toxin. Hyperosmotic solutions only partially counteract this effect (Puiseaux-Dao et al., 2001).

5.3. SYMPTOMS AND TREATMENT IN HUMANS

Two different poisoning have been identified in humans: “indirect” intoxication by ingestion of contaminated mussels and poisoning for direct contact.

In the first one symptoms are both neurological and gastro-intestinal and appear within 1–3 hours after mussels ingestion.

Neurological syndrome includes paresthesia of area around the mouth, the face and throat, muscular ache, ataxia, inversion of thermal perception, bradycardia, midriasis.

Gastro-intestinal syndrome includes abdominal pain, nausea, diarrhea.

Recovery is complete within 24 and 48 hours and no fatality has ever been recorded.

It has been observed that local anaesthetics, calcium administration and hyperosmotic solutions are useful in poisoning treatment, as they counteract brevetoxins action on membrane.

Following direct contact with contaminated aerosol, respiratory tract inflammation (cough, burning sensation) and conjunctivitis appear (Pierce, 1986; Morris, 1991; Marcaillou-Le Baut et al., 2001).

5.4. TOXICOSES IN ANIMALS

Brevetoxins were considered as responsible agent in many poisoning of cetaceans, like manatees and bottlenose dolphin but also of sea turtles (Landsberg and Steidinger, 1998; Van Dolah et al., 2003).

Die-off on manatees have been linked to NSP since 1965 in Florida, but various episodes have occurred in following years (1982 and 1996) in the same area. Timing of mortality events coincided with the presence of *K brevis* blooms and was often associated with fish and seabirds die-off.

Affected animals showed disorientation, inability to submerge or to maintain horizontal position, listlessness, flexing of the back, lip flaring and labored breathing. The only histological lesions observed were cerebral ones, while no other lesion was observed. The analysis of stomachs content showed a high amount of seagrasses and filter feeding tunicates; no measurable PbTX levels were found in tunicates (Layne, 1965; O'Shea et al., 1991).

In 1996 histopathological analysis of tissues showed consistent, severe congestion of nasopharyngeal tissues, bronchi, lungs, kidney and brain; hemorrhage of lungs, liver, kidney and brain were observed, whereas gastrointestinal tract showed no lesions. The presence of PbTX in lymphocytes and macrophages of affected tissues support the hypothesis that toxic effects in manatees is not due to acute neurotoxic effects alone, but rather may have resulted from chronic inhalation (Bossart et al., 1998).

First report of dolphin die-off due to NSP dates up to 1947, in Florida. Brevetoxins were also proposed as a causative agent in an unprecedented mortality of over 740 bottlenose dolphins in 1987 in New Jersey. Most

of stranded dolphins showed a wide range of pathological signs, involving chronic physiological stress, including fibrosis of the liver and lung, adhesion of abdominal and thoracic viscera, and secondary fungal and microbial infections associated with immune suppression (Gunter et al., 1948; Geraci, 1989; Tester et al., 1991; Mase et al., 2000).

Another bottlenose dolphin poisoning occurred in 2000, involving 120 animals, in Florida, coinciding with episodic peak of *K. brevis*. Stranded animals were in good physical condition, but histopathological examination showed significant upper respiratory tract lesions, with lymphoplasmacytic oropharyngitis and tracheitis, as well as lymphoplasmacytic interstitial pneumonia and lymphoid tissue depletion. PbTX was not found in spleen or lung, differently from what observed in manatees, whereas it was found in stomach content and liver. In these two organs PbTX3 was found; considering that PbTX2 is main product of K. brevis, it is thought that it is metabolized in the fish or that this metabolite is selectively retained by dolphins.

At present no lethal dose for dolphins and marine mammals has been determined, nor an acute or chronic adverse effect level (Van Dolah et al., 2003).

6. Amnesic Shellfish Poisoning (ASP)

Amnesic Shellfish Poisoning is a singular intoxication, as it is the only one not caused by dinoflagellates, but by a diatom, namely *Pseudo-nitzschia pungens*, which produce **domoic acid**. It is one of the deadly poisoning, even if fatalities are more rare than with STX and tetrodotoxin (Fig. 15).

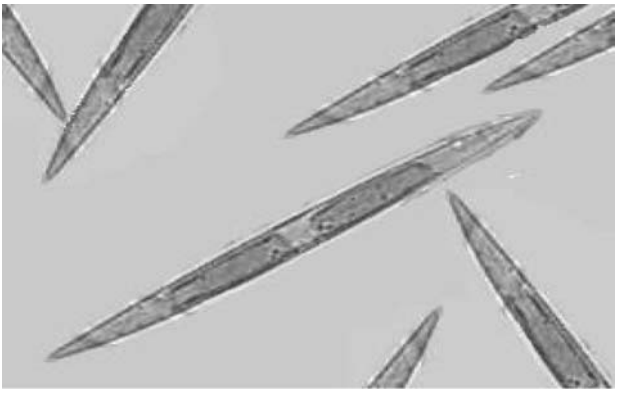


Figure 15. Domoic acid producing *Pseudo-nitzschia pungens*.

6.1. CHEMICAL STRUCTURE

Domoic acid (DA) is a tricarboxylic amino acid which was firstly isolated by red alga *Chondria armata domoi*. Side chain of the molecule contains two ethylenic bounds (Takemoto and Daigo, 1958; Amzil et al., 2001) (Fig. 16).

Domoic acid is thermo-stable, water soluble and instable in acidic environment; it has been shown that temperatures higher than 50°C (cooking temperatures) can transform domoic acid in epidomoic acid, which is the real responsible, together with isodomoic acid D, E and F, of ASP (Fig. 16). Anyway, several congeners of DA have been identified (Amzil et al., 2001).

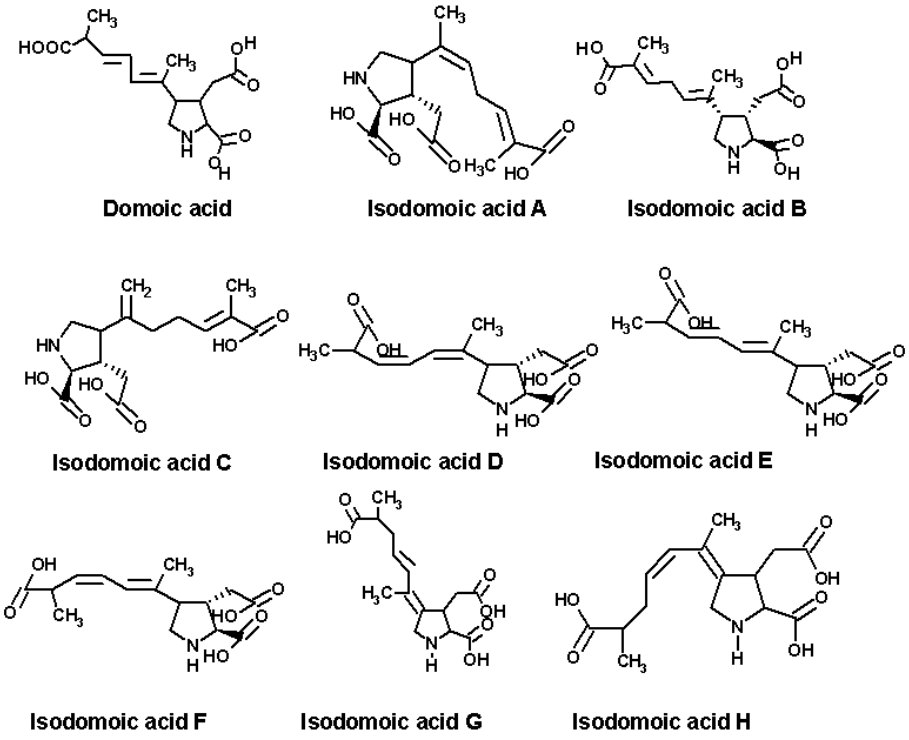


Figure 16. Chemical structure of domoic acid and its derivatives.

Chemical structure of domoic acid is similar of that of the endogenous neurotransmitter glutamate and of the excitatory neurotoxin kainic acid; this similarity is responsible for the mechanism of action of the toxin.

6.2. MECHANISM OF ACTION

Domoic acid acts at the central nervous system level. It is absorbed by gastrointestinal tract and slowly reaches CNS. As already said, it shares chemical similarity with glutamate and kainic acid, and its mechanism of action is based on binding to kainate and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) subtypes of glutamate receptors, while it does not interact with N-methyl-D-aspartate (NMDA) receptors (Debonnel et al., 1989).

Interestingly, the binding affinity of domoic acid to kainate receptor is higher than that of endogenous agonists (domoic acid>glutamate>AMPA).

Main difference among domoic and kainate acid and glutamate is the fact that while the action of glutamate rapidly disappears, avoiding over-stimulation of nervous cells, desensitation of receptors following exogenous agonist binding is negligible or absent, and when it occurs it is very slowly, that causing a continuous stimulation of the nervous cells (Puiseaux-Dao et al., 2001).

The interaction with the receptor lead to the opening of voltage-dependent calcium channels, allowing entrance of Ca^{2+} in the cell, as well as of other ions, i.e. Na^{+}.

The toxic effect resulting from this influx is at first an increase in nervous cells excitation. This effect is due to the disinhibition of some neural circuits, as domoic acid can inhibit GABA (inhibiting mediator) liberation in hippocampal areas through the activation of protein kinase (PK) C. Subsequently, high levels of calcium cause cell death and lesion in various cerebral areas, especially in areas where glutaminergic pathways are heavily concentrated. These receptors are preferentially distributed in CA1 and CA3 areas of hippocampus, which are responsible for learning and memory. These lesions can be responsible of main effect of domoic acid, complete and permanent loss of short-term memory. Anyway, it has been observed tat memory deficits occur at levels well below those causing structural damage (Cunha et al., 2000; Quintela et al., 2000; Puiseaux-Dao et al., 2001).

In vivo and in vitro studies have shown that DA activates AMPA/kainate receptors in striatum system, which cause release of excitatory amino acids activating NMDA receptors, finally leading to cell death (Larm et al., 1997; Puiseaux-Dao et al., 2001).

6.3. SYMPTOMS AND TREATMENT IN HUMANS

Diatoms were recognized as causative agents of ASP only in recent times, as they were not considered as an hazard for human health.

In 1987, over 100 people showed poisoning sings which could not be ascribed to any of known syndromes. Following researches made it possible to identify DA as active principle and *P. pungens* as producing organism. Interestingly, both two were already known, as domoic acid was used in Japan as vermifuge and the diatom was known, but no correlation among the two was ever seen before.

Poisoning symptoms appears rapidly, from 15 minute to 38 hours from mussels ingestion. After close to two days, some neurological alteration appears, presenting a different degree of severity.

Symptoms include gastrointestinal signs, e.g. nausea (77% of cases), vomiting (76%), diarrhea (42%), abdominal pain (51%), and neurological signs: dizziness, disorientation, lethargy, seizures, permanent loss of short-term memory.

Recovery occurs in a period ranging from one day to 4 months.

In 1987 outbreak, 4 out of the 100 people affected died after seizures appeared.

The analysis of brain of dead people revealed necrotic lesions and/or neuronal loss mainly at the hippocampal and amigdala areas, confirming the toxic effect of DA.

At present, no antidote exist for the treatment of poisoning, and all cares are symptomatic (Marcaillou-Le Baut et al., 2001).

6.4. TOXICOSES IN ANIMALS

Domoic acid has been identified as causative agent in pelicans and cormorants mass mortality in California in 1991 and in various and extensive die-offs of sea lions in the same region in 1998, 2000, 2006 and 2007.

Affected birds exhibited neurological symptoms similar to those reported in experimental animals, i.e. scratching and head weaving. In all instances the vector for toxins transfer was anchovy, but the toxin producing organism was a different member of *Pseuda-nitzschia* genus. At present, more than seven species are recognized as domoic acid producers (Work et al., 1993).

The first confirmed domoic acid poisoning in marine mammals occurred in sea lions in California in 1998. All animals were in good nutritional condition and displayed clinical symptoms, predominantly neurological: head weaving, scratching, tremors and convulsions. Affected animals were mainly adult females, 50% of them pregnant. Abortion was observed and

some pups born during the episode died. Highest levels of DA was found in urine and feces. Sea lions which died within 24 hours of stranding presented histologic lesions of brain, mainly neuronal necrosis, more severe in hippocampus and dentate gyrus. Heart was also affected, presenting myofiber necrosis and edema. Another similar episode occurred in 2000. In the same area and the same periods a die-off of sea otters was observed as well (Lefebvre et al., 1999; Gulland, 2000; Scholin et al., 2000; Bargu et al., 2002; Silvagni et al., 2005; California Wildlife Center, 2006).

Exposure to domoic acid has been proved also for whales, even if no clear toxic event was reported in these species. Lefebvre et al., 2002) found domoic acid in faeces and food (krill, anchovies and sardines) of whales.

7. Ciguatera Fish Poisoning (CFP)

Ciguatera Fish Poisoning is a well known poisoning linked to fish consumption, which was firstly described in 1555 by sailors in Caribbean areas. In 1866 Poey defined this intoxication "ciguatera" from the Cuban common name of a mussel known to cause the intoxication, "cigua" (Marcaillou-Le Baut et al., 2001; Puiseaux-Dao et al., 2001).

At present, the term ciguatera is used to describe both the poisoning and the phenomena affecting marine environment and leading to the poisoning itself, namely coral reef degradation.

Producing organism was found to be *Gambierdiscus toxicus* which produce **ciguatoxins** (CTX) (Fig. 17). Other toxins are included in the group of ciguatoxins, namely **maitotoxins** (MTX), which anyway have never been linked to toxic episodes in humans. Like ciguatoxins, maitotoxins are produced by *G. toxicus*.

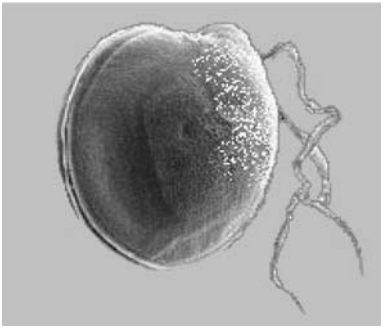


Figure 17. Gambierdiscus toxicus.

7.1. CHEMICAL STRUCTURE

7.1.1. *Ciguatoxins*

Ciguatoxins are highly stable toxins, as cooking, freezing and salting do not reduce their toxicity. They are soluble in organic polar solvents and in water (Fig. 18).

The study of molecular structure of the toxins lead to the identification of two different toxins, one from the Pacific area (P-CTX1) and one from the Caribbean (C-CTX1), which differ for the number of C in the molecules (60 in P-CTX1 and 62 in C-CTX1) and stability in acidic environment: P-CTX1 is acid labile, while C-CTX1 is stable in acidic pH (Vernoux, 1988) (Table 4).

Both two groups of toxins belong to polycyclic polyethers.

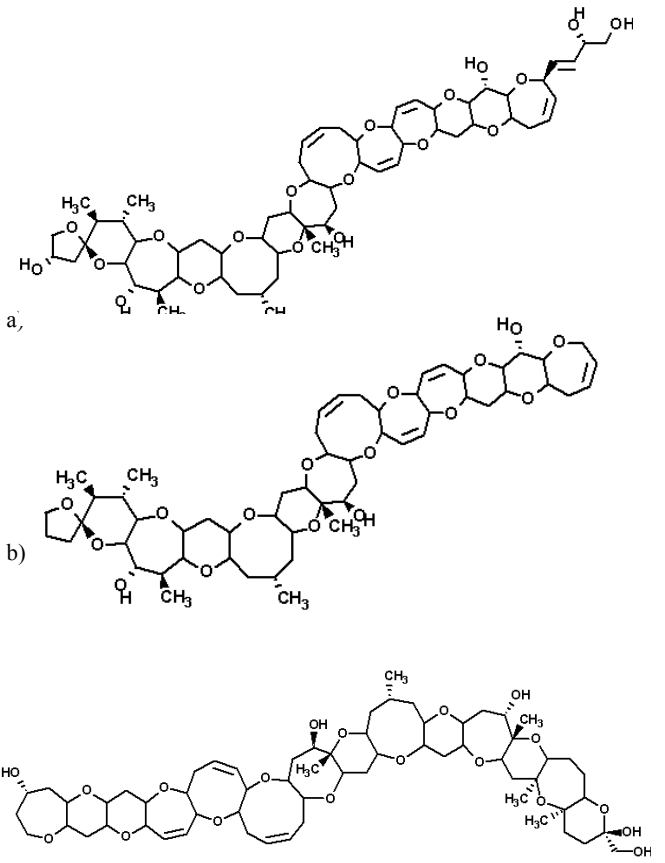


Figure 18. Molecular structure of CTXs: a) P-CTX; b) P-CTX3; c) C-CTX.

TABLE 4. Comparison of some characteristics of CTXs.

	C-CTX	PCTX
Vector fish	*Caranx latus*	*Gymnothorax javanicus*
Molecular formula	C62H92O19	C60H86O19
LD50	3,6 µg/kg	0,35 µg/kg
Number of cyclic ethers	14	13
Terminal cycle	C56 hemyacetal	C52 spiroacetal

These polyethers are chemically little reactive, and it has been found that side chains not involved in cycles formation are the reactive part of the molecules. A reduction in toxicity has been observed following chemical binding of hydroxyl function with various compounds or the formation of double bonds (Yasumoto and Oshima, 1979).

Among Pacific toxins, 9 different structure have been found, while 2 only have been identified for Caribbean group.

These 11 toxins are grouped in 3 types, and it has been found that some of them are conformation epimers at the spyro-acetalic or hemiacetalic asymmetric carbon (Table 5).

TABLE 5. Known chemical structure of CTXs divided by type.

Type	Toxins with known structure	
I	P-CTX1	
	P-CTX2 and P-CTX3	C52 epimers
	P-CTX4A and P-CTX4B	C52 epimers
II	P-CTX3c and P-CTX3B	C49 epimers
	P-CTX2A1	
	51 hydroxy-CTX3C	
III	C-CTX1 and C-CTX2	C56 epimers

One probable explanation for this huge variability in structural characteristics is metabolism: the accumulation along one or more steps of food chains of “basic” toxins make them experience some biotransformation which oxidize the molecule and make it more polar. Although metabolism is conceived to reduce toxicity, it has been seen that oxidized ciguatoxins are more toxic than parent compounds (Puiseaux-Dao et al., 2001).

7.1.2. *Maitotoxins*

Maitotoxins are another group of toxins produced by *G. toxicus* which have never been associated to CFP, as they have never been isolated in flesh

of fish, but only in viscera of herbivorous fish (Yasumoto et al., 1976; Yasumoto et al., 1984).

They are highly hygroscopic polycyclic polyethers, characterized by the absence of 2 sulphated groups (Fig. 19).

Despite molecular similarity with CTXs, their effects are different and no spontaneous or chemical transformation produced CTXs starting from MTXs (Puiseaux-Dao et al., 2001).

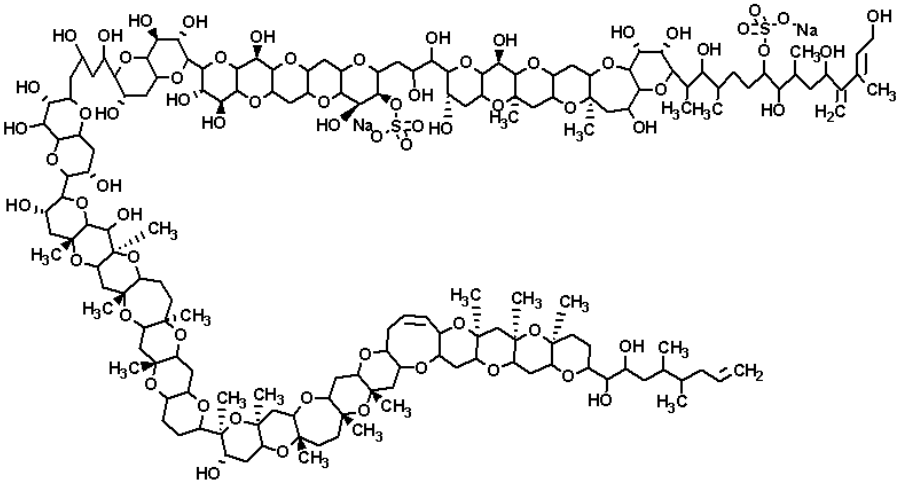


Figure 19. Chemical structure of maitotoxins.

7.2. MECHANISM OF ACTION

7.2.1. *Ciguatoxins*

Ciguatoxins share their mechanism of action with brevetoxins, because they bind to site 5 of voltage-dependent sodium channels. They activate the sodium channel, alter the selectivity of the channel for Na^+, delay or block channel inactivation and activate the channel at more negative membrane potential. This last action persistently increases membrane permeability to sodium.

All the aspects of mechanism of action have already been described in brevetoxins section.

7.2.2. *Maitotoxins*

Maitotoxins can not cross cell membrane and act by hydrolyzing membrane phosphoinositides and phospholipids, inducing calcium influx and the consequent increase in Ca^{2+} cytosolic concentration, and depolarizing all neural

and neuroendocrine membranes, causing an increase in neurotransmitters and hormones Ca-dependant liberation.

Phosphoinositides and phospholipids hydrolysis has proved to be dependant on extracellular calcium and is catalyzed by phospholipase C at high doses and by phospholipase A at low doses (Sladeczek et al., 1988; Gusovsky et al., 1989; Choi et al., 1990; Gusovsky and Daly, 1990; Lin et al., 1990; Meucci et al., 1992; Bressler et al., 1994).

7.3. SYMPTOMS AND TREATMENT IN HUMANS

7.3.1. *Ciguatoxins*

CFP is characterized by a sequence/association of neurological, gastrointestinal and cardio-vascular symptoms, presenting a high variability. Some occasional fatalities have been observed, following severe intoxications.

Although it can affect all ages, a certain, higher sensitivity seems to exist for the age range 30–49, interestingly, some occasional episode of toxin transfer through breast feeding was observed (Marcaillou-Le Baut et al., 2001).

In "classical" syndrome incubation lasts from 3 to 8 hours, even if in severe poisoning the delay time is 1 hour and in mild intoxication it can reach 12–20 hours (Bagnis, 1967; Bagnis et al., 1979).

The prodromic phase of ciguatera lasts about 2 hours and is characterized by gastrointestinal alterations involving face congestion, headache, salivation, and by neural symptoms: numbness of face, tongue and extremities (Pearce et al., 1983).

In the second phase, digestive symptoms are predominating, while neurological and, only in more severe cases, cardiac signs are only occasional (Bourdeau and Bagnis, 1989).

Gastroenteric signs are more intense than in starting phase, precocious and constant and include:

- Abdominal pain
- Vomiting
- Diarrhea.

Neurological signs are progressive and include:

- Tingling
- Burning or electric discharge sensation

- Thermal sensation inversion, which is an indicative, even if not specific, sign of the intoxication.
- Pruritus. This is another frequent and typical sign. It is a tardive sign, starting from extremities and diffusing to the all body, which is quite persistent and can also become permanent. A certain increase in the disturb has been observed at night, causing insomnia in affected people (Bourdeau and Bagnis, 1989).

Interestingly, pruritus can last for weeks after the poisoning has solved, and can be re-evoked by fish consumption (Bourdeau and Bagnis, 1989).

Some more occasional neurological signs can be recorded:

- Mydriasis or strabismus
- Paresis or ataxia of legs
- Articular and muscular pain.

These signs too can be re-induced even years after the poisoning by consumption of reef fish.

Finally, vertigos, teeth pain and cutaneous rashes have been recorded (Marcaillou-Le Baut et al., 2001).

Cardiovascular signs are recorded only in severe intoxication and include:

- Irregular heart beats
- Bradycardia
- Complete alteration of cardiac regulation.

As a general rule, gastroenteric and cardiovascular signs solve in a few days, while neurological signs last for weeks or more.

CFP has a very low mortality rate (0,1–4,5 %) and death occurs after respiratory failure or collapse (Marcaillou-Le Baut et al., 2001).

There seems to be a different trend in clinical symptoms between males and females, males showing more frequently gastro-intestinal signs and females muscular pain (Bagnis et al., 1979).

There is also a different profile in symptoms presentation between Pacific and Caribbean toxins, P-CTX being more neurological and C-CTX more gastroenteric.

A certain spontaneous detoxification has been observed in humans, but it is assumed to take long time, even if it has never been estimated. This can easily lead to accumulation phenomena in humans, and continuous consumption of little toxic fish can thus induce poisoning, similarly to consumption of one single, highly toxic fish (Lawrence et al., 1980; Chan, 1998).

Diagnostic of intoxication is based on clinical signs and on epidemiological data as well as on information obtained from the patient (recent fish consumption, species eaten, etc.) and on exclusion of other pathologies, i.e. bacterial and viral infections, other toxins, allergies (Marcaillou-Le Baut et al., 2001).

The treatment is symptomatic and includes:

Gastroenterical symptoms

– Spasmolitics
– Anti-diarrhoic drugs
– Anti emetics
– Gastric lavage

Neurological symptoms

– Calcium gluconate
– Vitamin B

Cardiovascular signs

– Atropine
– Cardiovascular analeptics

Pruritus

– Corticoids
– Anti histaminic drugs.

Mannitol has proved to have some efficacy against nervous signs in the treatment of poisoning, the most rapidly it is administered the best the effect. It probably acts by counteracting neuronal edema induced by Na entrance in neurons, followed by water influx in the cell (Palafox et al., 1998).

7.3.2. *Maitotoxins*

Maitotoxins poisoning is characterized mainly by neurological symptoms, including, in order of importance:

- Altered thermal perception
- Muscular pain
- Pruritus
- Urinary problems
- Articular pain
- Increased transpiration.

Gastrointestinal signs are quite rare (Marcaillou-Le Baut et al., 2001).

7.4. TOXICOSES IN ANIMALS

Evidence of the involvement on CTX in the morbidity and/or mortality of marine mammals remains speculative.

One interesting case is that concerning decline in population of monk seal in Hawaii. Population decline occurred has been primarily attributed to the poor survival rates among juveniles and pups and to slower growth rate of juvenile. It is yet unclear which are the reasons of this mortality rate, but it has been hypothized that ciguatera could play a role.

Indeed, preliminary survey of known prey fish species showed some positivity to the toxin; anyway no clear connection between CTX presence and population decline (Craig and Ragen, 1999; Van Dolah et al., 2003).

8. Tetrodotoxin

Consumption of fish contaminated by **tetrodotoxin** (TTX) or its derivatives is responsible for one of the most severe intoxication, as close to 60% of affected people die within 4 to 6 hours after ingestion.

Recent researches have provided strong evidence of the bacteriological origins of TTX*:*

- Puffer fish grown in culture do not produce tetrodotoxin until they are fed tissues from a toxin producing fish.
- The blue-ringed octopus found in Australian waters accumulates tetrodotoxin in a special salivary gland and infuses its prey with toxin by bite. This octopus contains tetrodotoxin-producing bacteria.
- Xanthid crabs collected from the same waters contain tetrodotoxin and paralytic shellfish toxin.

It is now clear that marine bacteria have long been in mutualistic symbiosis with marine animals and it is now known that the related toxins tetrodotoxin and anhydrotetrodotoxin are synthesized by several bacterial species, including strains of the family *Vibrionaceae*, *Pseudomonas sp*., and *Photobacterium phosphoreum*. Puffer fish took advantage of a single point mutation in their sodium channel receptors which rendered these fish immune from the effects of TTX. Following herbivores grazing, marine invertebrates and vertebrates accumulate these bacteria, provide them with a suitable host environment, and in return receive the protection of marine biotoxins, compliments of the prokaryotes (Johnson, 2002).

8.1. CHEMICAL STRUCTURE

TTX is a positively charged guanidinium group and a pyrimidine ring with additional fused ring systems, which contain hydroxyl groups which must certainly help stabilize the TTX-sodium channel binding complex at the aqueous interface (Johnson, 2002) (Fig. 20).

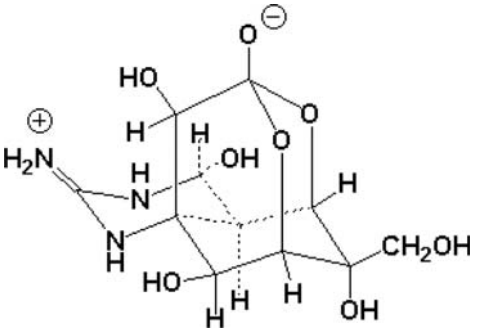


Figure 20. Chemical structure of TTX.

8.2. MECHANISM OF ACTION

TTX is an especially potent neurotoxin, specifically blocking voltage-gated sodium channels on the surface of nerve membranes. The TTX-Na Channel binding site is extremely tight (K_d = 10^{-10} nM). It is proposed that this binding results from the interaction of the positively charged guanidino group on the tetrodotoxin and negatively charged carboxylate groups on side chains in the mouth of the channel. TTX mimics the hydrated sodium cation, enters the mouth of the Na^+-channel peptide complex, binds to a peptide glutamate side group, among others, and then further tightens it

hold when the peptide changes conformation in the second half of the binding event. Following complex conformational changes, TTX is further electrostatically attached to the opening of the Na^+ gate channel (2d event occurs *in vivo* as the dehydration of the aqueo-sodium complex).

TTX's tenacious hold on the Na^+-channel complex is further demonstrated by the occupancy time of TTX v. hydrated-Na^+ at the complex. Hydrated sodium reversibly binds on a nanosecond time-scale, whereas TTX binds and remains on the order of tens of seconds. With the bulk of the TTX molecule denying sodium the opportunity to enter the channel, sodium movement is effectively shut down, and the action potential along the nerve membrane ceases. A single milligram or less of TTX is enough to kill an adult.

It has been proved that TTX does it not poison the host as the sodium ion channel in the host is different than that of the victim and is not susceptible to the toxin. Indeed it has been demonstrated for one of the puffer fish that the protein of the sodium ion channel has undergone a mutation that changes the amino acid sequence making the channel insensitive to tetrodotoxin. The spontaneous mutation that caused this structural change is beneficial to the puffer fish because it allowed it to incorporate the symbiotic bacteria and utilize the toxin it produces to its best advantage. A single point mutation in the amino acid sequence of the sodium-ion channel in this species renders it immune from being bound and blockaded by TTX (Johnson, 2002).

8.3. SYMPTOMS AND TREATMENT IN HUMANS

The first symptom of intoxication is a slight numbness of the lips and tongue, appearing between 20 minutes to three hours after eating poisonous pufferfish. The next symptom is increasing paraesthesia in the face and extremities, which may be followed by sensations of lightness or floating. Headache, epigastric pain, nausea, diarrhea, and/or vomiting may occur. Occasionally, some reeling or difficulty in walking may occur. The second stage of the intoxication is increasing paralysis. Many victims are unable to move; even sitting may be difficult. There is increasing respiratory distress. Speech is affected, and the victim usually exhibits dyspnea, cyanosis, and hypotension. Paralysis increases and convulsions, mental impairment, and cardiac arrhythmia may occur. The victim, although completely paralyzed, may be conscious and in some cases completely lucid until shortly before death. Death usually occurs within 4 to 6 hours, with a known range of about 20 minutes to 8 hours.

From 1974 through 1983 there were 646 reported cases of fugu (pufferfish) poisoning in Japan, with 179 fatalities. Estimates as high as 200 cases per year with mortality approaching 50% have been reported. Only a few cases have been reported in the United States, and outbreaks in countries outside the Indo-Pacific area are rare. Sushi chefs who wish to prepare fugu, considered a delicacy by many in Japan, must be licensed by the Japanese government.

The comparative toxicity of TTX is summarized by William H. Light. "Weight-for-weight, tetrodotoxin is ten times as deadly as the venom of the many-banded krait of Southeast Asia. It is 10 to 100 times as lethal as black widow spider venom (depending upon the species) when administered to mice, and more than 10,000 times deadlier than cyanide. It has the same toxicity as saxitoxin which causes paralytic shellfish poisoning A recently discovered, naturally occurring congener of tetrodotoxin has proven to be four to five times as potent as TTX. Except for a few bacterial protein toxins, only palytoxin, a bizarre molecule isolated from certain zoanthideans (small, colonial, marine organisms resembling sea anemones) of the genus *Palythoa*, and maitotoxin, found in certain fishes associated with ciguatera poisoning, are known to be significantly more toxic than TTX. Palytoxin and maitotoxin have potencies nearly 100 times that of TTX and Saxitoxin, and all four toxins are unusual in being non-proteins. Interestingly, there is also some evidence for a bacterial biogenesis of saxitoxin, palytoxin, and maitotoxin....[i]n living animals the toxin acts primarily on myelinated (sheathed) peripheral nerves and does not appear to cross the blood-brain barrier" (Johnson, 2002).

9. Pfiesteria and Estuary-Associated Syndrome

Possible estuary-associated syndrome (PEAS) is known to occur in brackish coastal waters along the mid-Atlantic coast of the U.S. and has been reported in a few scattered locations worldwide as well. This syndrome seems to be associated to *Pfiesteria* and *Pseudopfiesteria* exposure (Fig. 21), even if no clear connection between algae and syndrome have been found yet (Duncan et al., 2005).

At present the toxins responsible for fish lethality or neurologic symptoms has not yet been identified. Anyway, Moeller et al., 2001 have isolated and partially purified water soluble toxins contained in water from toxic *Pfiesteria* cultures.

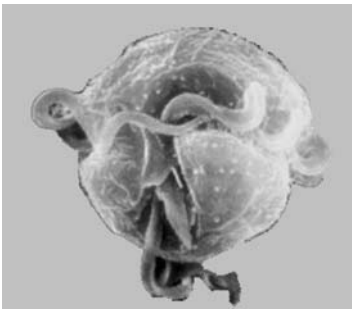


Figure 21. Pfiesteria piscicida.

As well as for the toxins chemical structure, the exact mechanism of action of *Pfiesteria* has not been identified.

Exposure to *Pfiesteria* toxins in the air, water, or fish at the site of an outbreak can cause skin and eye irritation as well as short-term memory loss, confusion, and other cognitive impairments in people. No toxic activity has been detected in shellfish harvested from sites of *Pfiesteria* blooms.

The reported human health effects (e.g. respiratory irritation, skin rashes, and possible neurocognitive disorders) from exposure to natural waters in the mid-Atlantic states are still being assessed. Illness from consuming shellfish and fish in areas of *Pfiesteria* and *Pseudopfiesteria* occurrence are unknown.

Pfiesteria and *Pseudopfiesteria* are associated with fish kills in mid-Atlantic states. Recent studies suggest that fish kills are due to: 1) motile cells attaching to fish and feeding on fish skin cells which could allow invasion of pathogens or weaken the animal's immunity; and/or 2) undescribed, water-soluble and lipid-soluble bioactive fractions being released to the environment. There are published studies purporting different causes and different pathways.

Interestingly, in the laboratory, toxicity of water in an aquarium is rapidly lost within a day if the fish that were provided as food are removed.

Pfiesteria piscicida has a very complex life cycle, and at certain stages of this cycle has a lethal toxic effect on fish, as demonstrated by massive fish kills in North Carolina estuaries and in the Chesapeake Bay (Birkhauser et al., 1975; Ashton et al., 1977; Andersen et al., 1993; Burkholder et al., 2001a; Samet et al., 2001). The organism's polymorphic life cycle (Figure 22) consists of three distinct life-form stages—flagellated, amoeboid, and encystedthat live in bottom sediment s or as free-swimming organisms in

the water column. These stages involve at least 24 size, shape and morphotypic variants, ranging from 5 to 450 μm in size. The stages include rhizopodial, filose and lobose amoebae; toxic and non-toxic zoospores (asexual flagellated spore); cysts of various structure; and gametes (mature sexual reproductive cells having a single set of unpaired chromosomes). Under laboratory conditions in the presence of live fish, its sediment-dwelling amoeboid and resting stages transform rapidly into free-swimming flagellate stages in response to unknown chemical cues secreted or excreted by fish. The induced (excysted) flagellate stages swarm into the water column and become toxic during their continued exposure to the fishderived (sometimes shellfish-derived) chemical stimulants.

The toxic zoospores gather together, alter their random swimming pattern into directed movement, doubling their swimming speed in the process, and commence predatory behavior directed toward targeted fish. The toxic zoospores produce a neurotoxin of unknown structure, soluble in water, and which may be liberated as an aerosol under some conditions. Fish are first narcotized by the toxin, die suddenly, and slough off tissues, which the attacking zoospores consume by sucking out the cell contents through the attached peduncle. The zoospores sometimes ingest other microscopic plant and animal prey at the same time. During this killing period, the zoospores reproduce both asexually (mitotic division) and by producing gametes that fuse to produce toxic planozygotes (actively swimming offspring formed by sexual reproduction, i.e. the union of two gametes). The presence of live fish is required both for completion of the sexual cycle and for toxin induction. Upon fish death or their retreat, the toxic zoospores and planozygotes transform into (mostly) nontoxic amoeboid stages that gather onto the floating fish carcasses on which they feed for extended periods, and follow the sinking fish remains to the bottom sediments. Not all toxic zoospores and planozygotes transform into amoebae. Some encyst and sink into bottom sediments; a lesser number revert to non-toxic zoospores that remain in the water column. The proposed 24 stages of the complex life cycle are based on laboratory observation (Samet et al., 2001).

It has been observed that the morbidity of *Pfiesteria* is dependent on food availability: *Pfiesteria* blooms were stimulated by inorganic nutrient enrichment (Burkholder et al., 1992; Glasgow et al., 1995; Burkholder et al., 2001b), and transition to a toxic stage was associated with the presence of fish tissues or secretions (Burkholder and Glasgow, 1997; Marshall et al., 2000). The fish appeared narcotized, displaying lethargic behavior, a poor fright response, lesions, hemorrhage, and ultimately death. Water samples

indicated that *P. piscicida* was present at concentrations ranging from 600–35,000 cells/ml in waters ranging in temperature from 9–31°C and in salinity from 0–30 psu during fish kills. Much lower concentrations of *P. piscicida* were found only hours after fish kills due to *P. piscicida*'s encystment following lack of food (due to fish death) and settlement into sediment (Burkholder et al., 1992).

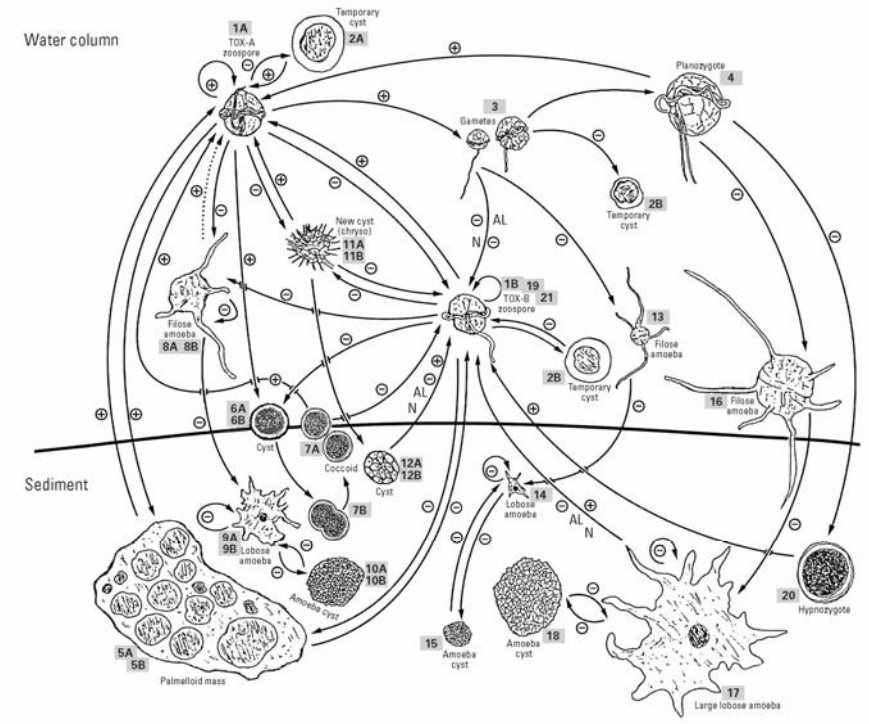


Figure 22. Pfiesteria piscicida life cycle. The pathways indicate the presence (+) versus the absence (–) of live finfish; AL = presence of cryptomonads and certain other algal prey; N = nutrient enrichment as organic and/or inorganic N and P; S = environmental stressor such as sudden shift in temperature or salinity, physical disturbance, or prey depletion. Dashed lines = hypothesized pathways. Stages have been conservatively numbered to facilitate description. TOX-B: zoospores temporarily non-toxic in the absence of live fish prey; TOX-A: zoospores which produce toxin when sufficient live fish are added. Zoospores can transform to filose and lobose amoebae. Planozygotes can transform to larger filose and lobose amoebae. Small filose and lobose amoebae can also be produced by gametes (Burkholder et al. (2001a), modified).

10. New toxins

10.1. AZASPIRACIDS

Azaspiracids are polycyclic polyethers molecules with 40 carbon atoms, with a different level of methylation, presenting a carboxylic acid and a cyclic imine function and a unique cyclic structure (Satake et al., 1998; Ofuji et al., 1999). Some human intoxication has been reported in France, Netherland and Italy (McMahon and Silke, 1998). The producing organism is *Protoperidinium crassipes*, which distributes uniformly in the mussels body (Figs. 23 and 24).

Poisoning is characterized by symptoms similar to DSP (diarrhea, nausea and vomiting) and recovery occurs in 3–5 days.

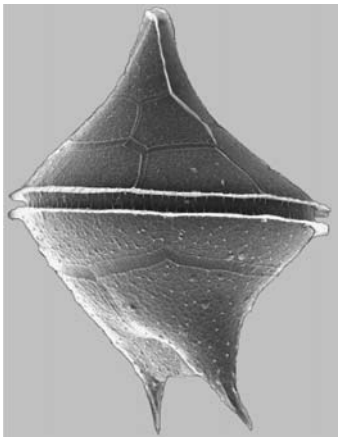


Figure 23. *Protoperidinium crassipes*.

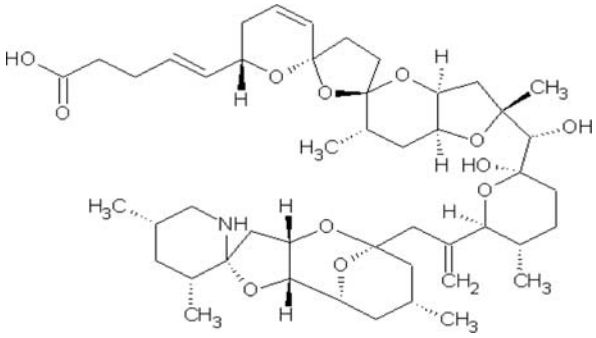


Figure 24. Chemical structure of azaspiracids.

In animals a neurotoxic syndrome is observed, causing death at low doses in two to three days due to progressive paralysis; high doses are lethal in few minutes, and death occurs for paralysis and violent seizures.

Histopathological analysis of rats administered oral or intraperitoneal toxin showed degeneration and desquamation of gut. This phenomena can recover after exposure ends, but recovery times are longer that for OA. Some pancreatic, thymus and cardiac necrosis has been observed, as well as lipidic accumulation in liver (Ito et al., 2000).

10.2. SPIROLIDES

Spirolides (from A to F) are macrocyclic polyethers produced by *Alexandrium ostenfeldii* (Fig. 25). At present their mechanism of action has not yet been clarified, but they seems to affect central nervous system (Hu et al., 1995a; Hu et al., 1995b; Hu et al., 1996a; Cembella, 1998; Cembella et al., 2000).

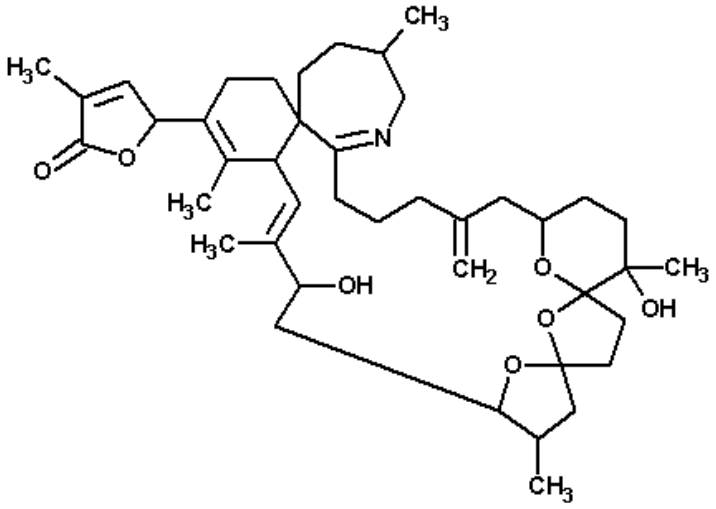


Figure 25. Spirolides chemical structure.

10.3. GIMNODINE

An ichthyotoxic toxin is **gymnodine**, produced by *Gymnodium mikimotoi*; its mechanism of action is not known, but the toxin is not haemolytic, cytotoxic and does not activate ionic channels (Seki et al., 1995; Miles et al., 1999).

10.4. PROROCENTROLIDES

Prorocentrum lima and *P. maculosum* produce not only okadaic acid, but other macrocyclic polyethers named **prorocentrolides** and **prorocentrolides** B, more polar than OA. The toxicological and pharmacological activities are not well defined, but it is clear that they have no inhibiting activity on phosphatases (Torigoe et al., 1988; Hu et al., 1996b).

10.5. COOLIATOXIN

Cooliatoxin is produced by *Coolia monotis*; it is a polyether considered as a monosulphated analogue of yessotoxins. The effect of cooliatoxin in animal is similar to that of yessotoxins, even if the delay time for symptoms appearance is longer (Holmes et al., 1995).

The respiratory distress responsible for animal's death is not a result of direct inhibition of phrenic nerve or of diaphragm activity, but is caused by an initial stimulation followed by block of non-myelized nerves activity, which induce a transitory contraction of smooth muscles and a positive inotrope effect on cardiac muscle (Holmes et al., 1995).

10.6. ZOOXANTELLATOXINS

Zooxantellatoxins are macrocyclic polyethers produced by symbiotic dinoflagellates *Symbiodinium*. Their cellular effects are similar to those of maitotoxins and are controlled by extracellular calcium:

- Increase in cytosolic concentration of calcium
- Hydrolysis of phoshoinositide via phospholipase C and of phospholipids, leading to arachidonic acid liberation
- Muscular contraction
- Sodium influx and potassium efflux from cell (Rho et al., 1995; Rho et al., 1997; Moriya et al., 1998)

As for maitotoxins, two mechanisms of action have been considered for zooxantellatoxins:

- Calcium channels activation dependent on membrane potential
- Activation of non-selective cationic channels

11. Toxicoses Due to "Unsual" Vectors

Some fish and marine vertebrate species are responsible for "unusual" syndromes, generally called "**sarcotoxisms**", which sometimes are caused by a combination of two or more toxins. Toxic compounds produced by benthic and/or epiphytes can affect various trophic pathways, depending on the complexity of affected ecosystem; this effect is thus maximal in tropical areas, where biodiversity if very high (fig. 4).

A summary of poisoning is given in table 6.

TABLE 6. General characteristics of sarcotoxisms.

Name	Species	Clinical characteristics	Toxin
Clupeotoxism	Herrings, anchovies, sardines	Short incubation time, digestive syndrome, pruritus, tachycardia, vertigos, cyanosis. Coma and death are not so rare	Palitoxine? Mixed toxins?
Allucinatory episodes	Acanthuridae Mugilidae, Mullidae, Estraciontidae, Pomacentridae, Serranidae, Siganidae, Sparidae	Short incubation time. Allucinations, vertigos, behaviour alterations, motory incoordination	Various, unknown toxins
Charcarotoxisms	Various shark species	Both nervous and digestive signs	Mixed toxins
Scombroidotoxism	Scrombridae, tuna, bonitos, mackerel	Short incubation time, rapid evolution, nervous and digestive signs. Regression within 8-12 hours	Histamine and biogenic amines
Chelonitoxism	Sea turtles	Digestive syndrome which can also be fatal	Unknown toxins Lyngbyatoxin?

Some of these poisoning are described in following paragraphs.

11.1. LYNGBYATOXIN AND APLYSIATOXIN

The marine cyanobacterium *Lyngbya majuscula* has been implicated in acute adverse health effects in humans over the last forty years with symptoms including dermatitis involving itching, rash, burning blisters and deep desquamation and, respiratory irritation and burning of the upper gastrointestinal tract on ingestion. It has been shown that the toxins **lyngbyatoxin A** (LA) and **debromoaplysiatoxin** (DAT) isolated from *L. majuscula* samples in Australia and USA are at least partially responsible for these symptoms. It should be noted that *L. majuscula* has been found to grow in more than 98 locations around the world in tropical, sub-tropical and temperate climates (Grauer and Arnold, 1961; Mynderse et al., 1977; Solomon and Stoughton, 1978; Cardellina et al., 1979; Fujiki et al., 1985; Izumi and Moore, 1987; Anderson et al., 1988; Marshall and Vogt, 1998; Osborne et al., 2001a; Osborne et al., 2001b).

An association was found between the level of water exposure and reporting of symptoms. Those with higher exposures were more likely to report skin and eye symptoms, which previously have been implicated with exposure to *L. majuscula*. Toxins of L. majuscula have been demonstrated both anecdotally and experimentally to be fast acting, thus eliminating the variable of duration of exposure (Grauer and Arnold, 1961; Solomon and Stoughton, 1978; Serdula et al., 1982; Izumi and Moore, 1987; Marshall and Vogt, 1998).

Episodes of human intoxications due to sea turtles consumption have been known in some Indo-Pacific areas (Halstead, 1970; Champetier De Ribes et al., 1998). The detection of lyngbyatoxin A in meat of turtles lead to the identification of this toxin as the causative agent of intoxication (Yasumoto (1998). Symptoms were characterized by inflammation of oral and esophageal area, difficulty of swallowing, acute gastritis, papule of the tongue, mouth ulcers, weakness, tachycardia, headache, dizziness, fever, salivation, stinking breath. In Madagascar some death in youth and newborn (due to ingestion of toxins with breast milk) were observed. *Lyngbya majuscola* has been proved to produce also **aplysiatoxin**, which induce hypersecretion of mucous from the caecum and large intestine, bleeding from the small intestine and death; the toxin has proved to be a tumor promoter by activating protein kinase C (Mynderse et al., 1977; Fujiki et al., 1981; Ito and Nagai, 1998; Ito and Nagai, 2000).

Lyngbiatoxin has been considered as a promoting agent for fibro-papillomatosis in sea turtles: tumor promoting agents have been shown to enhance viral synthesis, to enhance oncogene-induced transformation of

cells and to reduce immune responses by suppression of the immune-surveillance mechanism. All these actions ease the occurrence of fibro-papillomatosis (Arthur et al., 2008).

11.2. CARCHAROTOXIN

The consumption of sharks' flesh can sometime be the origin of human intoxications, which are recorded since 1993. These intoxications are characterized by neurological signs, mainly ataxia, while gastro enteric symptomatology is quite rare. This poisoning was for long time considered a form of ciguatera. Recent identification of **carcharotoxins** proved that these toxins have no similarity to ciguatoxins (Habermehl et al., 1994; Boisier et al., 1995).

It should be remembered that sharks can anyway contain more than one toxin, ciguatoxins included in Table 7.

TABLE 7. Shark intoxication: syndromes, sources and species involved.

Syndroms	**Source**	**Species involved**
Sarcotoxismes	Ciguatera	*Carcharinus galeocerdo* *Isurus sp.* *Prionace glauca*
Neurosensorial disturbs	Not known	*Somniosus* *Squalus*
Carcharotoxisme	Not known	*Carcharinus leucas* *Carcharinus ambonensis*
Toxicoses	Pollutants Ciguatera Neurosensorial problem Vitamin A	*Prionace* *Sclyliorhinus* *Sphyrna* *Carcharhinus* *Carcharodon*

11.3. PALITOXINS

Clupeotixism is a poisoning characterized by a high rate of fatalities following to sardines, herrings and anchovies consumption. Causative agent for the poisoning was found to **palitoxin**, a toxins produced by *Ostreopsis* spp.; indeed, the dinoflagellates was found in viscera of toxic fish (Usami et al., 1995; Molgò et al., 1997).

Being palitoxin isolated from other organisms than dinoflagellates, i.e. marine coelenterates, sea anemones, crabs and other marine invertebrates, the algal origin of the toxin is under discussion (Moore and Scheuer, 1971; Hirata et al., 1979; Beress et al., 1983; Mahnir and Kozlovskaja, 1992; Gleibs et al., 1995; Yasumoto et al., 1997).

Palitoxin is a polyhydroxylated complex molecules which is constant despite the producing organism (Quod et al., 2001).

Palitoxin was for long time considered as a form of ciguatera, but nowadays clinical symptoms allows to differentiate among the two poisoning (Puiseaux-Dao et al., 2001).

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