37. Marine Biotoxins

Aníbal Martínez, Alejandro Garrido-Maestu, Begoña Ben-Gigirey, María José Chapela, Virginia González, Juan M. Vieites, Ana G. Cabado

This chapter revises current knowledge on marine biotoxins that are known nowadays, considering all the distinct groups based on chemical structure and lipophilic or hydrophilic characteristics. Diarrheic shellfish poisoning (DSP), paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), azaspiracid shellfish poisoning (AZP), ciguatera fish poisoning (CFP) are some of the groups that will be reviewed in this chapter.

The recent development and application of advanced technologies from the generically defined *-omics* sciences coupled with bioinformatics platforms has been included in this chapter in order to understand the ecology and evolution of phytoplankton species and bloom dynamics. Dinoflagellate toxins are structurally and functionally diverse, and many present unique biological activities.

The literature and information regarding the biological activities and the potential application of these phycotoxins has been gathered in this book section.

Marine biotoxins are natural compounds mainly produced by marine microalgae, dinoflagellates, and diatoms. A small percentage of algae produce toxins that can harm human beings and also pose a deadly risk. Toxins are nonproteinaceous metabolites with different and complex structures, physical properties, and specific mechanisms of action. In general, they have low molecular weight ranging from 250 to 3000 Da. Shellfish and fish may become contaminated with these phycotoxins due to accumulation through marine food webs. Risk to humans often results in gastrointestinal disease, loss of short-term memory, neurological disorders, paralytic effects, or even death. In addition, the threat of marine biotoxins is not only a major cause of concern on human health and food safety, but it is also detrimental to the explotation of marine resources

around the world and the economy of shellfish producers and associated industries, including tourism. This chapter revises current knowledge on marine biotoxins that are known nowadays, considering all the distinct groups based on chemical structure and lipophilic or hydrophilic characteristics. Diarrheic shellfish poisoning (DSP), paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), azaspiracid shellfish poisoning (AZP), ciguatera fish poisoning (CFP) are some of the groups that will be reviewed in this chapter. Contamination by marine biotoxins often involves more than one group of toxins and monitoring programs typically cover a range of toxins. There has been an increased effort to develop rapid and feasible screening methods for marine biotoxins. Many methods to detect toxins and phytoplankton have several technical and, sometimes, ethical limitations and generally lack an adequate validation. Functional assays, rapid screening tests, generally based on immunological methods and molecular biology techniques, among others are attractive alternatives to toxins and microalgae characterization. In this chapter, different approaches to monitor and specific detection of shellfish toxins and dinoflagellates are reviewed taking into account the availability of resources. The potential use of several techniques for marine biotoxins or phytoplankton characterization, such as biosensors, applied immunology, PCR, sequencing, radioisotopic, flow cytometry, metabolomics, and proteomics will be explained along the chapter. Marine phytoplankton comprised both prokaryotic and eukaryotic species that share a common ability to photosynthesize and thus thrive in the upper, euphotic zone of the worlds' oceans. Together, the prokaryotic and eukaryotic phytoplankton support marine food webs, including valuable fisheries and play a key role in the regulation of global biogeochemical cycles. Identification and quantification of individual species remains a difficult task. The recent development and application of advanced technologies from the generically defined *-omics* sciences coupled with bioinformatics platforms has been included in this chapter in order to understand the ecology and evolution of phytoplankton species and bloom dynamics. Dinoflagellate toxins are structurally and functionally diverse, and many present unique biological activities. At present, marine biotoxins include an extraordinary potential source of new bioactive compounds for pharmaceutical or medical uses constituting an emergent field of interest. Sometimes, the toxin itself is directly used as a drug, although more often the toxin lead compound provides a design idea for the development of a drug molecule. The biological activities of these phycotoxins are harmful to the target organisms, since the function of the toxin is either to protect the toxic species from attack by a predator or to immobilize the potential preys. Despite the damage in the target organism, toxins have a great potential for not target organisms, in particular for therapeutic purposes in humans. For instance, the target of many marine neurotoxins is the Na^{+} channel, though the sites of interaction and, thus, the pharmacological effects differ among compounds. In this context, phycotoxins can be invaluable tools to recognize and identify ionic channels and the specific role of each channel subtype in controlling cell function, exocytosis, or the Ca^{2+}-dependent release of a given neurotransmitter. The literature and information regarding the biological activities and the potential application of these phycotoxins has been gathered in this book section.

37.1 Marine Toxins

Marine biotoxins are produced by certain species of toxic phytoplankton and can accumulate in various marine species: fish, crustacean, or molluscs such as mussels, clams, oysters, and scallops. In shellfish, toxins mainly accumulate in the digestive glands without causing adverse effects on the shellfish itself. However, when contaminated shellfish is consumed by humans this may lead to a severe intoxication or even death, causing public health and economic problems.

Currently, five groups of marine toxins are regulated in Europe: amnesic shellfish poisoning toxins (ASP), paralytic shellfish poisoning toxins (PSP), okadaic acid (OA) and pectenotoxin group (DSP), azaspiracid group, and yessotoxin group (EC/853/2004) [37.1]. Another group, neurotoxic shellfish poisoning (NSP) toxins (e.g., brevetoxin) is regulated in United States, New Zealand, and Australia. Many cases of tetradotoxin food poisoning are reported in Southeastern Asia as well as ciguatera fish poisoning (CFP) that was limited to tropical and subtropical areas. Other emerging toxins (Palytoxin, cyclic imines,) are not on the radar of the European Legislation or in other regions of the world. Scientists and legislative authorities are aware of these toxins but do not have sufficient amount of information related to their activity, estimation of the toxicity and risk assessment in humans [37.2].

37.2 Lipophilic Toxins

37.2.1 Okadaic Acid-Group (OA) Toxins and Pectenotoxin-Group Toxin (PTXs)

OA-group toxins are a class of marine biotoxins that include OA and dinophysis toxins (DTX1, DTX2, and DTX3). These toxins cause Diarrhetic Shellfish Poisoning (DSP), which is characterized by symptoms such as abdominal pain, diarrhea, nausea, and vomiting. These toxins are produced by planktonic dinoflagellates, *Dinophysis* genus, and the benthic *Prorocentrum* genus and can be found mainly in shellfish.

They were first reported in Netherlands in 1960s [37.3, 4] and in Japan in 1978 [37.5]. Nowadays, their distribution is considered worldwide, Europe, Japan, North and South America Australia, Indonesia, and New Zealand [37.6].

OA-group toxins are heat-stable polyether compounds [37.7]. While OA and DTX2 only differ by the position of one methyl group in the molecule, DTX1 has one additional methyl group. DTX3 represents a wide range of derivatives of OA, DTX1, and DTX2 that form *acylated* analogues when they are esterified with saturated and unsaturated fatty acids. Acylated analogs were reported to contribute considerably to the concentration of total OA-group toxins [37.7, 8].

OA and DTXs act by inhibiting serine/threonine phosphoprotein phosphatases PP1 and PP2A.These enzymes perform the dephosphorylation of numerous proteins; this function is closely related to many essential metabolic processes in eukaryotic cells [37.9, 10].

Pectenotoxins (PTXs) frequently co-occur with the OA-group. The presence of PTXs in shellfish was discovered due to high acute toxicity in mouse bioassay (MBA) after intraperitoneal (*i.p.*) injections of lipophilic extracts. Animal studies indicate that they are much less potent via the oral route and they do not induce diarrhea. PTXs have been detected in microalgae and/or bivalve mollusks in Australia, Italy, Japan, New Zealand, Norway, Portugal, and Spain and they are produced by *Dinophysis* spp.

PTXs are cyclic polyether macrolides that include over 15 analogs. PTX1 to PTX 5 were originally isolated from Japanese scallops, *Patinopecten yessoensis* [37.11]. More recently, other PTXs were found but only four analogs (PTX2, PTX12, PTX11, and PTX13) have been identified as actual biosynthetic products of the algae. Other PTXs seem to be either product of the shellfish metabolism or artifacts [37.12].

37.2.2 Yessotoxin-Group Toxins (YTXs)

Yessotoxins (YTXs) are a group of structurally related polyether toxins produced by the dinoflagellates *Protoceratium reticulatum*, and *Lingulodinium polyedrum*, and *Gonyaulax spinifera* [37.13]. YTXs had traditionally been included within the DSP group but the EU has excluded the YTXs from the DSP group in 2004, and nowadays, YTXs are considered as a separate group in the current regulation [37.1].

YTXs was first isolated in Japan, in 1986 [37.14] from the digestive gland of *P. yessoensis*, a scallop that gave its name to the toxin. More recently, YTXs have been reported worldwide, in Korea [37.15], Chile [37.16], and New Zealand [37.17]. In Europe, it has been described in mollusks in Norway, Italy, Spain, and Russia [37.13].

YTXs are disulfated polyethers, with a characteristic ladder-shape formed by 11 adjacent ether rings of different sizes and a terminal acyclic unsaturated side chain consisting of nine carbons and two sulfate ethers [37.13, 14]. The presence of the sulfo-ether group makes these molecules the most polar of the lipophylic toxins. More than 90 YTXs congeners have been reported but structures for most of them have not been determined and only 30 have been isolated [37.13, 18].

There is discrepancy between the toxicity via oral administration and *i.p.* injection and the precise mechanism of action is not yet known [37.13]. Several studies have been carried out to clarify this, it seems clear that YTXs do not inhibit phosphatases PP1 and PP2A [37.19]. They modulate Ca^{2+} homeostasis in human lymphocytes [37.20] and produce a cytotoxic effect during in vitro experiments in various cellular models, F-actin decrease, alter cell adhesion and cause apoptotic events [37.6].

37.2.3 Azaspiracid-Group Toxins (AZAs)

Azaspiracids (AZAs) are a group of marine biotoxins that accumulate in shellfish and represent an emerging human health risk [37.21]. They were originally isolated from blue mussels from Killary Harbour (Ireland) and produced an outbreak of shellfish poisoning in the Netherlands, in 1995. Afterward, seafood contamination by AZAs have been reported in different coastal localizations, including Europe, North and South America, Africa, and Japan [37.22–26].

There have been several attempts to identify the AZA-producing organism(s). *James* et al. [37.27] have detected the presence of AZA-1, 2, and 3 in extracts from the heterotrophic dinoflagellate *Protoperidium crassipes*. However, this organism is supposed to act as a vector and nowadays, *Azadium spinosum* is considered the organism that produces these compounds [37.28].

AZAs are nitrogen-containing polyether toxins involving a unique spiral ring assembly containing a heterocyclic amine and an aliphatic carboxylic acid moiety [37.6, 27, 29]. They have been identified as AZA1 to AZA5 and numerous natural analogs and artifacts of methanolic solution (methyl ester analogs). AZA4 and AZA5 seem to be a biotransformation product of primary compounds into shellfish.

AZA1 is highly potent and capable of producing an important degradation of the intestinal tract and finally the death of rodent. In vitro assays, AZAs elevate caspase activity, increase cellular concentrations of Ca^{2+} and cAMP, induce irreversible cytoskeletal rearrangements, deplete cellular ATP, inhibit neuronal ion flux and bioelectrical activity, inhibit cell–cell adhesion and stimulate cholesterol biosynthesis [37.21, 25]. Human symptoms of intoxication include nausea, vomiting, and stomach cramps, but thus far, no deaths have been attributed to AZAs [37.25].

37.3 Hydrophilic Toxins

37.3.1 Saxitoxin-Group Toxins (STXs)

Saxitoxin (STXs)-group toxins include various naturally occurring neurotoxic alkaloids that induce the so-called paralytic shellfish poisoning (PSP) in humans. PSP is characterized by symptoms varying from a slight tingling sensation or numbness around the lips to fatal respiratory paralysis [37.30]. The intake of toxins necessary to induce PSP symptoms varies greatly. This may be due to differences in susceptibility as well as a lack of precision in exposure assessments due to problems with sampling and analysis of contaminated shellfish at the time of intoxication.

They have been detected in bivalve mollusks from various areas of the world. Several decades ago, few localizations appeared to be affected by PSP; but nowadays they are found worldwide [37.31–33].

STXs are mainly produced by toxic dinoflagellates belonging to the genus Alexandrium: e.g., *A. tamarensis*, *A. minutum* (*syn. A. excavata*), *A. catenella*, *A. fraterculus*, *A. fundyense*, and *A. cohorticula*, *Gymnodimium, Pyrodinium* and they have also been identified in some cyanobacteria which may occur in fresh and brackish waters.

Most of STX-group toxins are water-soluble and heat-stable nonproteinaceous compounds whose basic structure is composed of a 3,4-propinoperhydropurine tricyclic system. Up to 57 different STX analogs have been described and they can be divided into subgroups based on substituent side chain such as carbamate, sulfate, hydroxyl, hydroxybenzoate, or acetate and also decarbamoyl variants [37.6]. STX, NeoSTX, GTX1, and dc-STX seem to be the most toxic analogs. These neurotoxins produce a blockade of ion conductance through the voltage-gated Na^{+} channel (VGSC) in nerves and muscles fibers [37.30].

37.3.2 Domoic Acid

Domoic acid (DA) is a potent neurotoxic amino acid that accumulates in high concentrations in shellfish, anchovies, and sardines that feed on the toxic phytoplankton. DA can affect marine animals, seabirds, and humans via consumption of this contaminated shellfish causing amnesic shellfish poisoning (ASP). DA was first reported in Canada in 1987 when it caused various human deaths after consuming DA-contaminated mussels [37.34]. Effects on both gastrointestinal tract and nervous system were observed including vomiting, nausea, diarrhea, abdominal cramps, and hemorrhagic gastritis; neurological symptoms are headache, dizziness, vision disturbances, disorientation, loss of short-term memory, motor weakness, seizures, profuse respiratory secretions, hiccoughs, unstable blood pressure, cardiac arrhythmia, and coma. Neurological symptoms may occur after a delay of a few hours or up to 3 days [37.35]. DA especially harms the hippocampus and amygdaloid nucleus in the brain and damages the neurons by activating α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate receptors, causing an influx of Ca^{2+} and cell degeneration [37.36, 37].

DA is produced by the red alga *Chondria armata* and also from the diatoms *Pseudo-nitzschia* spp. and

Nitzschia [37.38]. It is a water-soluble and heat-stable cyclic amino acid and it is structurally very similar to another known neurotoxin, the kainic acid [37.39]. Several isomers of DA (epi-domoic acid (epi-DA), (domoic acid C5′-diastereomer) and isodomoic acids A, B, C, D, E, F, G, and H (iso-DA A–H)) have been reported as well [37.7]. Iso-DA A, B, and C have not been detected in shellfish tissue. DA transforms into epi-DA through long-term storage [37.40] and degrades and transforms to epi-DA and iso-DAs through exposure to ultraviolet light [37.41–43]. In addition, the epimerization is also accelerated by heating [37.44].

37.4 Other Toxins

37.4.1 Ciguatoxins-Group Toxins

Ciguatoxins (CTXs) are a class of marine biotoxins that cause CFP. CTXs occur in fish as a result of biotransformation of precursor gambiertoxins produced by the benthic dinoflagellate *Gambierdiscus* spp. They are mainly associated to tropical and subtropical areas, but recently CTX-group toxins were identified for the first time in fish in Europe [37.45]. Various congeners of CTXs have been identified according to differences in their molecular structure: Pacific (P-CTX), Caribbean (C-CTX), and Indian (I-CTXs) [37.46].

Symptoms of the intoxication of ciguatera in humans include gastrointestinal and neurological effects [37.47]. Although, *Gambierdiscus toxicus* has been traditionally considered the main species responsible for CTXs production, nowadays it is known that *Gambierdiscus* spp. shows a high degree of complexity and it is composed of genetically and morphologically toxin producer species. Thus, distantly related groups co-occur across geography. Ten *Gambierdiscus* species were reported in the Atlantic and in the Pacific region [37.48, 49], and other new species were found in European Atlantic waters and in the Mediterranean Sea [37.50].

The congeners of CTXs are lipophilic polyethers compounds consisting of 13−14 rings fused by ether linkages into a rigid ladder-like structure. CTXs are colorless, tasteless, odorless, and relative heat stable, devoid of heteroatoms other than oxygen and bear few conjugated bonds. Nowadays, over 50 chemical congeners of CTXs have been identified whose toxicity can vary significantly: more than 100-fold among species of *Gambierdiscus* spp. compared with a 2 to 9-fold within species variation due to changing growth conditions [37.49]. The chemical structures of more than 20 P-CTXs analogs, main CTX in Pacific areas, have been identified, and structural modifications are seen in both termini of the toxin molecules, mostly by oxidation. Two CTXs from Caribbean sea (C-CTXs) were first isolated by *Vernoux* and *Lewis* in 1997 [37.51], and then were identified structurally. Other additional congeners were identified in 2002 [37.52]. More recently, four Indian Ocean CTXs (I-CTXs) have been reported [37.53], but their structural determination remains to be established.

The VGSC (Na_V) is the primary molecular target of CTXs, and their binding to the neurotoxin receptor site 5 of Na_V causes the opening of the ion pore, activation of the channels, and Na^+ entrance into the cells. Membrane depolarization and functional impairment of excitable cells are produced due to Na^+ entry into the cell. Secondary responses observed in cells exposed to CTXs include Ca^{2+} into the cell by reverse action of Na^+/Ca^{2+} exchangers eventually leading to muscular contraction and neurotransmitter release. Na^+ influx also affects the entrance of water into the cell, leading to cell swelling, blebbing, and cytotoxicity [37.45].

37.4.2 Brevetoxins (BTX)

Brevetoxin-(BTX) group toxins are neurotoxic polyether biotoxins which can accumulate in shellfish and fish. They are primarily produced by a dinoflagellate *Karenia brevis* (formerly called *Gymnodinium breve* and *Ptychodiscus brevis*) first identified in the Gulf of Mexico in 1947 [37.54–56]. However, other algae species (*Chattonella antiqua*, *Chattonella marina*, *Fibrocapsa japonica*, *Heterosigma akashiwo*) have also been reported to produce BTX-like toxins [37.57]. BTXs have been described in several localizations such as North America, New Zealand, Australia, Japan and Scotland [37.57–59]. *K. brevis* or *K. brevis*-like species have also been reported from Japan, New Zealand, West-Atlantic, Spain, Portugal, and Greece [37.57]. Nevertheless, no intoxication outbreaks in humans or occurrence of BTX-group toxins in shellfish or fish have been reported in Europe [37.55].

They are grouped into types A and B based on their molecular backbone structures. BTX-1 (or PbTx-1)13 (type A) and BTX-2 are considered to be the parent toxins from which other BTX-group toxins derive [37.60]. BTX A has a backbone of 10 fused cyclic ether rings; BTX B (most abundant in *K. brevis*) has a backbone of 11 fused cyclic ether rings [37.60–62]. It is important to highlight that BTX-group toxins are metabolized in shellfish and fish, yielding several metabolites of BTX-group toxins that finally affect to consumers [37.55, 63].

Ion channels are the principal target of BTXs by binding to receptor site 5 of the VGSC in cell walls [37.60] leading to an uncontrolled Na^+ influx into cells and depolarization of neuronal and muscle cell membranes. The response to BTXs in different cells, organs or in vivo models is dependent on various factors, such as the existence of different VGSC subtypes expressed.

37.4.3 Tetrodotoxin (TTXs)

TTX (11-[^{3}H]-tetrodotoxin) is a neurotoxin that has been identified from taxonomically diverse marine organism. Its name derives from *Tetraodontiformes*, an order that includes pufferfish, ocean sunfish or mola, triggerfish, among others. However, TTX has been described in a diverse range of phylogenetically unrelated organism, such as newts, frogs, nematodes, starfish, crabs, mollusk, and others. It has also been suggested that TTX is produced by symbiotic bacteria. Actually, there has been a debate between source of production of TTX from animals or bacteria, and nowadays it has not been firmly established a conclusion. TTX may serve as an antipredator defense, offensive weapon, or for within- and between-species communication [37.64].

Although TTX is associated to tropical waters, mainly South Asia and more specifically Japan, it has been also identified in Mexico and United States [37.65]. In Europe, TTX has been reported in migrant puffer fish in Greek waters [37.66] and in a gastropod *Charonia lampas lampas* in Portugal [37.67].

TTX is a heat-stable and water-soluble heterocyclic guanidine with a highly unusual structure containing a single guanidinium moiety attached to a highly oxygenated carbon backbone that consists of a 2,4-dioxaadamantane structure with five hydroxyl groups [37.68].

Tetrodotoxin binds to site 1 of the fast VGSC [37.33]. Site 1 is located at the extracellular pore opening of the ion channel. The binding of any molecules to this site temporarily disables the function of the channel. Saxitoxin, neosaxitoxin also bind the same site although TTX has different affinities for the variant Na^+ channel isoforms that confer resistance to various species [37.69]. TTX causes paralysis of voluntary muscles (including the diaphragm and intercostal muscles, stopping breathing), and reduced blood pressure, predominantly by vasodilatation. Early symptoms of this effect might include among others weakness, tingling of the lips, and dizziness [37.70]. Gastrointestinal symptoms are often severe and include nausea, vomiting, diarrhea, and abdominal pain. Cardiac arrhythmias may precede complete respiratory failure and cardiovascular collapse.

37.4.4 Palytoxin (PlTXs) and Analogs

Palytoxin (PlTX) is one of the most poisonous nonprotein substances known to date. PlTX was first isolated and purified from *Palythoa toxica* [37.71] and currently present a worldwide distribution [37.71, 72]. PlTXs have also been detected in other marine zoanthids (soft corals) of the genus *Palythoa* (e.g., *P. tuberculosa*, *P. vestitus*, *P. mammilosa*, *P. carobaeorum*, *P. aff. Margaritae*) and benthic dinoflagellates of the genus *Ostreopsis* (e.g., *Ostreopsis siamensis*, *O. mascarenensis*, *O. ovata*) [37.73]. Blooms of *Ostreopsis* spp. have also been reported in European countries such as Spain, France, Italy, and Greece [37.74].

The PlTX-group toxins are complex polyhydroxylated compounds with both lipophilic and hydrophilic areas. They are white, amorphous, hygroscopic solids. At least eight different PlTX analogs are known: PlTX, ostreocin-D, ovatoxin-A, homopalytoxin, bishomopalytoxin, neopalytoxin, deoxypalytoxin, and 42-hydroxypalytoxin [37.75].

The main accepted molecular action of palytoxin is blockage of the Na^+/K^+-ATPase pump [37.76–78]. This binding inhibits the active transport of Na^+ and K^+ across the cell membrane that is essential for cell homeostasis, by transforming the pump into a nonspecific permanently open ion channel [37.79]. Symptoms of PlTX-group toxins intoxication are not well defined, but include weakness and myalgia, fever, nausea, and vomiting.

37.4.5 Cyclic Imines (CIs)

Cyclic imines (CIs) are a recently discovered group of marine biotoxins formed by: spirolides (SPXs), gymnodimines (GYMs), pinnatoxins (PnTXs), pte-

riatoxins (PtTXs), prorocentrolides, and spiroprorocentrimine. These toxins are macrocyclic compounds with imine- (carbon–nitrogen double bond) and spiro-linked ether moieties. They are grouped together due to that imino group acts as functioning pharmacophore [37.80] and the similarities in their *i.p.*, toxicity in mice [37.6, 81].

SPXs are the largest group of cyclic imines and together with GYMs are the best characterized. SPXs were described for the first time in contaminated shellfish, in 1991, in Canada [37.82]. These toxins are metabolites of dinoflagellates *Alexandrium ostenfeldii* and *Alexandrium peruvianum* and are sometimes found in the presence of other toxins such as PSP toxins [37.83]. Up to 14 SPXs analogs have been described of which 13-desmethyl SPX C is the most commonly found in shellfish [37.81].

GYMs are produced by the dinoflagellates *Karenia selliformes* and first isolated in oysters from New Zealand [37.84]. PnTXs and PtTXs are the CI structurally and synthetically more related to SPXs. PTXs were first discovered in extracts from the digestive glands of pen shell, *Pinna attenuata* in China and Japan [37.85]. Seven PnTXs analogs (PnTXs A-G) have been characterized [37.81]. Pteriatoxins A, B, and C, were isolated from the Okinawan bivalve *Pteria penguin* in 2001 [37.86]. *Torigoe* et al. [37.87] isolated the Prorocentrolide A for the first time from *Prorocentrum lima* and then the Prorocentrolide B was first isolated from *P. maculosum* in 1996 [37.88]. Finally, Spiro-prorocentrimine was isolated from a laboratory-cultured benthic *Prorocentrum* species of Taiwan by *Lu* et al. [37.89]. At present, it is accepted that nicotinic and muscarinic acetylcholine receptors are their principal target.

37.4.6 Maitotoxins (MTXs) and Gambierol

Maitotoxins (MTXs) and gambierol are a group of water-soluble toxins produced by species of dinoflagellates of the genus *Gambierdiscus*, which grow on algae in tropical waters around the world. *Gambierdiscus* is known to produce both MTX and CTXs, which accumulate in the body of herbivorous fish and are transmitted through the tropical food chain to carnivorous species.

MTX is a polyketide-derived polycyclic ether integrated by four rigid polyether ladders connected by mobile hydrocarbon chains. Maitotoxin includes 32 ether rings, 22 methyls, 28 hydroxyls, and 2 sulfuric acid esters and has an amphipathic structure [37.90–92].

Maitotoxin activates Ca^{2+} permeable, nonselective cation channels, leading to an increase in levels of cytosolic Ca^{2+} ions. It is thought that maitotoxin leads to the formation of pores on these ion channels. Finally, a cell death cascade is activated, resulting in membrane blebbing and ultimately cell lysis [37.93]. Maitotoxin is known to activate cytosolic Ca^{2+}-activated proteases calpain-1 and calpain-2, contributing to necrosis [37.94].

Gambierol was described as a potent potassium voltage-gated channel blocker that exhibits potent acute lethal toxicity against mice (minimal lethal dose: 50 μg/kg, *i.p.*) and it is suspected to participate in the symptoms of CFP.

37.5 Biotechnological Techniques Used to Study Toxic Microalgae and Marine Biotoxins

The potential use of several techniques for marine biotoxins or phytoplankton characterization are explained along the chapter. In general, the sensitivity of the analytical methods and bioassays depends on the toxins studied. Combination of several approaches, including chromatography-based techniques, to identify individual toxins and the overall toxicity of the mixture, should provide an ideal alternative. Thus, a synergistic complement can be obtained by coupling biological and or functional assays with analytical techniques based on the physicochemical properties of the toxins.

37.5.1 Immunological Methods

The main advantage of the immunological techniques is the variety of immunoglobulins for the specific detection of different targets. These are characterized by sensitivity, specificity, fast performing, and automation. However, the corner stone for these methods seems to be the quality of the antibody presenting cross-reactivity limitations.

An unequivocal identification of phytoplancton and detection of phycotoxins can be obtained by using a species-specific antibody against the target organism

or group of toxins. Specific polyclonal or monoclonal antibodies can be reactive to one epitope and therefore more specific. Combination of some of them, allows the identification, counting, and examination of marine phytoplankton. Use of antibodies with fluorescent markers has made possible to apply different technologies, such as microscopy, flow cytometry, or flow cam. The light emitted by a labeled antibody can be detected by a fluorescent microscope, equipped with a UV light, a flow cytometer a confocal microscope or a flow cam. For instance, several monoclonal antibodies were obtained against different species of *Alexandrium* allowing the unequivocal identification of the dinoflagellate *A. minutum* [37.95]. Then, using immunofluorescence could help to identify different species in natural samples during coastal monitoring.

To discriminate and count small cells or a large number of samples, flow cytometry can be used. Designed to automate the rapid analysis and identification of cells, flow cytometry is a reliable method for the routine monitoring of the abundance of phytoplankton species, leading to an early detection of HABs. This is an optimal instrument for analyzing cells ranging from 0.5 to 20 μm in diameter (concentrations between 10^6 to 10^9 cells/L [37.96].

In this context, a modified flow cytometer, known as FlowCam, was developed to handle larger cells. It is an integrated system combining the capabilities of flow cytometry, microscopy, imaging, and fluorescence that counts, takes images and analyses the particles that range in size from 20 to 200. In addition, to monitor harmful species of microalgae, other potential applications of immunodetection include studies of plankton community structure and ocean optics. Other authors used Imaging FlowCytobot that combines video and flow cytometric technology to capture images of nano- and microplankton and to measure the chlorophyll fluorescence associated with each image. The images are of sufficient resolution to identify many organisms to genus or even species level [37.97].

The use of immunological assays has become a promising alternative for detecting small molecules such as marine biotoxins and many kits are commercially available.

Enzyme-Linked Immunosorbent Assays (ELISAs)

The enzyme-labeled immunosorbent assay ELISA is a powerful analytical tool for natural toxins detection. Specific antibodies recognize toxins and this bound complex is quantified by labeling the free component with a reporter enzyme, usually horseradish peroxidase (HRP) acting as an amplifier to produce many signals. The use of the 96-well microtitre polystyrene plate, the automation and the competitive principle are the most commercial immunoassays for natural toxins.

The ASP ELISA for the determination of DA was approved by the EU as an alternative to the official chemical method HPLC-UV detection [37.98]. This kit is a direct competitive immunoassay where free DA in the sample competes with DA-conjugated protein coated on plastic wells for binding to antidomoic acid antibodies in the solution. Other ELISA tests were developed as the Ridascreen Fast PSP, a competitive ELISA for the quantitative analysis of STX and related toxins. A monoclonal antibody with high affinity against brevetoxin B was used to develop a competitive ELISA for detection of brevetoxins in molluscs [37.99]. Production of monoclonal antibodies for immunoassays by immunizing mice with a synthetic hapten conjugate, instead of the natural toxins, allowed the detection of Pacific ciguatoxins using a direct sandwich ELISA [37.100, 101]. Recently, an improvement of the efficiency and simplification of a colorimetric competitive indirect immunoassay ELISA was carried out based on direct labeling via a covalent bond to the anti-OA antibody [37.102, 103] and a chemiluminiscent ELISA method for this toxin was also developed [37.104].

The challenge of designing antibodies with the optimal specificity and matching toxicity with toxic levels is further complicated by the structural and toxicological diversity within a toxin group together with a potential chemical conversion and biotransformation. However, since recombinant antibodies have been successfully constructed against PlTX [37.105], similar strategies could help the challenge for complex toxins groups. Lack of standardization and transferability of in house ELISA tests between laboratories should be overcome by increasing the focus on validation, based on new strategies for production of high-quality antibodies. Nevertheless, a comparative evaluation of ELISAs and EU reference methods for the detection of the hydrophilic toxins ASP and PSP was carried out in many seafood products [37.106]. The high correlation coefficient between official and immunoassays proves that these tests can be used as screening systems in a variety of species without matrix interference. However, the best sensitive bioassay will depend on the toxins evaluated. In this context, the advantages and disadvantages of ELISA and cytotoxicity assays to detect BTXs were recently reviewed [37.107].

Phage Display Technology

The phage display technology refers to the expression of peptides, proteins, or antibodies on the surface of filamentous bacteriophage viruses. This in vitro strategy provides a valuable system for easy and rapid generation of specific antibodies fragments directed against different antigens, including difficult targets as small molecules. Semisynthetic phage display libraries were used to select recombinant antibodies against PlTX. Some antibodies proved high affinity for immobilized and free PlTX in a competitive ELISA with a low detection limit, a very reproducible standard curve and a wide working range [37.105].

Biosensors

Biosensors are measuring systems based on a biological recognition component with three functioning parts: a biological element, cells, microorganisms, antibodies, the transducer, capable of measuring a biomolecular interaction and converting it into a signal, and the electronics, responsible for the display of the results easily interpreted.

Most of the biosensors developed for shellfish toxins are immunosensors and the optical biosensor with surface plasmon resonance (SPR) is the most widely used in the large majority of reported platforms [37.108]. It has been incorporated into a wide range of devices to measure the analytes present in a sample. For most biomolecules, the change in response is proportional to the mass of the material bound to the surface of the sensor chip. The measure of the binding response against time is called *sensorgram* [37.109]. Small molecular weight compounds, as many drugs and toxins, are very difficult to measure. Then, an inhibition assay approach that relies upon the immobilization of analytes to the chip surface and the injection of the binding partner over the chip surface was developed. Using this assay format, the level of binding to the surface is inversely proportional to the concentration of the target analyte present in the sample. There are three key elements that determine the way in which the methods perform.

- The binding protein, often an antibody, is essential in terms of delivering a specific molecular interaction with the target analyte.
- The surface chemistry is a very important parameter in an SPR assay. Small compounds can be immobilized via the production of an analyte–protein conjugate and this, in turn, is immobilized onto the surface on a sensor chip via an amine or carboxyl coupling strategy. While this approach can be relatively straightforward, it has two main disadvantages. Firstly, during the regeneration of the chip surface, the immobilized conjugate can alter its three-dimensional structure and, thereby, bring about a different level of interaction with antibodies during subsequent interaction measurements. Secondly, the lifespan of an immobilized conjugate on a chip surface tends to be very short. The sensor chips tend to be the most expensive part of the assay, resulting in a high costper test. A much better way to produce chip surfaces is through the direct immobilization of the analyte onto the sensor surface, being much more robust and stable than the conjugate approach.
- The type of method used for sample preparation has to be compatible with this analytical approach. In general, due to the advantages of SPR over conventional immunoassays, i. e., as they are nonequilibrium based and flow through based, in many cases the requirement for a highly purified sample extract can be avoided [37.96].

Some examples of biosensors for DA, OA, PSP, or TTX have been developed, tested and/or validated [37.96, 110–113]. However, one challenge for these assays is the ability to simultaneously measure multiple biotoxins in a single bioanalytical system as was performed in a Framework 7 Project entitled CONFFIDENCE (www.conffidence.eu). Within this project, the SPR methods developed for DSP, ASP, and PSP are being combined onto a single sensor chip using a multichannel biosensor developed as part of another European project Biocop (www.biocop.org).

Comparison of ELISA and SPR biosensor technology for detection of PSP toxins shows that the method of choice will depend on the end-user needs. Some authors point out the reduced manual labor and simplicitiy of SPR biosensor compared to ELISA, ease of sample extraction, and superior real time semiquantitave analysis [37.114], although lower costs and high sensitivity are some advantages of the ELISA.

DNA-biosensors have specific probes that target only DNA-sequences present in the organism of interest and can be used on-site and therefore circumvent the need to return samples into the laboratory. A DNA-biosensor was adapted by *Metfies* et al. to the electrochemical detection of the toxic dinoflagellate *A. ostenfeldii* [37.115]. The technical background of this device is explained in detail in the German patent application DE 10032 042 A1 (Elektrochemischer Ein-

wegbiosensor für die quantitative Bestimmung von Analytkonzentrationen in Flüssigkeiten). The device could facilitate the work that must be undertaken in the course of monitoring toxic algae by eliminating the need to count algae and reduce the toxins tests.

An immunosensor based on magnetic beads as support to immobilize OA on the surface of SPE and then to perform indirect competitive immunoassay using differential pulse voltammetry as a method of electrochemical detection [37.116]. Modifications of this automated flow-through amperometric immunosensor were performed for highly sensitive detection of OA [37.117, 118]. Other sensors include the molecularly imprinted polymer (MIP)-based sensors that use templates to provide polymers with specific recognition properties and chemosensors based on the photoinduced electron transfer (PET) sensing of the toxins by synthetic fluorophores.

Lateral Flow Immunochromatography

Lateral flow immunochromatographic assays are based on a competitive immunoassay format, similar to the home pregnancy test and provide a qualitative indication to the presence of OA and analogs, and DA. These methods also faced the same antibody cross-reactivity challenge.

37.5.2 Enzymatic, Colorimetric, and Other Tests

Several studies based on the mechanism of action of OA-group toxins, inhibition of the enzyme PP2A, were designed and adapted. Some systems as fluorimetry or colorimetry were the most common employed [37.119, 120], although other as electrochemical biosensors were also reported [37.102, 121].

Numerous colorimetric protein phosphatase inhibition assays have been recently reviewed [37.102, 103]. An enzymatic colorimetric test, OkaTest, based on the OA-group of toxins mechanism of action, was recently internationally validated to be used as an alternative or complementary to the reference method for monitoring the OA toxins group in molluscs [37.122] according to Commission Regulations (EC) No. 2074/2005 [37.123] and No. 15/2011 [37.124].

Recently a ligand binding assay was developed to detect cyclic imine in the frame of the Atlantox project, within the Atlantic Area Operational Programme (Atlantox 2008-1/003): www.atlantox.com. This assay, under patent, is based on the mechanism of action of these toxins that act on the nicotinic acetylcholine receptor.

A fast, simple, and sensitive capillary electrophoresis-based immunoassay with electrochemical detection was developed for STX and analogs in shellfish. The method was based on competitive reactions between HRP-labeled antigen and free antigen with a limited amount of antibody [37.125].

The feasibility of OA detection in real-time by using an acoustic wave platform with a microfluidic feature and an ELISA-like protocol was recently reported [37.126].

37.5.3 Receptor Binding Assay (RBA)

The receptor binding assay (RBA) is a high-throughput method for detection of toxins in extracts of seawater and shellfish. This method is based upon the competition between radiolabeled and unlabeled toxins for available receptor sites. The radioactive toxin is displaced from its receptor by toxin present in an unknown sample, thereby, reducing the total radioactivity. Thus, the amount of radioactively labeled toxin that is displaced is proportional to the amount of toxin in the unknown sample. Then, the toxin present in an unknown sample can be quantified by comparison to a standard curve obtained using pure toxin.

RBA was developed from the isolation of VGSC obtained from animal tissues, and was basically implemented by competitive measures of radioactivity using tritiated saxitoxin ($[^3H]$-STX) or brevetoxin (PbTx). Vieytes et al. published a rapid and sensitive method that used Na^+ channels obtained from rat brain membranes to detect PSP toxins. Na^+ channels were coated onto microtiter plates and were used to develop a direct solid-phase binding assay [37.127]. Years later, more developed method was described by *Doucette* et al. based on microplate scintillation technology. Good quantitative agreement of the assay with MBA and HPLC analysis of crude extracts of contaminated shellfish, as well as PSP toxin-producing algae, was observed [37.128]. Usup reported about binding properties of six saxitoxin congeners using $[^3H]$-STX and a preparation rich in Na^+ channels. In this study, EC50 values ranged from 4.38 nM for STX to 142 nM for GTX5 [37.129].

RBA has been refined in the last decade for the detection of PSP toxins. These methods suffer from limitations such as the availability of radio-labeled materials. Restrictions on the world-wide distribution of $[^3H]$-STX imposed by the international Chemical

Weapons Convention in 1997 served to other radiolabeled toxin development such as 11-[^{3}H]-tetrodotoxin (TTX) [37.130, 131]. In 2004, thanks to collaborative efforts among the US Food and Drug Administration (FDA), International Atomic Energy Agency (IAEA), and the National Oceanographic and Atmospheric Administration (NOAA), the worldwide unavailability of ^{3}H saxitoxin was solved temporarily. A relative stable [^{3}H] STX is now distributed worldwide by IAEA and was used in collaborative trials of the PSP toxins using receptor binding assay [37.132]. The assay used a competition among PSP toxins, in shellfish extracts, and a labeled ^{3}H STX diHCl for binding to VGSC in a rat brain membrane preparation. Quantification of binding was carried out using a microplate or traditional scintillation counter. The study focused on the ability to measure the PSP toxicity of samples below, near, or slightly above the regulatory limit of 800 µg STX diHCl equiv/kg [37.1]. The correlation with the MBA (OMA 959.08) yielded a correlation coefficient (r2) of 0.84, while correlation with the precolumn oxidation HPLC method (OMA 2005.06) yielded a (r2) of 0.92. This method has been accepted as official method of analysis AOAC (OMA 2011.27) [37.133].

Similar to PSP toxins method; BTX RBA is a functional bioassay in which an unknown quantity of nonradiolabeled BTX competes with radiolabeled BTX [^{3}H]PbTx-3, for the site 5 receptor of VGSC. Although, the assay using direct detection of a radiolabeled probe, bound to rat brain membranes, is a simple and robust format of pharmacological assays; other variations have been also tested using phospholipid vesicles [37.134] and membranes from other animals than rats [37.135, 136]. More recently other study has compared various extracts of BTX using four independent methods: RBA, radioimmunoassay (RIA), neuroblastoma (N2A) cytotoxicity assay, and liquid chromatography/mass spectrometry (LC-MS). As determined by LC/MS, the RBA, RIA, and N2A cytotoxicity assay detected 73, 83, and 51% of the total BTX concentration [37.137].

RBA designed for CTXs traditionally measures the binding competition between CTX in the sample and a [^{3}H]PbTx standard for the Na^+ channel receptor. The use of [^{3}H]PbTx is a necessity, as nowadays no tritiated CTXs is available. It should to be noted that RBA was successfully applied for monitoring programs on ciguatera risk in three islands of French Polynesia [37.138, 139].

In the case of ASP toxins, the RBA quantifies DA activity by the competitive displacement of radiolabeled kainic acid from a cloned glutamate receptor by DA in a sample. In 1997, *Van Dolah* et al. [37.140] reported the further development of the receptor assay using a cloned rat GLUR6 glutamate receptor. The limit of detection and selectivity of the assay were optimized through inclusion of the glutamate decarboxylase (GLDC) pretreatment step to eliminate potential interference due to high concentrations of endogenous glutamate in shellfish. However, as GLDC was difficult to obtain from suppliers, other variations were introduced [37.141].

Due to limitations of the standard AOAC MBA for estimation of toxins levels there was a need for alternative testing protocols. The relevance of RBA tools is high, but the need for specific equipment and licensing requirement for the use of radiolabeled compounds may lead to obvious limitations in the application of this assay. Some main disadvantages include the high dependency on the receptor source, it does not provide any information on the toxin profile, it cannot be easily automated, and it has been validated only for a few toxins. However, RBA is adequately sensitive and more specific than MBA [37.45]. Attempts to modify this assay to use label other than tritiated forms has led to the development of a competitive RBA based upon the use of BTX-B2 labeled with a chemiluminescent acridinium moiety. The acridinium brevetoxin-B2 seems to be a promising alternative to the conventional radioactive ligand in order to avoid constraints associated with RBA [37.46, 142].

37.6 Biotechnology Application for Phytoplankton Detection, Monitoring, and Toxins Production

Recent development and application of advanced technologies from the generically defined *-omics* sciences (genomics, transcriptomics, proteomics, and metabolomics) coupled with bioinformatics platforms has already provided deep and often revolutionary shifts in understanding the ecology and evolution of phytoplankton species and bloom dynamics [37.143].

Harmful algal blooms (HABs) are natural events that have become common in coastal waters worldwide during the last years, being recurrent in Euro-

pean coastal waters. The toxic microalgae responsible for these blooms are mainly dinoflagellates, which display heteromorphic life cycles, including motile planktonic stages, as well as immotile benthic resting cysts [37.144, 145].

Monitoring of harmful species is important to understand population dynamic activities related to the exploitation of coastal areas. To distinguish species by their morphological characteristics, cells must be examined by light, epifluorescence, or electron microscopy. This can be time consuming, and requires taxonomic expertise. Moreover, in some cases, morphology-based diagnostic tests lead to misidentification due to the existence of morphologically closely related species. Molecular methods, generally based on the DNA probe hybridization and PCR techniques, have been developed for the rapid and sensitive identification of HAB species [37.145–147]. The target DNA genes are generally within ribosomal RNA operons, which are repeated in high copy number in the microalgae genome and can be genus or species specific, as 18S, 28S, 5.8S, ITS1, and ITS2 among others.

37.6.1 Genomics

Conventional genomics is often based upon the sequencing and annotation of whole genomes, with subsequent bioinformatics focus on the structure and function of key groups of genes. However, the ability to perform genetics experiments with microalgae has been accelerated in recent years by the availability of genome sequences. The application of emerging genomics techniques to phytoplankton research is likely to become widespread in the future.

Sequencing

Currently, there are more marine cyanobacterial genome sequences available than eukaryotic phytoplankton genome sequences, primarily due to differences in genome size and complexity. The average cyanobacterial genome size is 2–3 million base pairs (Mbp), although they range up to 9 Mbp. In contrast, eukaryotic genomes tend to be orders of magnitude larger [37.148]. Completed genome projects have focused on species with relatively small genomes, ranging from 12 [37.149] up to 57 Mbp [37.150]. The recent availability of marine phytoplankton genome sequences has prompted a wealth of research in diverse fields including ecology, evolution, biochemistry, and biotechnology.

Concerning eukaryotic marine phytoplankton, it must be considered that it includes phylogenetic lineages resulting from primary, secondary, and tertiary endosymbiosis events. This phylogenetic and physiological diversity is translated into a wide range of potential genome sizes, structures, metabolic pathways, life histories, and evolutionary relationships. Of the four major lineages of eukaryotic phytoplankton, whole genome sequences are currently available for two; the heterokonts [37.150, 151] and the prasinophytes [37.149, 152]. Additional members of the heterokont and haptophyte lineages are in the pipeline for sequencing or draft genomes are being analyzed (e.g., the haptophyte *Emiliania huxleyi*; http://genome.jgi-psf.org/Emihu1/Emihu1.home.html) [37.148].

Genomics are more than simply genome sequencing including transcriptional analyses of both sequenced [37.153, 154] and unsequenced organisms [37.155], and also targeted metagenomics of ecologically important but uncultured phytoplankton [37.156].

One of the challenges in examining structural diversity in eukaryotic phytoplankton is the large number of novel genes identified in each newly sequenced genome that have no homology to genes with known or suspected functions. Often, they are similar only to genes of unknown function in closely related organisms. Depending on the genome, the number of novel genes ranges from 20 to nearly 40% highlighting the limitations of homology-based approaches. Differences in gene structure (e.g., novel splice sites, signal peptides, and untranslated regions) constitute a challenge to gene-calling algorithms designed for higher plants and metazoans. One solution has been the development of tiling arrays for sequenced genomes [37.148] that differs from traditional microarrays in the nature of the probes. Instead of probing for sequences of known or predicted genes that may be dispersed throughout the genome, tiling arrays probe intensively for sequences which are known to exist in a contiguous region. This is useful for characterizing regions that are sequenced, but whose local functions are largely unknown. Using tiling arrays *Mock* et al. [37.154] predicted 3470 new genes in the *Thalassiosira pseudonana* genome that were not previously identified using standard gene-calling algorithms. This 33% of increase in the total number of genes predicted in the *T. pseudonana* genome suggests that gene density and number are not fully captured using standard gene-finding algorithms. Sequenced genomes also highlight the phyloge-

netic and metabolic diversity that characterizes marine phytoplankton. Description of new lineages, such as the uncultured picohaptophytes suggests that probably many ecologically important lineages have yet to be discovered [37.156].

Investment in genetic techniques both available and robust in a diverse array of marine phytoplankton will greatly help in understanding gene function, especially for the large number of hypothetical and conserved hypothetical genes [37.148]. New approaches have emerged and will continue to emerge that rely on genome sequence data. For example, whole genome microarrays include a range of targets such as all annotated genes or even the whole genome. New directions in this area will use genome information from diverse keystone species to create microarrays for use in characterizing environmental samples; mRNA sequencing approaches such as Illumina sequencing generate only short reads and can take advantage of available genomes to examine gene expression. For example, mRNA from two conditions is used to generate cDNA libraries that are sequenced to obtain quantitative gene expression profiles that can be compared.

The application of these emerging genomics techniques to phytoplankton research is likely to become widespread in the future. One challenge beyond the genomics revolution is to apply these techniques in the marine environment to examine phytoplankton physiology in situ. This will mean synthesizing many different types of data simultaneously such as real-time physical and chemical environmental variables, community species composition, gene expression profiles, and protein signatures. This system-level approach has the potential to greatly expand our understanding of how phytoplankton acts in their environment [37.148].

PCR and Real-Time PCR (qPCR)

qPCR is a very promising technique in terms of sensitivity, specificity, enumeration capability, and costs reduction [37.145, 157]. Molecular approaches, when used together with other morphological characters, may help to solve taxonomical problems. The nuclear rDNA internal transcribed spacer regions and the 5.8S rRNA gene are widely used to characterize the phylogenetic aspects of a great variety of these organisms [37.147, 158–160].

Even though these applications are very valuable regarding human health, the use of PCR and qPCR for the direct detection and identification as well as monitoring of toxic phytoplankton is of special interest. Several studies have been developed for simplex and multiplex detection of different groups, genus and/or species, as *Alexandrium*, *Ostreopsis*, or different cyanobacteria [37.161–164]. It is important to highlight the fact that environmental samples often contain complex mixtures of organic matter, which may inhibit PCR/qPCR, thus an internal amplification control (IAC) should be included to avoid misinterpretation of false-negative results. Additionally, different strategies have been applied to overcome the problem of PCR/qPCR inhibition caused by contamination substances: the use of thermostable DNA polymerase, commercial nucleic acid extraction kits that remove inhibitors and facilitate purification, dilution of template DNA prior to PCR/qPCR assays and the use of bovine serum albumin in the PCR/qPCR assay [37.144, 163]. Several studies have been published applying PCR/qPCR for monitoring HAB worldwide, from the Mediterranean Sea to Australia [37.157, 164–166].

Regarding toxin quantification/detection, it is more often performed by other techniques, but different studies have also been performed applying PCR/qPCR. In 2011, *Murray* et al. developed a method targeting the unique core gene *sxtA* to identify saxitoxin-producing HABs in Australian marine water [37.165]. Their method was tested against different species and strains of *Alexandrium*, *Ostreopsis*, *Gymnodinium* among others. *Al-Tebrineh* et al. also developed a qPCR method capable of detecting SXT producing species, but they took advantage of the higher throughput of multiplex formats of qPCR and also targeted the genes coding for microcystin (*mcy*), nodularin (*nda*), cylindrospermopsin (*cyr*) in a multiplex qPCR experiment to specifically detect toxin producing species [37.163].

Microarrays

In a microarray study, gene-specific probes are spotted onto precise positions on a solid surface and targeted with an unknown DNA or RNA sample. Microarrays represent the scaling up, miniaturization, and automation of well-known hybridization techniques. Recently, a FP7 European research project called MIDTAL (Microarrays for the detection of toxic algae) aimed to construct a universal microarrayfor the detection of harmful algae and their toxins (htpp://www.midtal.com/). Results obtained showed the third generation of the MIDTAL microarray that includes between 140 and 163 probes for various toxic algal species including ASP, DSP, and PSP producers. The designed microarray has great potential to be used as a monitoring tool for toxic algae detecting multiple species simultaneously although improvements in RNA extraction

and tests are still needed [37.167]. The most obvious advantage of the microarray is its ability to identify species that cannot be identified with traditional methods, such as *Pseudo-nitzschia*, *Alexandrium*, and *Heterosigma* [37.168, 169].

Other Genomic Techniques

Other techniques different from sequencing and PCR have been also applied in phytoplankton studies. These include restriction fragment length polymorphism (RFLP), the first DNA-based technique used in phytoplankton studies; denaturing gradient gel electrophoresis (DGGE); single stranded conformation polymorphism (SSCP); random amplified polymorphic DNA (RAPD); amplified fragment length polymorphism (AFLP); Microsatellites or short sequence repeats (SSRs); molecular probes and single nucleotide polymorphisms (SNPs). Most of these techniques were quite useful to study phytoplankton especially when no sequences were available because they can be used without prior knowledge of the genome [37.170].

37.6.2 Transcriptomics

Transcriptomic studies gather efforts to isolate all the RNA transcripts, i. e., the transcriptome, from a sample of cells, and determine the presence and abundance of each particular species of gene-encoding RNA or mRNA. These studies help to elucidate genes involved in nutrient acquisition and metabolism (including those involved in synthesizing or regulating the synthesis of toxic molecules), aid in annotating genomes, and also have the potential to find novel transcripts or transcripts with unusual structures that may hint at the mechanism of gene regulation [37.143, 171, 172].

For many organisms with either large and/or complex genomes, transcriptome sequencing may be a more immediate and cost-effective route than whole genome sequencing to obtain ecological and evolutionary information.

An expressed sequence tag or EST is a short subsequence of a cDNA. They may be used to identify gene transcripts in gene discovery and sequence determination. The identification of ESTs has proceeded rapidly, with approximately 74.2 million ESTs now available in public databases (e.g., GenBank 1 January 2013, all species). The cDNAs used for EST generation are typically individual clones from a cDNA library. The resulting sequence is a relatively low quality fragment whose length is limited by current technology to approximately 500–800 nucleotides. Because these clones consist of DNA that is complementary to mRNA, the ESTs represent portions of expressed genes. They may be represented in databases as either cDNA/mRNA sequence or as the reverse complement of the mRNA, the template strand. ESTs can be mapped to specific chromosome locations using physical mapping techniques, such as radiation hybrid mapping, Happy mapping, or FISH. Alternatively, if the genome of the organism that originated the EST has been sequenced, one can align the EST sequence to that genome using a computer.

EST analysis of the dinoflagellate *A. minutum* revealed 192 genes that were differentially expressed between isolates that produced PSP toxins and those that did not [37.173]. In terms of the functional ecology of *A. minutum*, the 192 genes are putative candidates for genes involved in toxin synthesis and regulation or acclimation to intracellular PSP toxins. The sequences in the EST library also suggest that PSP toxins generated by this alveolate did not arise from a recent gene transfer from cyanobacteria, as was previously hypothesized [37.173]. Other dinoflagellate EST sequencing projects have yielded further insights into toxin production, predator–prey interactions, genome architecture, and trophic status. As sequencing costs drop and capabilities increase, transcriptome analyses of marine phytoplankton species and strains within species will soon become not only feasible but also common. This approach has also been recently applied to *A. ostenfeldii* [37.143, 174].

37.6.3 Proteomics

The field of proteomics is complementary to genomics and transcriptomics since it provides additional information on gene expression and regulation. Proteomics include the determination of protein expression levels and protein–protein interaction studies. Moreover, proteomics aim to identify posttranslational modifications of proteins, as well as the organization of proteins in multiprotein complexes and their localization in tissues [37.172].

In the past decade, genomics began to explore the diversity, cellular evolution and adaptive abilities of marine phytoplankton. Although this has provided the community with a huge amount of information, it is not necessarily being translated into biochemical expression or phenotype. Genomics demonstrates which genes are shared, but proteomics can show clearer relationships by illustrating functional similarities and phe-

notypic variances. With a well-designed experiment, researchers can examine the conditions under which a protein is expressed; its cellular location, the relative quantities, and what protein–protein interactions take place [37.175].

Chan et al. [37.176, 177] studied protein profiles under different environmental conditions found in *Prorocentrum triestinum*. He showed both constancy of protein profiles and variability of the relative abundance of some proteins under different and culture conditions. Differentially expressed protein patterns of *P. triestinum* indicated that *P. triestinum* growing under nitrogen-limited conditions possessed higher growth potential since its protein expression profiles are more similar to that of the blooming stage. These results are consistent with the observations that it is possible to rapidly trigger a bloom by the addition of a supply of nitrates. A more detailed analysis of the differential synthesis of proteins in these various stages and studies to elucidate the functions of these proteins will be critical for understanding the molecular mechanisms involved in blooming. Phytoplankton proteomic approaches that use whole genome sequences to examine protein expression by matching protein MS/MS spectra to those predicted from whole genome sequences are already available [37.150]. Cell surface proteins (CSPs) of *Alexandrium catenella* have been also studied in detail. Protein profiles at different toxin biosynthesis stages, were analyzed and 53 differentially expressed proteins were identified. These were involved in various biological processes, nine of which might be involved in the PST biosynthesis of *A. catenella* using the quantitative proteomic approach to compare protein profiles at different toxin biosynthesis stages.

Proteomics studies should help to reveal the toxin biosynthesis mechanisms and pathways in dinoflagellates [37.178]. Proteomics has provided valuable information on the effects and also the mode action of the dinoflagellate metabolite yessotoxin on a human liver cell line. Also, analysis of the plastid proteome of the Florida red tide dinoflagellate *K. brevis* detected an electron transfer protein (plastocyanin) inherited from green algae that may contribute to ecological success in iron use. Nevertheless, much promise remains to be fulfilled in the application of proteomics to studies of HAB ecophysiology and bloom dynamics [37.179].

37.6.4 Metabolomics

The term metabolomics is usually defined as the comprehensive and quantitative analysis of all small molecules in a biological system [37.180]. Metabolomics allows monitoring and mapping the dynamic range of bioactive secondary metabolites under different conditions. The most commonly used gas chromatography-time-of-flight mass spectrometry (GC-TOF-MS)-based method facilitates the identification and robust quantification of a few hundred metabolites. The merging of metabolomics and metagenomics has the potential to be a particularly powerful combination in shedding light on planktonic and microbial interactions in marine ecosystems. Statistical evaluation of data provides insight into the metabolic changes within the cells as well as into the released metabolites. The knowledge of the complex patterns of chemicals released by microalgae will reveal new mechanisms for processes such as: community function, food location, or complex defensive and allelopathic interactions [37.181].

A great number of metabolic pathways and regulatory mechanisms could be predicted based on the genomic sequences. Nevertheless, some of the genes might not be functional in vivo. Recent technical developments make metabolomics a suitable tool to define their genes function and regulation in microalgae. Analyses of diatom genomes have suggested tight connection between a number of novel pathways and regulatory steps in diatom metabolism. However, there are still several important open questions to be addressed. Given the massive recent acceleration in genome sequencing capacities we will shortly have an enormous resource of genetic information for marine phytoplankton. Combining this information with details on their metabolic complement will greatly enhance our understanding of the metabolic processes by and how they interact with their environment, as well as the evolution of their underlying metabolic pathways [37.182].

Metabolomics approach has already been successfully applied to the dinoflagellate endosymbiont *Symbiodinium* [37.179]. As an emerging technique, metabolomics have not been widely applied in toxic phytoplankton studies, but some research groups are developing databases for identification of marine biotoxins using metabolomics approaches [37.183].

37.7 Potential Pharmacological Uses of Phycotoxins

In the past few decades, extensive studies have been devoted to investigate the toxicology and pharmacology of biotoxins [37.184, 185]. A renewed interest is seen in marine alkaloids and their analogues, including the STX-group toxins (STXs), with regards to their use as therapeutic agents or as a drug lead [37.186]. STXs bioactivity studies and molecular modeling could lead to the design of unnatural analogs with improved pharmaceutical characteristics. Recently, a group of toxins isolated from marine cone snails (genus *Conus*), known as conotoxins, have been shown to contain over 2000 peptide analogs. Conotoxins are able to specifically target a broad range of ion channels and membrane receptors and are currently under investigation for possible clinical trials. In 2004, a synthetic version of a conotoxin analogue, ω-conotoxin MVIIA, also known as ziconotide (trade name Prialt) was the first marine natural product to be approved for use by the US FDA [37.186]. Ziconotide acts by targeting N-type voltage sensitive Ca^{2+} channels and is used for the treatment of chronic pain in spinal cord injury.

Ion channels triggered by voltage changes along the cell membrane are termed voltage-gated ion channels (VGICs), include channels that conduct Na^{+}, Ca^{2+} and K^{+}. Activation of Na^{+} or Ca^{2+} VGICs produces cell stimulation by membrane depolarization, while K^{+} channel activation results in inhibitory hyperpolarization. Toxins that bind VGICs can serve as powerful molecular probes to study the channel structure and function, as well as serve as models for studying evolutionary targets and drug discovery [37.187]. Some marine biotoxins are classified as neurotoxins due to their ability to interact with these channels [37.188]. As we will discuss later in this section many marine biotoxins have affinity by one or more VGICs and therefore could have an important pharmaceutical potential.

37.7.1 Lipophilic Toxins (LTs)

Okadaic Acid-Group (OA) Toxins and Pectenotoxin-Group Toxins

OA is a marine biotoxin that presents a great value in medical research and has facilitated the understanding of several cellular processes [37.189]. OA-group toxins have interest due to their high cytotoxic activity and the extraordinary ability to promote tumors [37.190, 191]. It is considered that their effect varies depending on the cell type and concentration used [37.192].

It has been a model for analyzing therapeutic effects of some drugs in treating neurodegenerative diseases [37.193]. OA acts as a potent neurotoxin that induces apoptotic events in various cell lines and promotes tumors in several organs. Mechanisms are dependent on cell type, the antioxidant status of each cell and the ability to metabolize OA [37.194–199].

OA-group toxins are specific inhibitors of PP1 and PP2A that are involved in the regulation of many cellular processes and modulate the extent of phosphorylation or dephosphorylation of proteins. Other cellular targets for OA have been suggested [37.189, 200–203]. OA has been used to analyze the mechanisms by which the conjugated linoleic acids can act as antitumor agents in breast cancer cells due to its activity as an inhibitor of PP2A [37.189]. In addition it has been reported that the extent of OA-induced injuries and the toxin organotrophicity are dose-related and may be determined by the administration route. After intravenous administration, OA acts as a hepatotoxin with undetectable effects on the intestine but also has an impact on cytoskeletal elements at sublethal doses [37.204].

PTXs are potent cytotoxic compounds against several human cancer cell lines. They are hepatotoxic whose mechanism of action has not yet been fully elucidated [37.205–207]. Among other effects, the induction of apoptosis in rat hepatocytes, in salmon and p53-deficient cell lines have been reported [37.208].

PTX2 is an inhibitor of actin and cytokinesis and it is known to be used as a chemotherapeutic agent against tumors type p53 [37.209]. It was demonstrated that a loss of p53 sensitizes tumor cells to actin damage. PTX2 was first identified as a cytotoxic entity in marine sponges, which depolymerizes actin filaments and it was found to be highly effective and more potent to activate an intrinsic pathway of apoptosis in p53-deficient tumor cells compared to those with functional p53 both in vitro and in vivo. Other agents that depolymerize or knot actin filaments were also found to be toxic to p53-deficient tumors. In p53-deficient cells, PTX2 triggers apoptosis through mitochondrial dysfunction, and this is followed by the release of proapoptotic factors and caspase activation. Furthermore, Bax activation and Bim induction was observed only in p53-deficient cells after PTX2 treatment. Therefore, these results suggest that Bim triggers apoptosis by activating Bax in p53-deficient tumors upon actin damage, and that actin inhibitors may be potent chemotherapeutic agents against p53-deficient tumors.

Recent studies demonstrated that this congener do not induce diarrhea or any other signs of disease by oral administration. Body distribution of PTX2 and PTX2 seco acid (PTX2SA) was analyzed in mice after oral and intraperitoneally administration. Cytotoxicity assays in vitro have been the first step to the discovery of many drugs used in antitumor therapy. However, more information is needed about its toxicity to humans and their pharmacokinetics [37.210].

Yessotoxins-Group Toxins

YTXs are polyether compounds that were discovered due to their high acute toxicity during MBA by *i.p.*, injection for lipophilic toxin analysis of mollusks [37.211, 212]. There are no published reports of human poisonings caused by YTXs and no available data on chronic exposure, carcinogenicity, or genotoxicity [37.213]. Various authors have suggested that the target organ following *i.p.* administration is the heart [37.214]; however, other authors consider that YTX is involved in neurological disorders and its main target is the thymus [37.215]. *Tereo* et al. have reported severe microscopic alterations in cardiac muscle [37.214]. Difference between these studies is not clear and has been proposed that discrepancies may be due to different purities of the toxins and the use of different strains of mice [37.213]. YTX administration to human lymphocytes leads to an increase in cytosolic Ca^{2+} concentration [37.20] and in specific activity of cyclic nucleotide phosphodiesterase 3′, 5′ [37.216]. Other studies have reported that cellular Ca^{2+} homeostasis is not related to its cytotoxic effect [37.217]. Furthermore, YTX causes dose-dependent decrease in the levels of GMPc and AMPc after incubation due to the activity of phosphodiesterases. These effects depend on Ca^{2+} and can be changed by phosphodiesterases [37.216, 218].

YTX induced a decrease in F-actin filaments in several cell models as cerebellar granule cells (CGCs), mouse fibroblast, rat L3, mouse myoblast cell lines, and MCF-7 human breast adenocarcinoma cells [37.217, 219]. However, this seems not to be a universal effect, since no effects over F-actin levels were detected in M17 neuroblastoma cell line, in rabbit enterocytes, or in human Caco-2 cells [37.220, 221]. YTXs cause selective disruption of the E-cadherin-catenin in epithelial cells and could potentially suppress tumor functions of E-cadherin [37.222]. Pathway degradation of E-cadherin is affected by YTX at long incubation times and low doses. Since E-cadherin has been linked to tumor spreading and metastasis, it has been a concern if the disruption of E-cadherin by YTX could affect tumor expansion in YTX-contaminated mollusk consumers. However, in vivo experiments demonstrated that although the resulting molecule of E-cadherin disruption had increased, YTXs did not induce any effect on E-cadherin system in in vivo experiments [37.223].

YTX-induced apoptotic events in different cellular lines, including cancer cells, primary cell cultures, and cell lines. There are reports related to YTX-induced apoptosis in rat hepatocytes, rat glioma cells, HeLa S3 cells, rat cerebellar neurons, BE(2) neuroblastoma cell line, L6 myoblast cell line, BC3H1 myoblast cell line, mouse fibroblast NIH3T3 cell line, CaCo-2 cells, MCF-7 cancer cells, and HepG2 cell cultures. In this sense, YTX could be used in therapeutic applications, for instance, as an antitumor drug [37.224]. Thus, the European patent application EP1875906 [37.225] considers to use YTX as an antitumor drug, and protoceratin I, the major compound with cytotoxic activity against human tumor cell lines isolated from *P. cf. reticulatum*, was proved to be identical to homo YTX [37.226].

It has also been investigated the activity of various YTXs analogs on MCF-7 tumor cell lines; structural modification in homoYTX do not change the activity of the toxin, while the side chain structure of carbon 9 seems essential. Thus, carboxiYTX and hidroxihomoYTX present less activity than YTX [37.227]. These data have been confirmed by kinetic studies between phosphodiesterases and YTX, hidroxiYTX and carboxiYTX, proving the lower affinity between these proteins and the last two analogs [37.228].

Azaspiracid-Group Toxins

Molecular targets of these toxins are not well known, but some studies were focused on the study of second messengers: increased intracellular Ca^{2+} and AMPc [37.229–232], effects of reorganization of the actin cytoskeleton [37.233–236] and effects on adhesion by activation of adhesion protein fractionation (claudins and cadherins) [37.234, 237, 238].

Although, symptoms observed during episodes of AZAs poisoning were similar to those typically observed with DSP, administration of toxic AZAs materials to mice led to great differences with the DSP. Mice injected with low doses of AZAs did not present diarrhea [37.239], and in a few hours develop slowly progressive paralysis, difficulty in breathing, and finally death [37.240]. A 200 mg/kg *i.p.* dose appears to be the minimal lethal dose in mice to AZA1 while AZA3 and AZA2 showed a higher toxicity (110 and 140 mg/kg, respectively) [37.240, 241]. Damages in liver, pancreas,

spleen, thymus and stomach were observed. Moreover, after administration of AZA sublethal doses by gavage, alterations were observed in the small intestine, affecting epithelial cells with a degree of damage progressively increasing from 4 to 24 h after administration [37.242, 243]. AZA cytotoxicity was first observed by *Flanagan* et al. [37.244] in hepatoblastoma and bladder carcinoma cells exposed to contaminated crude mussel extract. *Twiner* et al. [37.235] observed that AZA1 causes cytotoxicity in kidney, lung and immune, neuronal, and pituitary cells. However, neurons appear to be particularly sensitive to AZAs, causing irreversible morphological changes with relatively short exposure times and cytotoxicity with longer exposures to these toxins [37.231, 236]. It also requires longer exposure times to achieve complete cytotoxicity in primary cultures of cerebellar granule cells [37.200]. Also, they induce apoptosis through caspase activation [37.210, 245].

AZAs are implicated in upregulation on gene expression and protein levels of low-density lipoprotein receptor (LDLR). This effect seems to be a response to the decreased level in intracellular cholesterol caused by AZA1 [37.25]. It was shown that T lymphocytes exposed to AZA1 up regulated gene expression and protein levels of LDLR due to decreased levels of intracellular cholesterol. Although premature to determine decisively, yet consistent with currently available in vitro data, AZA1 appears to be targeting a membrane protein such as a claudin, cadherin, or LDLR, in turn eliciting an effect on intracellular signaling molecules and the cytoskeleton, ultimately resulting in cytotoxicity.

37.7.2 Hydrophilic Toxins

Saxitoxin-Group Toxins (PSP)

STX-group toxins are closely related water-soluble tetrahydropurine compounds acting by interfering with VGSC functioning. The toxin acts from the exterior of the cells by getting access to the extracellular cavity of the channel and binding to the so-called site 1 of the α-subunit in the Na^+ channel [37.246]. Site 1 is shaped by a short portion (SS2) of the amino acid stretches connecting the S5 and S6 trans-membrane helices in the four domains of the α subunit, giving rise to a cavity that accommodates the toxin. STX then forms hydrogen bonds and electrostatic interactions with the side chains of several amino acids that participate to the ion selectivity filter of the channel. The interaction of one STX molecule with the site 1 of the α-subunit in the Na^+ channel essentially plugs the channel and blocks its ion flux. The loss of Na^+ conductance in excitable cells prevents membrane depolarization and the transmission of the action potential, representing the molecular basis of the toxic effects of STX. As a consequence of VGSC blockade, a progressive loss of neuromuscular function ensues, leading to the reported neurotoxic symptoms that can result in death by asphyxia. Skeletal analogs of STX with a fused-type tricyclic ring system, designated FDSTX, were synthesized as candidate Na^+ ion channel modulators [37.247]. Their inhibitory activity on Na^+ ion channels was examined by means of cell-based assay. Two of the analogs showing moderate inhibitory activity were further evaluated by the use of the patch-clamp method in cells that expressed $Na_V1.4$ (a TTX-sensitive Na^+ channel subtype) and $Na_V1.5$ (a TTX-resistant Na^+ channel subtype). These compounds showed moderate inhibitory activity toward $Na_V1.4$, and weaker inhibitory activity toward $Na_V1.5$. Uniquely, however, the inhibition of $Na_V1.5$ by one of the analogs was *irreversible*.

Some STXs have demonstrated pharmaceutical potential as a long-term anesthetic in the treatment of anal fissures and for chronic tension-type headache. The recent elucidation of the STX biosynthetic gene cluster in cyanobacteria and the identification of new STX analogs will present opportunities to further explore the pharmaceutical potential of these intriguing alkaloids [37.186]. A specific suite of analogs can be isolated from a single STXs-producing organism, which is directly a result of the evolution of genes present within the organism's genome. Naturally occurring STXs can also be precursors for extracellular metabolic or chemical transformations into new analogs. Knowledge of these transformations may have important implications for the detection, toxicity, and removal of STXs from a contaminated source. Other medicinal uses for STXs may become more established by screening the bioactivity of less toxic analogs, since their use as a potential local anesthetic has long been known. The characterization of STX biosynthesis genes [37.165, 248] and their potential use in combinatorial biosynthesis, together with the constant discovery of novel analogs (either natural or transformed), is likely to expand the possibilities for their pharmaceutical use.

STX also has a huge pharmaceutical potential for its ability to induce anesthesia through interaction with the VGSC [37.186]. It has been suggested that site 1 blockers prolong the duration of anaesthesia in a synergistic manner when combined with other local anaesthetics. In spite of this, the push for STX to enter clinical tri-

als has been hindered by its systematic toxicity. The use of STX as a slow release, prolonged anesthetic was recently demonstrated using a novel controlled release system in male rats. Liposomal formulations of STX, either alone or in conjunction with dexamethasone and/or bupivacaine, were able to block the sciatic nerve in rats for long periods with no damaging myotoxic, cytotoxic, or neurotoxic effects and little associated inflammation. Liposome formulations of STX for slow and site-directed release for prolonged anaesthesia have been postulated as a putative treatment of localized and severe joint pain.

GTX2 and GTX3 also have clinical potential and have been utilized for the treatment of anal fissures by their direct injection into both sides of the fissure. A success rate of 98% with remission after 15 and 28 days for acute and chronic conditions respectively was observed. Both toxins have also been used in the treatment of chronic tension type headache, with 70% of patients responding to treatment. These studies recognize that several STXs have potential as future pharmaceutical leads; therefore, more investigation is needed in this field [37.6].

NEO-STX has being developed as a local anesthetic. Doses of 100 μg have been used by local infiltration in anesthetized adult humans without adverse effect. *Wylie* et al. hypothesized that similar doses could cause significant respiratory, neuromuscular, and cardiovascular impairment [37.249].

Domoic Acid (ASP)

DA belongs to the kainoid class of compounds, which is a class of excitatory neurotransmitters. Pharmacological studies reported that DA isomers are not as toxic as DA because they bind less strongly to the kainate receptor proteins than DA itself; however, 5′-epi-domoic acid has a similar toxicity [37.35]. *Mayer* and *Hamann* [37.250] reviewed the available literature on pharmacological and toxicological studies with marine natural products including articles reporting on the bioactivity and/or pharmacology of 106 marine chemicals. They included DA in the group of compounds that affect the immune system. Moreover they reported that DA at in vitro concentrations that were toxic to neuronal cells (1 mM) was shown to trigger a limited activation of rat brain microglia, an immune cell type that contributes to circa 10% of the total glial population in the central nervous system, and the concomitant release of two potentially neurotoxic mediators, namely TNF-α and matrix metalloproteinase-9.

DA acts as a potent glutamate receptor agonist. Although mechanisms of domoic acid neurotoxicity are poorly defined, an excitotoxic mechanism is believed to be involved. DA inhibits astrocytes glutamate uptake in a dose-dependent manner, leading to accumulation of glutamate in the extracellular compartment and thus producing neurotoxicity [37.251].

In vitro studies have shown that, depending on its concentration, DA causes necrotic or apoptotic cell death in mouse cerebellar granule neurons (CGN). Necrosis is observed at higher concentrations of DA (10 μM) and involves activation of both AMPA/kainate and *N*-metyl-D-aspartic acid (NMDA) receptors, the latter activated by DA-induced glutamate release. In contrast, apoptotic damage predominates at lower concentrations of DA (e.g., 100 nM) and is solely due to activation of AMPA/kainate receptors [37.252]. Both, necrosis and apoptosis have been shown to be mediated by oxidative stress. Upon oral administration, DA is poorly absorbed, has a short half-life in blood, and poorly permeates the blood–brain barrier, limiting the access of low levels of DA in the central nervous system, but this might not be the case during development, a period of suggested particular sensitivity to DA neurotoxicity. The authors evaluated if a prolonged exposure to low DA levels would result in neurotoxicity and/or in altered susceptibility to a subsequent exposure in cultured mouse CGNs. Neurons from wild-type mice and from mice lacking the glutamate cysteine ligase (GCL) modifier subunit ($Gclm^{-/-}$) (already known to be highly susceptible to DA neurotoxicity) were compared. They found that prolonged exposure to low-level DA in vitro provides protection against toxicity of a higher DA acute exposure, apparently due to the ability of chronic low dose of DA to up-regulate glutathione (GSH) synthesis. Genetic conditions leading to low GSH levels, which already increase susceptibility to DA neurotoxicity, could potentially negate such protective mechanisms [37.252].

37.7.3 Other Toxins

Ciguatoxins (CTXs)

CFP is the most frequently reported seafood-toxin illness in the world. It produces gastrointestinal, cardiovascular, neurologic, and neuropsychiatric symptoms which can last from days to even months. CTXs derive from benthic dinoflagellates and are transferred through the food web from the algae to herbivorous and carnivorous fish, and finally to humans.

CTXs are among the most potent natural substances known. One of the Pacific Ocean ciguatoxins, P-CTX-1, poses a health risk at concentrations as low as 0.08–0.1 μg/kg although CTXs rarely accumulates in fish at levels that are lethal to humans [37.253]. CTXs activate the VGSCs in cell membranes, which increases Na^+ ion permeability and depolarizes the nerve cell. This depolarization is believed to cause the array of neurological signs associated with CFP. Voltage-clamp studies suggested that CTXs cause spontaneous, and enhance evoked, action potentials by lowering activation thresholds and delayed repolarization of VGSCs. Several studies further elucidated cellular effects and molecular mechanisms of CTXs actions including selective binding and competition with BTX for site 5 on VGSCs, elevation of intracellular Ca^{2+} levels, stimulation of spontaneous and evoked neurotransmitter release from synaptosomes and motor nerve terminals, axonal and Schwann cell edema, induction of TTX-sensitive leakage current in dorsal root ganglion neurons and blockade of voltage-gated potassium channels [37.254]. Neurological symptoms are consistent with CTXs' interactions with VGSCs; however, all the symptoms produced during CFP may not be exclusively due to VGSCs [37.46]. The chronic fatigue syndrome that may last for weeks or months has been related to high nitric oxide (NO) production. Penil and muscular relaxation, as well as L-type Ca^{2+} channel activation, were shown to be NO-mediated via the nitric oxide synthetase pathway.

CTX also caused rapid hypothermia in mice, which lose their ability to regulate their own body temperature and became poikilothermic. This reduction in body core temperature was found to be due to neuroexcitatory actions of ciguatera on regions of the brain stem receiving vagal afferents and ascending pathways associated with visceral and thermoregulatory responses [37.255]. These authors reported a complete review on the pharmacological effects of CTXs, including cardiovascular and gastrointestinal effects, effects on other smooth muscle, actions on ganglionic and neuromuscular transmission and on VGIC, and other Na^+-dependent actions. Despite the multiple pharmacological effects no medical applications of these toxins have been reported so far.

Brevetoxin-Group Toxins

BTXs are lipid soluble cyclic polyethers (M.W. ≈ 900) and potent neurotoxins that open VGSCs leading to Na^+ influx into the cell. BTXs exert their toxicity by interacting with neurotoxin receptor site 5 associated with domain IV of the α subunit of the VGSC. BTXs binding to tissues that contain VGSCs on excitable cells results in membrane depolarization, repetitive firing, and increase in Na^+ currents [37.256]. At the Na^+ channel, at least four actions of BTXs have been identified leading to the depolarization of excitable cell membranes: shift of the voltage dependence, inhibition of inactivation, increase of mean open times and multiple subconductance levels. A description of these actions and the mammalian BTXs toxicity was recently review [37.63].

BTXs exert a variety of actions in vivo affecting both the peripheral as well as the central nervous systems, probably due to their high lipid solubility and ability to penetrate the blood–brain barrier. Humans exposed to the toxin usually exhibit neurotoxic shellfish poisoning symptoms, which include nausea, difficulties in movement, and seizures [37.257]. Cellular effects associated with exposure to BTXs have been observed in the immune system of many species, although the mechanisms of action of BTXs on immune cells and immune competence are not well understood. Several potential mechanisms for BTX immunotoxicity have been suggested, including the inhibition of cathepsin, apoptosis, release of inflammatory mediators, and oxidative stress [37.58]. In vitro experiments have demonstrated possible DNA damage, chromosomal aberrations and effects on cellular growth [37.256, 258]. Other immune system effects include mast cell degranulation [37.259] and histamine release which may contribute to observed airway responses following the inhalation of aerosolized BTXs.

There are over 10 different BTXs isolated in seawater blooms and *K. brevis* cultures, as well as multiple analogs and derivatives from the metabolism of shellfish and other organisms [37.58]. *Cao* et al. [37.260], quantified the potencies and efficacies of an array of lipophilic VGSCs gating modifiers by measuring Na^+ influx in murine neocortical neurons. An assay using a Na^+-sensitive fluorescent dye to primary cultures of neurons afforded a highly quantitative assessment of the increments in neuronal Na^+ level produced by gating modifier toxins. The relative efficacies differ substantially; the rank order of efficacy of several naturally occurring BTXs and semisynthetic analogs as Na^+ channel gating modifiers was PbTx-1 > PbTx-desoxydioxolane > PbTx-2 = PbTx-3 < PbTx-3α-naphthoate. *K. brevis* cultures also produce shorter ring structures, hemi-BTXs and brevenals, likely to be incomplete products of the BTX biosynthetic pathway. These smaller cyclic ether ring

structures exhibit reduced biological activity, yet they are still consistent with a site of action common to the BTXs [37.63]. An interesting discovery was the finding that brevenal (produced both in *K. brevis* laboratory cultures and in the environment during *K. brevis* blooms), is a brevetoxin antagonist. This is a case of a toxin producing organism that also secretes its own antagonist. Brevenal is synthesized by *K. brevis* in significant amounts and it acts at a different receptor site on nerve cells than the brevetoxins. It is a new and important natural product which could treat chronic obstructive pulmonary disease and cystic fibrosis [37.58].

Taupin [37.261] studied the activity of four generic formulae of BTX derivatives – formulae I–IV – and their compounds on the growth of neurites. He reported that BTX derivatives stimulate neurite growth, particularly the growth of minor processes from which the axons form, on neurons in primary cultures. The activity is mediated by VGNC and the *N*-methyl-D-aspartate-mediated intracellular Ca^{2+} pathway. They proposed the use of BTX derivatives for enhancing neuronal growth and for the treatment of neurodegenerative and neurological disorders, such as Alzheimer's disease, amyotrophic lateral sclerosis, cerebral strokes, traumatic brain, and spinal cord injuries.

Tetrodotoxin

TTX is a very well-known marine neurotoxin due to its frequent involvement in fatal food poisoning, unique chemical structure, and specific action of blocking Na^+ channels of excitable membranes [37.262]. The toxicity of TTX relates to two factors: paralysis of skeletal muscle, including the diaphragm and intercostal muscles, leading to respiratory failure, and reduced blood pressure, predominantly due to vasodilatation. Early signs of this effect might include weakness, tingling of the lips and dizziness [37.70]. Pharmacological activity of TTX on various animals, their organs and tissues has been extensively studied, and it was found that TTX strongly inhibits nerve conduction and has inhibitory effects on Na^+-K^+-ATPase activity. Recently, TTX has been shown to block L-type Ca^{2+} current (ICa) in canine cardiac cells [37.263]. These authors studied the TTX-sensitivity of Ca in isolated canine ventricular myocytes as a function of channel phosphorylation, extracellular pH, and the redox potential of the bathing medium using the whole cell voltage clamp technique. They demonstrated that TTX inhibits L-type Ca channels in the heart by binding to its selectivity filter.

TTX has been extensively used to elucidate the role of specific VGSCs subtypes in a wide range of physiological and pathophysiological processes in the nervous system. VGSCs play a key role in pain and TTX-sensitive subtypes have received much attention over the past few years because these channels have been strongly implicated in normal and pathological pain. Since TTX blocks this subset of VGSCs in a highly selective manner, this toxin may have a potential role in relieving pain [37.264, 265]. *Nieto* et al. [37.266] evaluated the effect of the acute systemic administration of low, nontoxic, doses of TTX on the expression of different signs (heat hyperalgesia, mechanical- and cold allodynia) of paclitaxel-induced neuropathic pain in mice. They reported that low doses of TTX can be useful to prevent and treat this pain since inhibited hyperresponsiveness to thermal and mechanical stimuli. Their findings suggest that TTX-sensitive subtypes of Na^+ channels play a role in the pathogenesis of chemotherapy-induced neuropathic pain.

Hagen et al. [37.267] evaluated the analgesic activity of TTX administered subcutaneously in a randomized, placebo-controlled, parallel design study, in patients with moderate or severe unrelieved cancer pain. This trial suggested that TTX could potentially relieve moderate to severe, treatment-resistant cancer pain in a large proportion of patients, and often for prolonged periods following treatment. In a later study [37.268] it was assessed the long-term safety and efficacy of subcutaneous TTX treatment in reducing the intensity of chronic cancer-related pain. They conducted a multicenter open-label longitudinal trial, in which 30 µg TTX was administered subcutaneously for 4 days in a heterogeneous cohort of patients with persistent pain. Onset of pain relief in no more than half the patients was typically cumulative over days, and after administration ended, the analgesic effect subsided over the course of a few weeks. Toxicity was usually mild or moderate and remained so through subsequent treatment cycles, with no evidence of cumulative toxicity or tolerance. They concluded that long-term treatment with TTX is associated with acceptable toxicity and, in a substantial minority of patients, resulted in a sustained analgesic effect. *Berde* et al. [37.70] reported that prolonged duration percutaneous sciatic nerve blockade could be achieved in rats using combinations of site 1 Na^+ channel toxins with either bupivacaine or epinephrine. Addition of either bupivacaine or epinephrine increases the LD_{50} of TTX in rats, i. e., reduces systemic toxicity from TTX, and also increases the potency of TTX by producing sensory blockade, thereby substantially improving the therapeutic index of TTX.

Thus, combination of formulations seems desirable from the standpoint of both efficacy and safety. TTX, formulated as Tectin, underwent Phase III trials as an injectable systemic analgesic for chronic cancer pain. *Berde* et al. [37.70] examined dose–duration relationships and sciatic nerve histology following local nerve blocks with combinations of Tectin with bupivacaine 0.25% solutions, with or without epinephrine 5 μg/mL in rats. Three-way combinations of TTX, bupivacaine and epinephrine produced significant prolonged sciatic nerve blockade in rats, compared to bupivacaine plain or bupivacaine + epinephrine. Their studies indicate that clinically relevant combinations of Tectin with commercially available bupivacaine 0.25% and epinephrine 5 μg/mL warrant further study for prolonged-duration local anesthesia. Moreover they suggest that a single-injection approach to prolonged local anesthesia would provide good pain relief as a component of multimodal analgesic regimens, reducing postoperative opioid requirements and improving the course of postoperative recovery and acute rehabilitation.

A review by *Nieto* et al. [37.269] focuses on the preclinical and clinical evidence supporting a potential analgesic role for TTX. In addition they compiled data on the contribution of specific TTX-sensitive VGSCs to pain. They conclude that the therapeutic use of TTX as an analgesic agent seems hopeful although further preclinical and clinical research is needed to clarify its potential use during painful conditions. *Wang* et al. [37.270] examined the effect of TTX, applied topically to the rat cornea, on the duration of corneal anesthesia, administered with either proparacaine (PPC) or the chemical permeation enhancer octyl-trimethyl ammonium bromide (OTAB). The effect of test solutions on corneal healing was also studied. Combination of TTX and PPC resulted in corneal anesthesia that was 8 to 10 times longer in duration than that from either drug administered alone, whereas OTAB did not prolong anesthesia. The rate of corneal healing was moderately delayed after coadministration of TTX and PPC. They concluded that coadministration of TTX and PPC significantly prolonged corneal anesthesia, but in view of delayed corneal reepithelialization, caution is suggested in the use of the drug combination.

Bragadeeswaran et al. [37.262] isolated three TTX-producing bacteria (*Kytococcus sedentarius*, *Cellulomonas fimi* and *Bacillus* species) from the pufferfish *Arothron hispidus*. Their partially purified filtrates exhibited hemolytic activity on chicken and human erythrocytes and also presented ATPase, Mg^{2+}-ATPase, Na^+K^+-ATPase, and AchE enzymatic activities. The authors also evaluated if the viability of leukemia cell line (P388) was adversely affected upon adding the crude microbial extracts and found a dose-dependent growth inhibition. These TTX-producing bacteria hold promise for the development of effective antitumor compounds.

Palytoxin and Analogs

PlTXs are a group of complex, extremely potent, and marine natural products. PlTX is a large (molecular weight: 2681), water-soluble polyalcohol first isolated from zoanthids (genus *Palythoa*) even though dinoflagellates of the genus *Ostreopsis* are postulated as the PlTX origin [37.77]. PlTX has been found in a variety of marine organisms ranging from dinoflagellates to fish and it is implicated in seafood poisoning with potential danger to public health. The most commonly reported complications of PlTX poisoning appears to be rhabdomyolysis, a syndrome injuring skeletal muscle, causing muscle breakdown, and leakage of large quantities of intracellular (myocyte) contents into blood plasma. Other symptoms associated with PlTX poisoning in humans are characterized by abdominal cramps, nausea, vomiting, diarrhea, paresthesia, bradycardia, renal failure, cyanosis, and respiratory distress [37.79]. The latter two precede death in fatal cases.

Toxicological studies with these compounds show repeatedly low LD_{50} values in different mammals, revealing an acute toxic effect on several organs, as demonstrated by different routes of exposure. Acute oral administration in mice induced histological and ultrastructural changes in several organs, such as liver, pancreas, cardiac, and skeletal muscle cells. The recognition of its wide distribution coupled with the poisoning effects that these toxins can have on animals, and especially on humans, have concerned the scientific community [37.79]. The toxin is known to affect cellular functions by selective binding to the Na^+-K^+ ATPase and converting the pump into a nonselective channel [37.195, 271]. The induced ion flux alterations are reported to target pathways involved in cytoskeletal dynamics within different cellular models [37.77]. Na^+-K^+ ATPase also functions as a signal transducer to relay messages from the plasma membrane to the intracellular organelles. The toxicological effects of acute oral administration of 42-OH-PlTX were evaluated in mice, as well as its in vitro delayed hemolytic effects [37.272]. The compound showed a toxicity profile comparable with that of the parent compound palytoxin (LD_{50} = 651 and 767 mg/kg, respectively), both

as lethality and acute pathological effects. Also the in vitro hemolytic activity of 42-OH-PlTX was similar to that of PlTX in agreement with the common mechanism of action on the Na^+/K^+ pump, to which the two compounds bind with the same affinity.

Franchini et al. [37.204] used the Frog Embryo Teratogenesis Assay-Xenopus (FETAX) to verify the effects of PlTX in mortality, delayed growth, and embryo malformation. The toxicological effects of PlTX evaluated by FETAX assay revealed evident impacts on embryo mortality, teratogenesis and growth. The histological analysis of the surviving young larvae revealed structural changes compared with controls in the nervous and muscle tissue, even if some specimens did not show any significant histopathological modifications. A general reduction in the size of the main inner visceral organs (i. e., intestine, pancreas, and liver) was reported, but no morphological changes. Severe injury to the heart structure was observed in some specimens.

PlTX is a novel skin tumor promoter, which has been used to probe the role of different types of signaling mechanisms in carcinogenesis. The multistage mouse skin model indicates that tumor promotion is an early, prolonged, and reversible phase of carcinogenesis. Understanding the molecular mechanisms underlying tumor promotion is therefore important for developing strategies to prevent and treat cancer [37.273]. PlTX stimulates a wide range of cellular responses that are likely to play a role in carcinogenesis. Stimulation of arachidonic acid metabolism and the production of prostaglandins modulation of the epidermal growth factor receptor, and modulation of mitogen-activated protein (MAP) kinase cascades are the best studied [37.273]. MAP kinases are a family of serine/threonine kinases that relay a variety of signals to the cellular machinery that regulates cell fate and function. This author [37.274] investigated how palytoxin stimulates MAP kinase activity and how MAP kinases mediate the response of cells to PlTX concluding that MAP kinases appear to be important mediators of PlTX-stimulated signals. The central role of MAP kinases in regulating a variety of critical cellular functions, ranging from enzyme activity to gene expression and ultimately to proliferation and apoptosis, may help explain how palytoxin can stimulate the variety of effects that are characteristic of tumor promoters. Other author suggests that PlTX conjugates could be used as prodrugs in cancer chemotheraphy due to its high cytotoxicity in vitro indicating that targeting the prodrug to a tumor, and there releasing the toxin, selective destruction of neoplastic cells could be achieved [37.275]. Many findings on the involvement of PlTX and analogs acting on different cellular targets and modulating the actin cytoskeleton within different cellular models were published [37.77, 276, 277]. *Louzao* et al. suggest that also cytoskeleton is necessary for PlTX activity and that this may find important applications in biomedical research, with particular interest toward the discovery and development of cytotoxic compounds providing novel antitumor therapy [37.77].

Cyclic Imines

CIs toxins are a very diverse group of marine biotoxins containing an imino functional group as part of its cyclic structure. In many of them, it was found that the toxicity is greatly diminished if imino group is reduced or destroyed [37.278]. Nowadays, there is no evidence of human poisoning caused by cyclic imines but the toxicity observed in experimental animals requires a deeper study of their activity and distribution [37.279]. Only the toxicity of spirolides (SPXs) and gymnodimines (GYMs) has been studied in depth. Spiroimine moiety has been established as the common pharmacophore unit of CIs group, although structural change in other parts of the molecules can also affect the toxicity [37.280]. Primary studies on mechanism of action of CIs suggested that it could be related to the interference on transmission of nervous impulses at neuromuscular junction level. It has been demonstrated that SPXs and GYMs act as potent antagonists of nicotinic (nAChRs) and muscarinic (mAChRs) acetylcholine receptors [37.281–283]. *Kharrat* et al. [37.281] demonstrated that GYM A broadly interferes in neuromuscular transmission by directly blocking muscle-type nAChRs. GYM A interacts with a wide range of muscular and neuronal types of nAChRs, larger than other well characterized toxins that also act on nicotinic receptors [37.281]. *Bourne* et al. [37.282] confirmed the interaction of SPX and GYM with several muscle and neuronal types of nAChRs by competition binding experiments against labeled α-bungarotoxin and epibatidine. No agonist effect was observed with SPX or GYM, but both toxins showed a dose-dependent antagonism in muscle- and neuronal-type receptors. The study concluded that SPX and GYM toxins display high affinity and potent antagonism but limited selectivity for the muscle type versus neuronal subtypes of nAChR [37.282].

SPXs effects on mAChRs were studied by *Wandscheer* et al. in a human neuronal model. They observed that 13-desmethyl SPX C caused a dose- and time-dependent inhibition on neuroblastoma to acetylcholine

extracellular stimulation. They demonstrated by competitive binding assays that the 13-desmethyl SPX is a competitive antagonist of mAChRs and also causes an internalization of the M3-type muscarinic receptor that might contribute to the inhibitory effect of the toxin on the Ca^{2+} response to acetylcholine stimulation [37.283].

Maitotoxins and Gambierol

Many bioactive compounds have been isolated from *Gambierdiscus* spp., i. e., maitotoxins (MTXs), gambierols, and gambieric acid. MTX increases intracellular Ca^{2+} and is considered one of the most potent marine biotoxins when injected intraperitoneally (ip) in mice, with an LD_{50} (24 h) of 50 ng/kg. However, MTX has no proven role in human intoxication due to its low capacity for accumulation in fish flesh and low oral potency [37.46]. At cellular level, MTX causes an increase in Ca^{2+} and Na^{+} by activating Ca^{2+}-permeable nonselective cation channels, resulting in potent membrane depolarization. This causes, in excitable cells, the opening of voltage-gated Ca^{2+} and Na^{+} channels. The massive influx of ions could exceed cell buffering mechanisms affecting cell viability [37.284]. Due to its ability to activate Ca^{2+}-permeable nonselective cation channels, MTX is considered a powerful tool in the study of Ca^{2+}-dependent mechanisms. Another MTX effect is the formation of cytolytic/oncotic pores that finally conduce to cell membrane blebbing and oncotic cell death. For this reason, MTX could be a unique tool to explore oncotic/necrotic cell death [37.284].

Gambierol has moderate inhibitory activity against VGSCs and modified Ca^{2+} homeostasis. High concentrations of this toxin activate Na^{+} channels in human neuroblastoma cells, and, as a consequence, gambierol induces a cytosolic Ca^{2+} increment [37.285]. *Cagide* et al. tested the effect of gambierol on the shape and F-actin cytoskeleton of neuroblastoma. Cells remained with spread morphology and a well-defined actin cytoskeleton, without showing evidence of alterations. Moreover, gambierol was able to produce depolarization, as well as a Ca^{2+} influx, in neuroblastoma cells and scarcely reduced their metabolism [37.286]. *Alonso* et al. [37.287] designed and synthesized truncated skeletal analogs of gambierol with inhibitory activity against voltage-gated potassium (Kv) channels. They examined the effect of these compounds in an in vitro model of Alzheimer's disease obtained from triple transgenic ($3 \times$ Tg-AD) mice, which expresses amyloid beta ($A\beta$) accumulation and tau hyperphosphorylation. In vitro preincubation of the cells with the compounds resulted in significant inhibition of K^{+} currents, a reduction in the extra- and intracellular levels of $A\beta$, and a decrease in the levels of hyperphosphorylated tau, reducing these two cellular pathologies. Thus, these compounds could be useful chemical probes for understanding the function of Kv channels and for elucidating the molecular mechanism of $A\beta$ metabolism modulated by NMDA receptors. Moreover, since both $A\beta$ peptides and phosphorylated tau proteins are supposed to interact with NMDA receptors and to be implicated in the glutamate enhancement of AD pathology, they could have a potential pharmacological use.

Gambieric acids isolated from the culture medium of *G. toxicus* were described as potent antifungal polyether compounds [37.46].

References

37.1 European Parliament: Regulation (EC) No 853/2004 of the European Parliament and of the council of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs, Off. J. Eur. Union L **139**, 55–205 (2004)

37.2 M. Andjelkovic: Emerging marine biotoxins – Report from the seminar, Semin. Emerg. Toxins, Vigo-Pontevedra (European Union Reference Labratory for Marine Biotoxins, Vigo Pontevedra Spain 2012)

37.3 M. Kat: *Dinophysis acuminata* blooms in the Dutch coastal area related to diarrhetic mussel poisoning in the Dutch Waddensea, Sarsia **68**(1), 81–84 (1983)

37.4 M. Kat: Diarrhetic mussel poisoning in the Netherlands related to the dinoflagellate *dinophysis acuminata*, Antonie Van Leeuwenhoek J. Microbiol. **49**(4–5), 417–427 (1983)

37.5 T. Yasumoto, Y. Oshima, M. Yamaguchi: Occurrence of a new type of shellfish poisoning in the Tohoku district, Bull. Jpn. Soc. Sci. Fish. **44**, 1249–1255 (1978)

37.6 A. Otero, M.J. Chapela, M. Atanassova, J.M. Vieites, A.G. Cabado: Biological activities and potential applications of marine biotoxins. In: *Marine Biomaterials*, ed. by S.-K. Kim (CRC/Taylor Francis, Boca Raton 2013)

37.7 European Food Safety Authority: Marine biotoxins in shellfish – Domoic acid. Scientific Opinion of the Panel on Contaminants in the Food chain, EFSA Journal **1181**, 1–62 (2009)

37.8 P. Vale, M.A. Sampayo: Esterification of DSP toxins by Portuguese bivalves from the Northwest coast determined by LC-MS – A widespread phenomenon, Toxicon **40**(1), 33–42 (2002)

37.9 P. Cohen: The structure and regulation of protein phosphatases, Annu. Rev. Biochem. **58**, 453–508 (1989)

37.10 T.A. Haystead, A.T.R. Sim, D. Carling, R.C. Honnor, Y. Tsukitani, P. Cohen, D.G. Hardie: Effects of the tumor promoter okadaic acid on intracellular protein phosphorylation and metabolism, Nature **337**, 78–81 (1989)

37.11 T. Yasumoto, M. Murata, Y. Oshima, M. Sano, G.K. Matsumoto, J. Clardy: Diarrhetic shellfish toxins, Tetrahedron **41**(6), 1019–1025 (1985)

37.12 J. Blanco, G. Alvarez, E. Uribe: Identification of pectenotoxins in plankton, filter feeders, and isolated cells of a *Dinophysis acuminata* with an atypical toxin profile, from Chile, Toxicon **49**(5), 710–716 (2007)

37.13 B. Paz, A.H. Daranas, M. Norte, P. Riobo, J.M. Franco, J.J. Fernandez: Yessotoxins, a group of marine polyether toxins: An overview, Mar. Drugs **6**(2), 73–102 (2008)

37.14 M. Murata, M. Kumagai, J.S. Lee, T. Yasumoto: Isolation and structure of yessotoxin, a novel polyether compound implicated in diarrhetic shellfish poisoning, Tetrahedron Lett. **28**, 5869–5872 (1987)

37.15 K.J. Lee, J.S. Mok, K.C. Song, H. Yu, J.H. Jung, J.H. Kim: Geographical and annual variation in lipophilic shellfish toxins from oysters and mussels along the South coast of Korea, J. Food Prot. **74**(12), 2127–2133 (2011)

37.16 G. Alvarez, E. Uribe, R. Diaz, M. Braun, C. Marino, J. Blanco: Bloom of the Yessotoxin producing dinoflagellate *Protoceratium reticulatum* (Dinophyceae) in Northern Chile, J. Sea Res. **65**(4), 427–434 (2011)

37.17 T. Yasumoto, A. Takizawa: Fluorometric measurement of yessotoxins in shellfish by high-pressure liquid chromatography, Biosci. Biotechnol. Biochem. **61**(10), 1775–1777 (1997)

37.18 C.O. Miles, A.L. Wilkins, A.D. Hawkes, A.I. Selwood, D.J. Jensen, R. Munday, J.M. Cooney, V. Beuzenberg: Polyhydroxylated amide analogs of yessotoxin from *Protoceratium reticulatum*, Toxicon **45**(1), 61–71 (2005)

37.19 H. Ogino, M. Kumagai, T. Yasumoto: Toxicological evaluation of yessotoxin, Nat. Toxins **5**, 255–259 (1997)

37.20 L.A. De la Rosa, A. Alfonso, N. Vilariño, M.R. Vieytes, L.M. Botana: Modulation of cytosolic calcium levels of human lymphocytes by yessotoxin, a novel marine phycotoxin, Biochem. Pharmacol. **61**(7), 827–833 (2001)

37.21 M.J. Twiner, R. El-Ladki, J. Kilcoyne, G.J. Doucette: Comparative effects of marine algal toxins azaspiracid-1,-2, and-3 on T. Jurkat lymphocyte cells, Chem. Res. Toxicol. **25**, 747–754 (2012)

37.22 G. Alvarez, E. Uribe, P. Avalos, C. Marino, J. Blanco: First identification of azaspiracid and spirolides in *Mesodesma donacium* and *Mulinia edulis* from Northern Chile, Toxicon **55**(2/3), 638–641 (2010)

37.23 A. López-Rivera, K. O'Callaghan, M. Moriarty, D. O'Driscoll, B. Hamilton, M. Lehane, K.J. James, A. Furey: First evidence of azaspiracids (AZAs): A family of lipophilic polyether marine toxins in scallops (*Argopecten purpuratus*) and mussels (*Mytilus chilensis*) collected in two regions of Chile, Toxicon **55**(4), 692–701 (2009)

37.24 H. Taleb, P. Vale, R. Amanhir, A. Benhadouch, R. Sagou, A. Chafik: First detection of azaspiracids in mussels in North West Africa, J. Shelfish Res. **25**, 1067–1070 (2006)

37.25 M.J. Twiner, N. Rehmann, P. Hess, G.J. Doucette: Azaspiracid shellfish poisoning: A review on the chemistry, ecology, and toxicology with an emphasis on human health impacts, Mar. Drugs **6**(2), 39–72 (2008)

37.26 R. Ueoka, A. Ito, M. Izumikawa, S. Maeda, M. Takagi, K. Shin-ya, M. Yoshida, R.W.M. van Soest, S. Matsunaga: Isolation of Azaspiracid-2 from a marine sponge *Echinoclathria* sp., as a potent cytotoxin, Toxicon **53**(6), 680–684 (2009)

37.27 K.J. James, C. Moroney, C. Roden, M. Satake, T. Yasumoto, M. Lehane, A. Furey: Ubiquitous 'benign' alga emerges as the cause of shellfish contamination responsible for the human toxic syndrome, azaspiracid poisoning, Toxicon **41**, 145–151 (2003)

37.28 K. Toebe, A.R. Joshi, P. Messtorff, U. Tillmann, A. Cembella, U. John: Molecular discrimination of taxa within the dinoflagellate genus Azadinium, the source of azaspiracid toxins, J. Plankton Res. **35**(1), 225–230 (2013)

37.29 European Food Safety Authority: Opinion of the scientific panel on contaminants in the food chain on a request from the European commission on marine biotoxins in shellfish – Azaspiracids, Eur. Food Saf. Auth. J. **723**, 1–52 (2008)

37.30 European Food Safety Authority: Marine biotoxins in shellfish – Saxitoxin group, Eur. Food Saf. Auth. J. **1019**, 1–46 (2009)

37.31 L. McCauley, D.L. Erdner, S. Nagai, M. Richlen, D.M. Anderson: Biogeographic analysis of the globally distributed harmful algal bloom species *Alexandrium minutum* (Dinophyceae) based on rRNA gene sequences and microsatellite markers, J. Phycol. **45**, 454–463 (2009)

37.32 WHOI (Ed.): Harmful algae (2007), http://www.whoi.edu/redtide/page.do?pid=18103 (accessed January 31, 2012)

37.33 S.M. Etheridge: Paralytic shellfish poisoning: Seafood safety and human health perspectives, Toxicon **56**, 108–122 (2010)

37.34 K.A. Lefebvre, A. Robertson: Domoic acid and human exposure risks: A review, Toxicon **56**(2), 218–230 (2010)

37.35 Y. He, A. Fekete, G. Chen, M. Harir, L. Zhang, P. Tong, P. Schmitt-Kopplin: Analytical approaches for an important shellfish poisoning agent: Domoic Acid, J. Agric. Food Chem. **58**(22), 11525–11533 (2010)

37.36 O.M. Pulido: Domoic acid toxicologic pathology: A review, Mar. Drugs **6**(2), 180–219 (2008)

37.37 J.S. Ramsdell: The molecular and integrative basis to domoic acid toxicity. In: *Phycotoxins Chemistry and Biochemistry*, ed. by L.M. Botana (Blackwell Publishing, Oxford 2007) pp. 223–250

37.38 Y. Kotaki: Ecobiology of amnesic shellfish toxin producing diatoms. In: *Seafood and Freshwater Toxins, Pharmacology, Physiology and Detection*, ed. by L.M. Botana (CRC/Taylor Francis, Boca Raton 2008) pp. 383–396

37.39 J.L.C. Wright, K. Boyd, A.S.W. de Freitas, M. Falk, R. Foxall, W.D. Jamieson, A.W. Laycock, A.W. McCulloch, A.G. McInnes, P. Odense, V.P. Pathak, M.A. Quilliam, M.A. Ragan, P.G. Sim, J.A. Thibault, J.A. Walter, M. Gilgan, D.J.A. Richard, D. Dewar: Identification of domoic acid, a neuroexcitatory amino acid, in toxic mussels from eastern Prince Edward Island, Can. J. Chem. **67**, 481–490 (1989)

37.40 M.A. Quilliam, P.G. Sim, A.W. McCulloch, A.G. McInnes: High-performance liquidchromatography of domoic acid, a marine neurotoxin, with application to shellfish and plankton, Int. J. Environ. Anal. Chem. **36**(3), 139–154 (1989)

37.41 Y. Djaoued, M. Thibodeau, J. Robichaud, S. Balaji, S. Priya, N. Tchoukanova, S.S. Bates: Photocatalytic degradation of domoic acid using nanocrystalline TiO_2 thin films, J. Photochem. Photobiol. **193**, 271–283 (2008)

37.42 J.L.C. Wright, M. Falk, A.G. McInnes, J.A. Walter: Identification of isodomoic acid-d and two new geometrical-isomers of domoic acid in toxic mussels, Can. J. Chem. **68**(1), 22–25 (1990)

37.43 J.L.C. Wright, M.A. Quilliam: Methods for domoic acid, the amnesic shellfish poisoning (ASP) toxin. In: *Manual on Harmful Marine Microalgae*, ed. by G.M. Hallegraeff, D. Anderson, A.D. Cembella (UNESCO, Paris 1995) pp. 115–135

37.44 M. Quilliam: Chemical methods for lipophillic shellfish toxins. In: *Manual on Harmful Marine Microalgae*, ed. by G. Hallegraeff, D.M. Anderson, A.D. Cembella (UNESCO, Paris 2003) pp. 211–245

37.45 European Food Safety Authority: Scientific Opinion on marine biotoxins in shellfish – Emerging toxins: Ciguatoxin group, Eur. Food Saf. Auth. J. **8**(6), 1627–1638 (2010)

37.46 A. Caillaud, P. de la Iglesia, H.T. Darius, S. Pauillac, K. Aligizaki, S. Fraga, M. Chinain, J. Diogene: Update on methodologies available for ciguatoxin determination: Perspectives to confront the onset of ciguatera fish poisoning in Europe, Mar. Drugs **8**(6), 1838–1907 (2010)

37.47 G.K. Isbister, M.C. Kiernan: Neurotoxic marine poisoning, Lancet Neurol. **4**(4), 219–228 (2005)

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

37.49 R.W. Litaker, M.W. Vandersea, M.A. Faust, S.R. Kibler, A.W. Naua, W.C. Holland, M. Chinain, M.J. Holmes, P.A. Tester: Global distribution of ciguatera causing dinoflagellates in the genus *Gambierdiscus*, Toxicon **56**, 711–730 (2010)

37.50 K. Aligizaki, G. Nikolaidis: Morphological identification of two tropical dinoflagellates of the genera *Gambierdiscus* and *Sinophysis* in the Mediterranean Sea, J. Biol. Res.-Thessalon. **9**, 75–82 (2008)

37.51 J.P. Vernoux, R.J. Lewis: Isolation and characterisation of Caribbean ciguatoxins from the horse-eye jack (*Caranx latus*), Toxicon **35**, 889–900 (1997)

37.52 I. Pottier, J. Vernoux, A. Jones, R. Lewis: Analysis of toxin profiles in three different fish species causing ciguatera fish poisoning in Guadeloupe, French West Indies, Food Addit. Contam. **19**, 1034–1042 (2002)

37.53 B. Hamilton, M. Hurbungs, J.P. Vernoux, A. Jones, R.J. Lewis: Isolation and characterization of Indian Ocean ciguatoxin, Toxicon **40**, 685–693 (2002)

37.54 C.C. Davis: Gymnodinium Brevis Sp. Nov. A cause of discolored water and animal mortality in the gulf of Mexico, Bot. Gaz. **109**(3), 358–360 (2002)

37.55 European Food Safety Authority: Panel on contaminants in the food chain (CONTAM), Scientific opinion on marine biotoxins in shellfish – emerging toxins: Brevetoxin group, Eur. Food Saf. Auth. J. **8**(7), 1–29 (2010)

37.56 G. Gunter, F.G.W. Smith, R.H. Williams: Mass mortality of marine animals on the lower west coast of Florida, November 1946–January 1947, Science **105**(2723), 256–257 (1947)

37.57 FAO (Ed.): Marine biotoxins. In: *Food and Nutrition Paper*, Vol. 80, Available from http://www.fao.org/docrep/007/y5486e/y5486e00.HTM (2004)

37.58 L.E. Fleming, B. Kirkpatrick, L.C. Backer, C.J. Walsh, K. Nierenberg, J. Clark, A. Reich, J. Hollenbeck, J. Benson, Y.S. Cheng, J. Naar, R. Pierce, A.J. Bourdelais, W.M. Abraham, G. Kirkpatrick, J. Zaias, A. Wanner, E. Mendes, S. Shalat, P. Hoagland, W. Stephan, J.A. Bean, S. Watkins, T. Clarke, M. Byrne, D.G. Baden: Review of Florida red tide and human health effects, Harmful Algae **10**, 224–233 (2011)

37.59 F. Van Dolah: Marine algal toxins: Origins, health effects, and their increased occurrence, Environ. Health Perspect. **108**(Suppl. 1), 133–141 (2000)

37.60 D.G. Baden, A.J. Bourdelais, H. Jacocks, S. Michelliza, J. Naar: Natural and derivative brevetoxins: Historical background, multiplicity, and effects, Environ. Health Perspect. **113**(5), 621–625 (2005)

37.61 J.H. Landsberg: The effects of harmful algal blooms on aquatic organisms, Rev. Fish. Sci. **10**(2), 113–390 (2002)

37.62 S.M. Plakas, R.W. Dickey: Advances in monitoring and toxicity assessment of brevetoxins in molluscan shellfish, Toxicon **56**(2), 137–149 (2010)

37.63 J.S. Ramsdell: The molecular and integrative basis to brevetoxin toxicity. In: *Seafood and Freshwater Toxins: Pharmacology, Physiology and Detection*, ed. by L.M. Botana (CRC/Taylor Francis, Boca Raton 2008) pp. 519–550

37.64 B.L. Williams: Behavioral and chemical ecology of marine organisms with respect to tetrodotoxin, Mar. Drugs **8**(3), 381–398 (2010)

37.65 B.D. Gessner, B. McLaughlin: Epidemiologic impact of toxic episodes: Neurotoxic toxins. In: *Seafood and Freshwater Toxins. Pharmacology, Physiology and Detection*, ed. by L.M. Botana (CRC/Taylor Francis, Boca Raton 2008) pp. 77–103

37.66 Y. Bentur, J. Ashkar, Y. Lurie, Y. Levy, Z. Azzam, M. Litmanovich: Lessepsian migration and tetrodotoxin poisoning due to *Lagocephalus sceleratus* in the eastern Mediterranean, Toxicon **52**, 964–968 (2008)

37.67 P. Rodriguez, A. Alfonso, C. Vale, C. Alfonso, P. Vale, A. Tellez, L.M. Botana: First toxicity report of tetrodotoxin and 5,6,11-trideoxyTTX in the trumpet shell *Charonia lampas lampas* in Europe, Anal. Chem. **80**, 5622–5629 (2008)

37.68 R. Chau, J.A. Kalaitzis, B.A. Neilan: On the origins and biosynthesis of tetrodotoxin, Aquat. Toxicol. **104**(1/2), 61–72 (2011)

37.69 C.H. Lee, P.C. Ruben: Interaction between voltage-gated sodium channels and the neurotoxin, tetrodotoxin, Channels **2**, 407–412 (2008)

37.70 C.B. Berde, U. Athiraman, B. Yahalom, D. Zurakowski, G. Corfas, C. Bognet: Tetrodotoxin-bupivacaine-epinephrine combinations for prolonged local anesthesia, Mar. Drugs **9**(12), 2717–2728 (2011)

37.71 R.E. Moore, P.J. Scheuer: Palytoxin – New marine toxin from a coelenterate, Science **172**(3982), 495 (1971)

37.72 J.R. Deeds, M.D. Schwartz: Human risk associated with palytoxin exposure, Toxicon **56**(2), 150–162 (2010)

37.73 European Food Safety Authority: Panel on contaminants in the food chain (CONTAM); Scientific opinion on marine biotoxins in shellfish – Palytoxin group, Eur. Food Saf. Auth. J. **7**(12), 1393–1340 (2009)

37.74 P. Ciminiello, C. Dell'Aversano, E. Dello Iacovo, E. Fattorusso, M. Forino, L. Grauso, L. Tartaglione, C. Florio, P. Lorenzon, M. De Bortoli, A. Tubaro, M. Poli, G. Bignami: Stereostructure and biological activity of 42-hydroxy-palytoxin: A new palytoxin analogue from hawaiian *Palythoa* subspecies, Chem. Res. Toxicol. **22**(11), 1851–1859 (2009)

37.75 P. Riobo, J.M. Franco: Palytoxins: Biological and chemical determination, Toxicon **57**(3), 368–375 (2011)

37.76 D.W. Hilgemann: From a pump to a pore: How palytoxin opens the gates, Proc. Natl. Acad. Sci. USA **100**(2), 386–388 (2003)

37.77 M.C. Louzao, I.R. Ares, E. Cagide, B. Espina, N. Vilarino, A. Alfonso, M.R. Vieytes, L.M. Botana: Palytoxins and cytoskeleton: An overview, Toxicon **57**(3), 460–469 (2011)

37.78 A.M. Rodrigues, A.C.G. Almeida, A.F.C. Infantosi: Infantosi: Effect of palytoxin on the sodium-potassium pump: Model and simulation, Phys. Biol. **5**(3), 036005 (2008)

37.79 V. Ramos, V. Vasconcelos: Palytoxin and analogs: Biological and ecological effects, Mar. Drugs **8**(7), 2021–2037 (2010)

37.80 B. Krock, U. Tillmann, U. John, A.D. Cembella: Characterization of azaspiracids in plankton size-fractions and isolation of an azaspiracid-producing dinoflagellate from the North Sea, Harmful Algae **8**(2), 254–263 (2009)

37.81 A. Otero, M.J. Chapela, M. Atanassova, J.M. Vieites, A.G. Cabado: Ciclyc imines chemistry and mechanism of action: A review, Chem. Res. Toxicol. **24**(11), 1817–1829 (2011)

37.82 A. Cembella, B. Krock: Cyclic imine toxins: Chemistry, biogeography, biosynthesis and pharmacology. In: *Seafood and Freshwaters Toxins: Pharmacology, Physiology and Detection*, ed. by L.A. Botana (CRC/Taylor Francis, Boca Raton 2008) pp. 561–580

37.83 T.M. Hu, J.M. Curtis, Y. Oshima, M.A. Quilliam, J.A. Walter, W.M. Watsonwright, J.L.C. Wright: Spirolide-B and Spirolide-D, two novel macrocycles isolated from the digestive glands of shellfish, J. Chem. Soc.-Chem. Commun. **20**, 2159–2161 (1995)

37.84 T. Seki, M. Satake, L. Mackenzie, H.F. Kaspar, T. Yasumoto: Gymnodimine, a new marine toxin of unprecedented structure isolated from New Zealand oysters and the dinoflagellate, *Gymnodinium* sp., Tetrahedron Lett. **36**(39), 7093–7096 (1995)

37.85 S.Z. Zheng, F.L. Huang, S.C. Chen, X.F. Tan, J.B. Zuo, J. Peng, R.W. Xie: The isolation and bioactivities of pinnatoxin, Chin. J. Mar. Drugs **33**(9), 33–35 (1990), in Chinese

37.86 N. Takada, N. Umemura, K. Suenaga, D. Uemura: Structural determination of pteriatoxins A, B and C, extremely potent toxins from the bivalve *Pte-*

ria penguin, Tetrahedron Lett. **42**(20), 3495–3497 (2001)

37.87 K. Torigoe, M. Murata, T. Yasumoto: Prorocentrolide, a toxic nitrogenous macrocycle from a marine dinoflagellate, *Prorocentrum lima*, J. Am. Chem. Soc. **110**, 7876–7877 (1988)

37.88 T.M. Hu, A.S.W. de Freitas, J.M. Curtis, Y. Oshima, J.A. Walter, J.L.C. Wright: Isolation and structure of prorocentrolide B, a fast-acting toxin from *Prorocentrum* maculosum, J. Nat. Prod. **59**(11), 1010–1014 (1996)

37.89 C.-K. Lu, G.-H. Lee, R. Huang, H.-N. Chou: Spiro-prorocentrimine, a novel macrocyclic lactone from a benthic *Prorocentrum* sp. of Taiwan, Tetrahedron Lett. **42**(9), 1713–1716 (2001)

37.90 Y. Kishi: Complete structure of maitotoxin, Pure Appl. Chem. **70**(2), 339–344 (1999)

37.91 M. Murata, H. Naoki, S. Matsunaga, M. Satake, T. Yasumoto: Structure and partial stereochemical assignments for maitotoxin, the most toxic and largest natural non-biopolymer, J. Am. Chem. Soc. **116**(16), 7098–7107 (1994)

37.92 M. Sasaki, N. Matsumori, T. Maruyama, T. Nonomura, M. Murata, K. Tachibana, T. Yasumoto: The complete structure of maitotoxin, Part I: Configuration of the C1–C14 side Chain, Angew. Chem. Int. Ed. Engl. **35**(15), 1672–1675 (1996)

37.93 M. Estacion, W.P. Schilling: Maitotoxin-induced membrane blebbing and cell death in bovine aortic endothelial cells, BMC Physiology **1**, 2–12 (2001)

37.94 K.K.W. Wang, R. Nath, K.J. Raser, I. Hajimohammadreza: Maitotoxin induces calpain activation in SH-SY5Y neuroblastoma cells and cerebrocortical cultures, Arch. Biochem. Biophys. **331**(2), 208–214 (1996)

37.95 M. Carrera, E. Garet, A. Barreiro, E. Garcés, D. Pérez, C. Guisande, A. González-Fernández: Generation of monoclonal antibodies for the specific immunodetection of the toxic dinoflagellate *Alexandrium minutum* Halim from Spanish waters, Harmful Algae **9**, 272–280 (2010)

37.96 A. González-Fernández, E. Garet Fonández, H. Kleivdal, C. Elliot, K. Campbell: Immunological methods for detection of toxic algae and phycotoxins: Immunofluorescence, ELISAS and other innovative techniques. In: *New Trends in Marine and Freshwater Toxins: Food Safety Concerns*, ed. by A.G. Cabado, J.M. Vieites (NovaScience, New York 2012) pp. 267–303, Chapter 9

37.97 L. Campbell, R.J. Olson, H.M. Sosik, A. Abraham, D.W. Henrichs, C.J. Hyatt, E.J. Buskey: First harmful *Dinophysis* (Dinophyceae, Dinophysiales) bloom in the US is revealed by automated imaging flow cytometry, J. Phycol. **46**, 66–75 (2010)

37.98 European Commission: Commission Regulation (EC): No 1244/2007 of 24 October 2007 amending Regulation (EC) No 2074/2005 as regards implementing measures for certain products of animal origin intended for human consumption and laying down specific rules on official controls for the inspection of meat, Off. J. Eur. Union L **281**, 12–18 (2007)

37.99 Y. Zhou, Y.-S. Li, F.-G. Pan, Y.-Y. Zhang, S.-Y. Lu, H.-L. Ren, Z.-H. Li, Z.-S. Liu, J.-H. Zhang: Development of a new monoclonal antibody based direct competitive enzyme-linked immunosorbent assay for detection of brevetoxins in food samples, Food Chem. **118**(2), 467–471 (2010)

37.100 T. Tsumuraya, K. Takeuchi, S. Yamashita, I. Fujii, M. Hirama: Development of a monoclonal antibody against the left wing of ciguatoxin CTX1B: Thiol strategy and detection using a sandwich ELISA, Toxicon **60**(3), 348–357 (2012)

37.101 T.I. Tsumuraya, I. Fujii, M. Hirama: Production of monoclonal antibodies for sandwich immunoassay detection of Pacific ciguatoxins, Toxicon **56**(5), 797–803 (2010)

37.102 A. Sassolas, G. Catanante, A. Hayat, L.D. Stewart, C.T. Elliott, J.L. Marty: Improvement of the efficiency and simplification of ELISA tests for rapid and ultrasensitive detection of okadaic acid in shellfish, Food Control. **30**(1), 144–149 (2013)

37.103 A. Sassolas, A. Hayat, G. Catanante, J.-L. Marty: Detection of the marine toxin okadaic acid: Assessing seafood safety, Talanta **105**, 306–316 (2013)

37.104 M.M. Vdovenko, C. Hung, I.Y. Sakharov, F.-Y. Yu: Determination of okadaic acid in shellfish by using a novel chemiluminescent enzyme-linked immunosorbent assay method, Talanta **116**, 343–346 (2013)

37.105 E. Garet, A.G. Cabado, J.M. Vieites, Á. González-Fernández: Rapid isolation of single-chain antibodies by phage display technology directed against one of the most potent marine toxins: Palytoxin, Toxicon **55**(8), 1519–1526 (2010)

37.106 E. Garet, A. González-Fernández, J. Lago, J.M. Vieites, A.G. Cabado: Comparative evaluation of enzyme-linked immunoassay and reference methods for the detection of shellfish hydrophilic toxins in several presentations of seafood, Toxicon **58**, 1410–1415 (2010)

37.107 M. Dechraoui Bottein, J. Maucher Fuquay, R. Munday, A.I. Selwood, R. van Ginkel, C.O. Miles, J.I. Loader, A.L. Wilkins, J.S. Ramsdell: Bioassays methods for detection of *N*-palmitoylbrevetoxin-B2 (BTX-B4), Toxicon **55**, 497–506 (2010)

37.108 J. Homola, E. Brynda, P. Tobiska, I. Tichy, J. Skvor: Optical biosensors using surface plasmon resonance, Proc. SPIE Photonics Devices Syst. **401**, 6130–6135 (1999)

37.109 J. Abery: SPR technology increases efficiency for discovery and development, Biopharm. Int. **1** (2001)

37.110 R.C. Stevens, S.D. Soelberg, B.-T.L. Eberhart, S. Spencer, J.C. Wekell, T.M. Chinowsky, V.L. Trainer, C.E. Furlong: Detection of the toxin domoic acid from clam extracts using a portable

surface plasmon resonance biosensor, Harmful Algae **6**(2), 166–174 (2007)

37.111 E.S. Fonfría, N. Vilariño, K. Campbell, C. Elliott, S.A. Haughey, B. Ben-Gigirey, J.M. Vieites, K. Kawatsu, L.M. Botana: Paralytic shellfish poisoning detection by surface plasmon resonance-based biosensors in shellfish matrixes, Anal. Chem. **79**(16), 6303–6311 (2007)

37.112 A.D. Taylor, J. Ladd, S. Etheridge, J. Deeds, S. Hall, S. Jiang: Quantitative detection of tetrodotoxin (TTX) by a surface plasmon resonance (SPR) sensor, Sens. Actuators B **130**(1), 120–128 (2008)

37.113 B.J. Yakes, S. Prezioso, S.A. Haughey, K. Campbell, C.T. Elliott, S.L. DeGrasse: An improved immunoassay for detection of saxitoxin by surface plasmon resonance biosensors, Sens. Actuators B **156**(2), 805–811 (2011)

37.114 K. Campbell, A.C. Huet, C. Charlier, C. Higgins, P. Delahaut, C.T. Elliott: Comparison of ELISA and SPR biosensor technology for the detection of paralytic shellfish poisoning toxins, J. Chromatogr. B **877**(32), 4079–4089 (2009)

37.115 K. Metfies, S. Huljic, M. Lange, L.K. Medlin: Electrochemical detection of the toxic dinoflagellate *Alexandrium ostenfeldii* with a DNA-biosensor, Biosens. Bioelectron. **20**(7), 1349–1357 (2005)

37.116 A. Hayat, L. Barthelmebs, J.-L. Marty: Enzyme-linked immunosensor based on super paramagnetic nanobeads for easy and rapid detection of okadaic acid, Anal. Chim. Acta **690**(2), 248–252 (2011)

37.117 R.B. Dominguez, A. Hayat, A. Sassolas, G.A. Alonso, R. Munoz, J.-L. Marty: Automated flow-through amperometric immunosensor for highly sensitive and on-line detection of okadaic acid in mussel sample, Talanta **99**, 232–237 (2012)

37.118 A. Hayat, L. Barthelmebs, A. Sassolas, J.-L. Marty: Development of a novel label-free amperometric immunosensor for the detection of okadaic acid, Anal. Chim. Acta **724**, 92–97 (2012)

37.119 T. Ikehara, S. Imamura, A. Yoshino, T. Yasumoto: PP2A inhibition assay using recombinant enzyme for rapid detection of okadaic acid and its analogs in shellfish, Toxins **2**(1), 195–204 (2010)

37.120 D.O. Mountfort, T. Suzuki, P. Truman: Protein phosphatase inhibition assay adapted for determination of total DSP in contaminated mussels, Toxicon **39**(2/3), 383–390 (2001)

37.121 G. Volpe, E. Cotroneo, D. Moscone, L. Croci, L. Cozzi, G. Ciccaglioni, G. Palleschi: A bienzyme electrochemical probe for flow injection analysis of okadaic acid based on protein phosphatase-2A inhibition: An optimization study, Anal. Biochem. **385**(1), 50–56 (2009)

37.122 H. Smienk, E. Dominguez, A. Otero, A.G. Cabado, J. Vieites: Quantitive determination of okadatic acid toxins group by a colorimetric phosphatase inhibition assay: Collaborative study, J. AOAC Int. **96**(1), 77–85 (2013)

37.123 European Council: (EC) CR (2005) Commission Regulation (EC) No 2074/2005 of 5 December 2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004 of the European Parliament and of the Council and for the organisation of official controls under Regulation (EC) No 854/2004 of the European Parliament and of the Council and Regulation (EC) No 882/2004 of the European Parliament and of the Council, derogating from Regulation (EC) No 852/2004 of the European Parliament and of the Council and amending Regulations (EC) No 853/2004 and (EC) No 854/2004, Off. J. Eur. Union L **338**, 27–59 (2005)

37.124 European Commission: Commision Regulation (EU): No 15/2011 of 10 January 2011 amending Regulation (EC) No 2074/2005 as regards testing methods for detecting marine biotoxins in live bivalve molluscs., vol. 11.1.2011, Off. J. Eur. Union **6**, 3–6 (2011)

37.125 X. Zhang, Z. Zhang: Capillary electrophoresis-based immunoassay with electrochemical detection as rapid method for determination of saxitoxin and decarbamoylsaxitoxin in shellfish samples, J. Food Compos. Anal. **28**(1), 61–68 (2012)

37.126 F. Fournel, E. Baco, M. Mamani-Matsuda, M. Degueil, B. Bennetau, D. Moynet, D. Mossalayi, L. Vellutini, J.P. Pillot, C. Dejous, D. Rebière: Love wave biosensor for real-time detection of okadaic acid as DSP phycotoxin, Sens. Actuators B **170**, 122–128 (2012)

37.127 M.R. Vieytes, A.G. Cabado, A. Alfonso, C. Louzao, A. Botana, L. Botana: Solid-phase radioreceptor assay for paralytic shellfish toxins, Anal. Biochem. **211**, 87–93 (1993)

37.128 G.J. Doucette, M.M. Logan, J.S. Ramsdell, F.M. Van Dolah: Development and preliminary validation of a microtiter plate-based receptor binding assay for paralytic shellfish poisoning toxins, Toxicon **35**(5), 625–636 (1997)

37.129 G. Usup, C.P. Leaw, M.-Y. Cheah, A. Ahmad, B.K. Ng: Analysis of paralytic shellfish poisoning toxin congeners by a sodium channel receptor binding assay, Toxicon **44**(1), 37–43 (2004)

37.130 G.J. Doucette, C.L. Powell, E.U. Do, C.Y. Byon, F. Cleves, S.G. McClain: Evaluation of 11-[3H]-tetrodotoxin use in a heterologous receptor binding assay for PSP toxins, Toxicon **38**(11), 1465–1474 (2000)

37.131 C.L. Powell, G.J. Doucette: A receptor binding assay for paralytic shellfish poisoning toxins: Recent advances and applications, Nat. Toxins **7**(6), 393–400 (1999)

37.132 B.A. Suárez-Isla: Paralytic shellfish toxins – pharmacology, and toxicology biological detection methods. In: *Seafood and Freshwater Toxins, Pharmacology, Physiology and Detection*, ed. by L.A. Botana (CRC/Taylor Francis, Boca Raton 2008) pp. 197–205

37.133 F.M. Van Dolah, S.E. Fuego, T.A. Leighfield, C.M. Mikulsk, G.J. Doucette: Determination of paralytic shellfish toxins in shellfish by receptor binding assay: Collaborative study, J. AOAC Int. **95**(3), 795–812 (2012)

37.134 V.L. Trainer, D.G. Baden, W.A. Catterall: Detection of marine toxins using recontituted sodium channels, J. AOAC Int. **78**, 570–573 (1995)

37.135 R.A. Edwards, A.M. Stuart, D.G. Baden: Brevetoxin binding in three phylogenetically diverse vertebrates, Toxic Marine Phytoplankton, 4th Int. Conf., Lund, Swedan, ed. by E. Graneli (Elsevier, New York 1990), Illus. Maps 290–293

37.136 V.L. Trainer, D.G. Baden: High affinity binding of red tide neurotoxins to marine mammal brain, Aquat. Toxicol. **46**, 139–148 (1999)

37.137 M.L.J. Twiner, M.-Y. Bottein Dechraoui, Z. Wang, C.M. Mikulski, M.S. Henry, R.H. Pierce, G.J. Doucette: Extraction and analysis of lipophilic brevetoxins from the red tide dinoflagellate Karenia brevis, Anal. Biochem. **369**(1), 128–135 (2007)

37.138 M. Chinain, H. Darius, A. Ung, M. Fouc, T. Revel, P. Cruchet, S. Pauillac, D. Laurent: Ciguatera risk management in French Polynesia: The case study of Raivavae Island (Australes Archipelago), Toxicon **56**(5), 674–690 (2010)

37.139 H.T. Darius, D. Ponton, T. Revel, P. Cruchet, A. Ung, M.T. Fouc, M. Chinain: Ciguatera risk assessment in two toxic sites of French Polynesia using the receptor-binding assay, Toxicon **50**, 612–626 (2007)

37.140 F.M. Van Dolah, T.A. Leighfield, B.L. Haynes, D.R. Hampson, J.S. Ramsdell: A microplate receptor assay for the amnesic shellfish poisoning toxin, domoic acid, utilizing a cloned glutamate receptor, Anal. Biochem. **245**(1), 102–105 (1997)

37.141 K.A. Baugh, S. Spencer, J.C. Wekell, V.L. Trainer, K.A. Steidinger, J.H. Landsberg, C.R. Tomas, G.A. Vargo: An alternative method for domoic acid determination in seawater particulates: A receptor binding assay using glutamate dehydrogenase. In: *Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission*, (Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO, Paris 2004)

37.142 T. Yasumoto: Search for ciguatoxin producing clones of *Gambierdiscus toxicus* on Okinawan coastal reef, Ciguatera and Related Biotoxins Workshop, New Caledoria, France, 2000 (Elsevier, Paris 2008)

37.143 D.M. Anderson, A.D. Cembella, G.M. Hallegraeff: Progress in understanding harmful algal blooms: Paradigm shifts and new technologies for research, monitoring, and management, Annu. Rev. Mar. Sci. **4**, 143–176 (2012)

37.144 A. Penna, C. Battocchi, E. Garcés, S. Anglès, E. Cucchiari, C. Totti, A. Kremp, C. Satta, M. Grazia Giacobbe, I. Bravo: Detection of microalgal resting cysts in European coastal sediments using a PCR-based assay, Deep Sea Res. Part II **57**(3), 288–300 (2010)

37.145 L. Galluzzi, A. Cegna, S. Casabianca, A. Penna, N. Saunders, M. Magnani: Development of an oligonucleotide microarray for the detection and monitoring of marine dinoflagellates, J. Microbiol. Methods **84**(2), 234–242 (2011)

37.146 M.W. Vandersea, S.R. Kibler, W.C. Holland, P.A. Tester, T.F. Schultz, M.A. Faust, M.J. Holmes, M. Chinain, R.W. Litaker: Development of semi-quantitative PCR assays for the detection and enumeration of *Gambierdiscus* species (*Gonyaulacales*, *Dinophyceae*), J. Phycol. **48**(4), 902–915 (2011)

37.147 T. Nishimura, S. Sato, W. Tawong, H. Sakanari, K. Uehara, M.M.R. Shah, S. Suda, T. Yasumoto, Y. Taira, H. Yamaguchi: Genetic diversity and distribution of the ciguatera-causing dinoflagellate *Gambierdiscus* spp. (Dinophyceae) in coastal areas of Japan, PLoS ONE **8**(4), e60882 (2013)

37.148 T.A. Rynearson, B. Palenik: Learning to read the oceans: Genomics of marine phytoplankton, Adv. Mar. Biol. **60**, 1–39 (2011)

37.149 B. Palenik, J. Grimwood, A. Aerts, P. Rouze, A. Salamov, N. Putnam, C. Dupont, R. Jorgensen, E. Derelle, S. Rombauts, K. Zhou, R. Otillar, S.S. Merchant, S. Podell, T. Gaasterland, C. Napoli, K. Gendler, A. Manuell, V. Tai, O. Vallon, G. Piganeau, S. Jancek, M. Heijde, K. Jabbari, C. Bowler, M. Lohr, S. Robbens, G. Werner, I. Dubchak, G.J. Pazour, Q. Ren, I. Paulsen, C. Delwiche, J. Schmutz, D. Rokhsar, Y. Van de Peer, H. Moreau, I.V. Grigoriev: The tiny eukaryote *Ostreococcus* provides genomic insights into the paradox of plankton speciation, Proc. Natl. Acad. Sci. USA **104**(18), 7705–7710 (2007)

37.150 C.J. Gobler, D.L. Berry, S.T. Dyhrman, S.W. Wilhelm, A. Salamov, A.V. Lobanov, Y. Zhang, J.L. Collier, L.L. Wurch, A.B. Kustka, B.D. Dill, M. Shah, N.C. VerBerkmoes, A. Kuo, A. Terry, J. Pangilinan, E.A. Lindquist, S. Lucas, I.T. Paulsen, T.K. Hattenrath-Lehmann, S.C. Talmage, E.A. Walker, F. Koch, A.M. Burson, M.A. Marcoval, Y.-Z. Tang, G.R. LeCleir, K.J. Coyne, G.M. Berg, E.M. Bertrand, M.A. Saito, V.N. Gladyshev, I.V. Grigoriev: Niche of harmful alga *Aureococcus anophagefferens* revealed through ecogenomics, Proc. Natl. Acad. Sci. USA **108**(11), 4352–4357 (2011)

37.151 E.V. Armbrust, J.A. Berges, C. Bowler, B.R. Green, D. Martinez, N.H. Putnam, S.G. Zhou, A.E. Allen, K.E. Apt, M. Bechner, M.A. Brzezinski, B.K. Chaal, A. Chiovitti, A.K. Davis, M.S. Demarest, J.C. Detter, T. Glavina, D. Goodstein, M.Z. Hadi, U. Hellsten, M. Hildebrand, B.D. Jenkins, J. Jurka, V.V. Kapitonov, N. Kroger, W.W.Y. Lau, T.W. Lane, F.W. Larimer, J.C. Lippmeier, S. Lucas, M. Medina, A. Montsant, M. Obornik, M.S. Parker, B. Palenik, G.J. Pazour, P.M. Richardson, T.A. Rynearson,

M.A. Saito, D.C. Schwartz, K. Thamatrakoln, K. Valentin, A. Vardi, F.P. Wilkerson, D.S. Rokhsar: The genome of the diatom *Thalassiosira pseudonana*: Ecology, evolution, and metabolism, Science **306**(5693), 79–86 (2004)

37.152 A.Z. Worden, J.-H. Lee, T. Mock, P. Rouze, M.P. Simmons, A.L. Aerts, A.E. Allen, M.L. Cuvelier, E. Derelle, M.V. Everett, E. Foulon, J. Grimwood, H. Gundlach, B. Henrissat, C. Napoli, S.M. McDonald, M.S. Parker, S. Rombauts, A. Salamov, P. Von Dassow, J.H. Badger, P.M. Coutinho, E. Demir, I. Dubchak, C. Gentemann, W. Eikrem, J.E. Gready, U. John, W. Lanier, E.A. Lindquist, S. Lucas, K.F.X. Mayer, H. Moreau, F. Not, R. Otillar, O. Panaud, J. Pangilinan, I. Paulsen, B. Piegu, A. Poliakov, S. Robbens, J. Schmutz, E. Toulza, T. Wyss, A. Zelensky, K. Zhou, E.V. Armbrust, D. Bhattacharya, U.W. Goodenough, Y. Van de Peer, I.V. Grigoriev: Green evolution and dynamic adaptations revealed by genomes of the marine picoeukaryotes micromonas, Science **324**(5924), 268–272 (2009)

37.153 A.E. Allen, J. LaRoche, U. Maheswari, M. Lommer, N. Schauer, P.J. Lopez, G. Finazzi, A.R. Fernie, C. Bowler: Whole-cell response of the pennate diatom *Phaeodactylum tricornutum* to iron starvation, Proc. Natl. Acad. Sci. USA **105**(30), 10438–10443 (2008)

37.154 T. Mock, M.P. Samanta, V. Iverson, C. Berthiaume, M. Robison, K. Holtermann, C. Durkin, S.S. BonDurant, K. Richmond, M. Rodesch, T. Kallas, E.L. Huttlin, F. Cerrina, M.R. Sussmann, E.V. Armbrust: Whole-genome expression profiling of the marine diatom *Thalassiosira pseudonana* identifies genes involved in silicon bioprocesses, Proc. Natl. Acad. Sci. USA **105**(5), 1579–1584 (2008)

37.155 P. von Dassow, H. Ogata, I. Probert, P. Wincker, C. Da Silva, S. Audic, J.-M. Claverie, C. de Vargas: Transcriptome analysis of functional differentiation between haploid and diploid cells of *Emiliania huxleyi*, a globally significant photosynthetic calcifying cell, Genome Biol. **10**(10), R114 (2009)

37.156 M.L. Cuvelier, A.E. Allen, A. Monier, J.P. McCrow, M. Messie, S.G. Tringe, T. Woyke, R.M. Welsh, T. Ishoey, J.-H. Lee, B.J. Binder, C.L. DuPont, M. Latasa, C. Guigand, K.R. Buck, J. Hilton, M. Thiagarajan, E. Caler, B. Read, R.S. Lasken, F.P. Chavez, A.Z. Worden: Targeted metagenomics and ecology of globally important uncultured eukaryotic phytoplankton, Proc. Natl Acad. Sci. USA **107**(33), 14679–14684 (2010)

37.157 K.B. Andree, M. Fernández-Tejedor, L.M. Elandaloussi, S. Quijano-Scheggia, N. Sampedro, E. Garcés, J. Camp, J. Diogène: Quantitative PCR coupled with melt curve analysis for detection of selected *Pseudo-nitzschia* spp. (Bacillariophyceae) from the northwestern Mediterranean Sea, Appl. Environ. Microbiol. **77**(5), 1651–1659 (2011)

37.158 A. Penna, M. Vila, S. Fraga, M.G. Giacobbe, F. Andreoni, P. Riobó, C. Vernesi: Characterization of *Ostreopsis* and *Coolia* (Dinophyceae) isolates in the western Mediterranean Sea based on morphology, toxicity and internal transcribed spacer 5.8S rDNA sequences, J. Phycol. **41**(1), 212–225 (2005)

37.159 B. Genovesi, M.-S. Shin-Grzebyk, D. Grzebyk, M. Laabir, P.-A. Gagnaire, A. Vaquer, A. Pastoureaud, B. Lasserre, Y. Collos, P. Berrebi: Assessment of cryptic species diversity within blooms and cyst bank of the *Alexandrium tamarense* complex (Dinophyceae) in a Mediterranean lagoon facilitated by semi-multiplex PCR, J. Plankton Res. **33**(3), 405–414 (2011)

37.160 D.G. Borkman, T.J. Smayda, C.R. Tomas, R. York, W. Strangman, J. Wright: Toxic *Alexandrium peruvianum* (Balech and de Mendiola) Balech and Tangen in Narragansett Bay, Rhode Island (USA), Harmful Algae **19**, 92–100 (2012)

37.161 F. Perini, A. Casabianca, C. Battocchi, S. Accoroni, C. Totti, A. Penna: New approach using the real-time PCR method for estimation of the toxic marine dinoflagellate *Ostreopsis* cf. *ovata* in marine environment, PLoS One **6**(3), e17699 (2011)

37.162 S. Nagai: Development of a multiplex PCR assay for simultaneous detection of six *Alexandrium* species (*Dinophyceae*), J. Phycol. **47**(3), 703–708 (2011)

37.163 J. Al-Tebrineh, L.A. Pearson, S.A. Yasar, B.A. Neilan: A multiplex PCR targeting hepato- and neurotoxigenic cyanobacteria of global significance, Harmful Algae **15**, 19–25 (2012)

37.164 M.-È. Garneau, A. Schnetzer, P.D. Countway, A.C. Jones, E.L. Seubert, D.A. Caron: Examination of the seasonal dynamics of the toxic dinoflagellate *Alexandrium catenella* at Redondo Beach, California, by quantitative PCR, Appl. Environ. Microbiol. **77**(21), 7669–7680 (2011)

37.165 S.A. Murray, M. Wiese, A. Stuken, S. Brett, R. Kellmann, G. Hallegraeff, B.A. Neilan: *sxtA*-Based quantitative molecular assay to identify saxitoxin-producing harmful algal blooms in marine waters, Appl. Environ. Microbiol. **77**(19), 7050–7057 (2011)

37.166 A. Penna, E. Bertozzini, C. Battocchi, L. Galluzzi, M.G. Giacobbe, M. Vila, E. Garces, A. Luglié: M. Magnani: Monitoring of HAB species in the Mediterranean Sea through molecular methods, J. Plankton Res. **29**(1), 19–38 (2007)

37.167 S.M. Dittami, C. Proux, S. Rousvoal, A.F. Peters, J.M. Cock, J.-Y. Coppee, C. Boyen, T. Tonon: Microarray estimation of genomic inter-strain variability in the genus Ectocarpus (Phaeophyceae), BMC Mol. Biol. **1**, 2 (2011)

37.168 J.U. Kegel, Y. Del Amo, L. Costes, L.K. Medlin: Testing a microarray to detect and monitor toxic microalgae in Arcachon Bay in France, Microarrays **2**(1), 1–23 (2013)

37.169 S.M. Dittami, Y. Pazos, M. Laspra, L.K. Medlin: Microarray testing for the presence of toxic algae monitoring programme in Galicia (NW Spain), Environ. Sci. Pollut. Res. **20**(10), 6778–6793 (2013)

37.170 A. de Bruin, B.W. Ibelings, E. Van Donk: Molecular techniques in phytoplankton research: From allozyme electrophoresis to genomics, Hydrobiologia **491**(1–3), 47–63 (2003)

37.171 T.I. McLean: Eco-omics: A review of the application of genomics, transcriptomics, and proteomics for the study of the ecology of harmful Algae, Microb. Ecol. **65**, 901–915 (2013)

37.172 A. Jamers, R. Blust, W. De Coen: *Omics* in algae: Paving the way for a systems biological understanding of algal stress phenomena?, Aquat. Toxicol. **92**(3), 114–121 (2009)

37.173 I. Yang, U. John, S. Beszteri, G. Gloeckner, B. Krock, A. Goesmann, A.D. Cembella: Comparative gene expression in toxic versus non-toxic strains of the marine dinoflagellate *Alexandrium minutum*, BMC Genomics **11**, 248 (2010)

37.174 N. Jaeckisch, I. Yang, S. Wohlrab, G. Glöckner, J. Kroymann, H. Vogel, A. Cembella, U. John: Comparative genomic and transcriptomic characterization of the toxigenic marine dinoflagellate *Alexandrium ostenfeldii*, PLoS ONE **6**(12), e28012 (2011)

37.175 B.L. Nunn, A.T. Timperman: Marine proteomics, Mar. Ecol. Prog. Ser. **332**, 281–289 (2007)

37.176 L.L. Chan, I.J. Hodgkiss, S. Lu, S.C.L. Lo: Use of two-dimensional gel electrophoresis proteome reference maps of dinoflagellates for species recognition of causative agents of harmful algal blooms, Proteomics **4**(1), 180–192 (2004)

37.177 L.L. Chan, I.J. Hodgkiss, J.M.F. Wan, J.H.K. Lum, A.S.C. Mak, W.H. Sit, S.C.L. Lo: Proteomic study of a model causative agent of harmful algal blooms, *Prorocentrum triestinum* II: The use of differentially expressed protein profiles under different growth phases and growth conditions for bloom prediction, Proteomics **4**(10), 3214–3226 (2004)

37.178 L. Wang, S.A. Shankarappa, R. Tong, J.B. Ciolino, J.H. Tsui, H.H. Chiang, D.S. Kohane: Topical drug formulations for prolonged corneal anesthesia, Cornea **32**(7), 1040–1045 (2013)

37.179 D.M. Anderson, A.D. Cembella, G.M. Hallegraeff: Progress in understanding harmful algal blooms: Paradigm shifts and new technologies for research, monitoring, and management, Annu. Rev. Mar. Sci. **4**, 143–176 (2012)

37.180 O. Fiehn: Combining genomics, metabolome analysis, and biochemical modelling to understand metabolic networks, Comp. Funct. Genomics **2**(3), 155–168 (2001)

37.181 A. Ianora, G. Romano, Y. Carotenuto, F. Esposito, V. Roncalli, I. Buttino, A. Miralto: Impact of the diatom oxylipin 15S-HEPE on the reproductive success of the copepod *Temora stylifera*, Hydrobiologia **666**(1), 265–275 (2011)

37.182 A.R. Fernie, T. Obata, A.E. Allen, W.L. Araujo, C. Bowler: Leveraging metabolomics for functional investigations in sequenced marine diatoms, Trends Plant Sci. **17**(7), 395–403 (2012)

37.183 P. Hess: Specific, Quantitative and metabolomic techniques for the analysis of marine biotoxins, Paper presented at the Marine and Freshwater Toxins Analysis, 4th H. Symp. AOAC Task Force Meed. Baiona (2013)

37.184 D.-Z. Wang: Neurotoxins from marine dinoflagellates: A brief review, Mar. Drugs **6**(2), 349–371 (2008)

37.185 D.-Z. Wang, Y. Gao, L. Lin, H.-S. Hong: Comparative proteomic analysis reveals proteins putatively involved in toxin biosynthesis in the marine dinoflagellate *Alexandrium catenella*, Mar. Drugs **11**(1), 213–232 (2013)

37.186 M. Wiese, P.M. D'Agostino, T.K. Mihali, M.C. Moffitt, B.A. Neilan: Neurotoxic alkaloids: Saxitoxin and its analogs, Mar. Drugs **8**(7), 2185–2211 (2010)

37.187 M.R. Watters: Marine neurotoxins as a starting point to drugs. In: *Seafood and Freshwater Toxins: Pharmacology, Physiology and Detection*, ed. by L.M. Botana (CRC/Taylor Francis, Boca Raton 2008) pp. 889–896

37.188 K.D. Cusick, G.S. Sayler: An Overview on the marine neurotoxin, saxitoxin: Genetics, molecular targets, methods of detection and ecological functions, Mar. Drugs **11**(4), 991–1018 (2013)

37.189 J.J. Fernández, M.L. Candenas, M.L. Souto, M.M. Trujillo, M. Norte: Okadaic acid, useful tool for studying cellular processes, Curr. Med. Chem. **9**, 229–262 (2002)

37.190 H. Fujiki, M. Suganuma: Unique features of the okadaic acid activity on class of tumor promoters, J. Cancer Res. Clin. Oncol. **125**, 150–155 (1999)

37.191 M.M. Islam, S. Begum, L. Lin, A. Okamura, M. Du, S. Fujimura: Synergistic cytotoxic effect between serine-threonine phosphatase inhibitors and 5-fluorouracil: A novel concept for modulation of cytotoxic effect, Cancer Chemother. Pharmacol. **49**, 111–118 (2002)

37.192 V. Valdiglesias, J. Méndez, E. Pásaro, E. Cemeli, D. Anderson, B. Laffon: Assessment of okadaic acid effects on cytotoxicity, DNA damage and DNA repair in human cells, Mutation Res. Fundam. Mol. Mech. Mutagen. **689**(1/2), 74–79 (2010)

37.193 J. He, Y. Yang, H. Xu, X. Zhang, X.M. Li: Olanzapine attenuates the okadaic acid-induced spatial memory impairment and hippocampal cell death in rats, Neuropsychopharmacology **30**, 1511–1520 (2005)

37.194 A.G. Cabado, F. Leira, J.M. Vieites, M.R. Vieytes, L. Botana: Caspase-8 activation initiates okadaic acid-induced apoptosis in neuroblastoma. In: *Molluscan Shellfish Safety*, ed. by B.R.A. Villalba, J.L. Romalde, R. Beiras (Consellería de Pesca e

Asuntos Marítimos da Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, Santiago de Compostela 2003) pp. 107–117
37.195 J. Lago, F. Santaclara, J.M. Vieites, A.G. Cabado: Collapse of mitochondrial membrane potential and caspases activation are early events in okadaic acidtreated Caco-2 cells, Toxicon **46**(5), 579–586 (2005)
37.196 A. Franchini, D. Malagoli, E. Ottaviani: Targets and effects of yessotoxin, okadaic acid and palytoxin: A differential review, Mar. Drugs **8**(3), 658–677 (2010)
37.197 V. Valdiglesias, B. Laffon, E. Pásaro, E. Cemeli, D. Anderson, J. Méndez: Induction of oxidative DNA damage by the marine toxin okadaic acid depends on human cell type, Toxicon **57**(6), 882–888 (2011)
37.198 V. Valdiglesias, B. Laffon, E. Pásaro, J. Méndez: Evaluation of okadaic acid-induced genotoxicity in human cells using the micronucleous test and gamma H2AX analysis, J. Toxicol. Environ. Health Part A: Current Issues **74**(15/16), 980–992 (2011)
37.199 V. Valdiglesias, B. Laffon, E. Pásaro, J. Méndez: Okadaic acid induces morphological changes, apoptosis and cell cycle alterations in different human cell types, J. Environ. Monit. **13**(6), 1831–1840 (2011)
37.200 C. Vale, L.M. Botana: Marine toxins and the cytoskeleton: Okadaic acid and dinophysistoxins, FEBS Journal **275**(24), 6060–6066 (2008)
37.201 F. Leira, C. Alvarez, J.M. Vieites, M.R. Vieytes, L.M. Botana: Characterization of distinct apoptotic changes induced by okadaic acid and yessotoxin in the BE(2)-M17 neuroblastoma cell line, Toxicol. In Vitro **16**(1), 23–31 (2002)
37.202 A.G. Cabado, F. Leira, M.R. Vieytes, J.M. Vieites, L.M. Botana: Cytoskeletal disruption is the key factor that triggers apoptosis in okadaic acid-treated neuroblastoma cells, Arch. Toxicol. **78**, 74–85 (2005)
37.203 F. Santaclara, J. Lago, J.M. Vieites, A.G. Cabado: Effect of okadaic acid on integrins and structural proteins in BE(2)-M17 cells, Arch. Toxicol. **79**(10), 582–586 (2005)
37.204 A. Franchini, D. Malagoli, E. Ottaviani: Targets and effects of yessotoxin, okadaic acid and palytoxin: A differential review, Mar. Drugs **8**(3), 658–677 (2010)
37.205 C. Lamas, I. Valverde, J. Lago, J.M. Vieites, A.G. Cabado: In vitro evaluation of apoptotic markers triggered by PTX-2 in Caco-2 and neuroblastoma cells, Egypt. J. Nat. Toxins **3**, 17–33 (2006)
37.206 C. Lamas, I. Valverde, J. Lago, J.M. Vieites, A.G. Cabado: Comparison between cytotoxic effects induced by PTX-2 in vitro in two human cell lines, Egypt. J. Nat. Toxins **3**, 1–16 (2006)
37.207 Z.H. Zhou, M. Komiyama, K. Terao, Y. Shimada: Effects of pectenotoxin-1 on liver cells in vitro, Nat. Toxins **2**, 132–135 (1994)
37.208 B. Espiña, J.A. Rubiolo: Marine toxins and the cytoskeleton: Pectenotoxins, unusual macrolides that disrupt actin, FEBS Journal **275**(24), 6082–6088 (2008)
37.209 H.D. Chae, T.S. Choi, B.M. Kim, J.H. Jung, Y.J. Bang, D.Y. Shin: Oocyte-based screening of cytokinesis inhibitors and identification of pectenotoxin-2 that induces Bim/Bax-mediated apoptosis in p53-deficient tumors, Oncogene **24**, 4813–4819 (2005)
37.210 N. Vilariño, B. Espina: Pharmacology of pectenotoxins. In: *Seafood and Freshwater Toxins, Pharmacology, Physiology and Detection*, ed. by L.M. Botana (CRC/Taylor Francis, Boca Raton 2008) pp. 361–380
37.211 T. Yasumoto, M. Murata, Y. Oshima, G.K. Matsumoto, J. Clardy: Diarrhetic shellfish poisoning. In: *Seafood Toxins*, ed. by E.P. Ragelis (American Chemical Society, Washington 1984) pp. 207–214
37.212 European Union Reference Laboratory for Marine Biotoxins: EU Harmonised Standard Operating Procedure for detection of lipophilic toxins by mouse bioassay (2009), pp. 1–14
37.213 European Food Safety Authority: Opinion of the Scientific Panel on Contaminants in the Food chain on a request from the European Commission on marine biotoxins in shellfish – yessotoxin group, EFSA Journal **907**, 1–62 (2008)
37.214 K. Terao, E. Ito, M. Oarada, M. Murata, T. Yasumoto: Histopathological studies on experimental marine toxin poisoning–5. The effects in mice of yessotoxin isolated from *Patinopecten yessoensis* and of a desulfated derivative, Toxicon **28**, 1095–1104 (1990)
37.215 A. Franchini, E. Marchesini, R. Poletti, E. Ottaviani: Acute toxic effect of the algal yessotoxin on Purkinje cells from the cerebellum of Swiss CD1 mice, Toxicon **43**, 347–352 (2004)
37.216 A. Alfonso, L. de la Rosa, M.R. Vieytes, T. Yasumoto, L.M. Botana: Yessotoxin a novel phycotoxin, activates phosphodiesterase activity. Effect of yessotoxin on cAMP levels in human lymphocytes, Biochem. Pharmacol. **65**, 193–208 (2003)
37.217 A. Tubaro, V. Dell'Ovo, S. Sosa, C. Florio: Yessotoxins: A toxicological overview, Toxicon **56**(2), 163–172 (2010)
37.218 A. Alfonso: Yessotoxins, pharmacology and mechanism of action: Biological detection. In: *Seafood and Freshwater Toxins, Pharmacology, Physiology and Detection*, ed. by L.M. Botana (CRC/Taylor Francis, Boca Raton 2008) pp. 315–327
37.219 A. Perez-Gomez, A. Ferrero-Gutierrez, A. Novelli, J.M. Franco, B. Paz, M.T. Fernández-Sánchez: Potent neurotoxic action of the shellfish biotoxin yessotoxin on cultured cerebellar neurons, Toxicol. Sci. **90**(1), 168–177 (2006)
37.220 F. Leira, C. Alvarez, A.G. Cabado, J.M. Vieites, M.R. Vieytes, L.M. Botana: Development of a F

actin-based live-cell fluorimetric microplate assay for diarrhetic shellfish toxins, Anal. Biochem. **317**, 129–135 (2003)

37.221 I.R. Ares, M.C. Louzao, M.R. Vieytes, T. Yasumoto, L.M. Botana: Actin cytoskeleton of rabbit intestinal cells is a target for potent marine phycotoxins, J. Exp. Biol. **208**(22), 4345–4354 (2005)

37.222 G. Ronzitti, F. Callegari, C. Malaguti, G.P. Rossini: Selective disruption of the E-cadherin-catenin system by an algal toxin, Br. J. Cancer **90**, 1100–1107 (2004)

37.223 F. Callegari, S. Sosa, S. Ferrari, M.R. Soranzo, S. Pierotti, T. Yasumoto, A. Tubaro, G.P. Rossini: Oral administration of yessotoxin stabilizes E-cadherin in mouse colon, Toxicology **227**, 145–155 (2006)

37.224 M.S. Korsnes, A. Espenes: Yessotoxin as an apoptotic inducer, Toxicon **57**(7/8), 947–958 (2011)

37.225 L.M. Botana, A. Alfonso, M.R. Vieytes, M.I. Loza-Garcia: Therapeutic use of yessotoxins as human tumor cell growth inhibitors, European Patent EP1875906 (2008)

37.226 P. Ciminiello, E. Fattorusso: Yessotoxins chemistry, metabolism and chemical analysis. In: *Seafood and Freshwater Toxins. Pharmacology, Physiology and Detection*, ed. by L.M. Botana (Taylor Francis, Boca Raton 2008) pp. 287–314

37.227 S. Ferrari, P. Ciminiello, C. Dell'Aversano, M. Forino, C. Malaguti, A. Tubaro, R. Poletti, T. Yasumoto, E. Fattorusso, G.P. Rossini: Structure–activity relationships of yessotoxins in cultured cells, Chem. Res. Toxicol. **17**, 1251–1257 (2004)

37.228 M.J. Pazos, A. Alfonso, M.R. Vieytes, T. Yasumoto, L.M. Botana: Kinetic analysis of the interaction between yessotoxin and analogues and immobilized phosphodiesterases using a resonant mirror optical biosensor, Chem. Res. Toxicol. **18**, 1155–1160 (2005)

37.229 A. Alfonso, Y. Román, M.R. Vieytes, K. Ofuji, M. Satake, T. Yasumoto, L.M. Botana: Azaspiracid-4 inhibits Ca^{2+} entry by stored operated channels in human T lymphocytes, Biochem. Pharmacol. **69**(11), 1627–1636 (2005)

37.230 A. Alfonso, M.R. Vieytes, K. Ofuji, M. Satake, K.C. Nicolaou, M.O. Frederick, L.M. Botana: Azaspiracids modulate intracellular pH levels in human lymphocytes, Biochem. Biophys. Res. Commun. **346**(3), 1091–1099 (2006)

37.231 Y. Román, A. Alfonso, M.C. Louzao, L.A. de la Rosa, F. Leira, J.M. Vieites, M.R. Vieytes, K. Ofuji, M. Satake, T. Yasumoto, L.M. Botana: Azaspiracid-1, a potent, nonapoptotic new phycotoxin with several cell targets, Cell Signal. **14**, 703–716 (2002)

37.232 Y. Román, A. Alfonso, M.R. Vieytes, K. Ofuji, M. Satake, T. Yasumoto, L.M. Botana: Effects of azaspiracids 2 and 3 on intracellular cAMP, $[Ca^{2+}]$, and pH, Chem. Res. Toxicol. **17**(10), 1338–1349 (2004)

37.233 A. Furey, S. O'Doherty, K. O'Callaghan, M. Lehane, K.J. James: Azaspiracid poisoning (AZP) toxins in shellfish: Toxicological and health considerations, Toxicon **56**(2), 173–190 (2010)

37.234 G. Ronzitti, P. Hess, N. Rehmann, G.P. Rossini: Azaspiracid-1 alters the E-cadherin pool in epithelial cells, Toxicol. Sci. **95**(2), 427–435 (2007)

37.235 M.J. Twiner, P. Hess, M.Y. Dechraoui, T. McMahon, M.S. Samons, M. Satake, T. Yasumoto, J.S. Ramsdell, G.J. Doucette: Cytotoxic and cytoskeletal effects of azaspiracid-1 on mammalian cell lines, Toxicon **45**(7), 891–900 (2005)

37.236 N. Vilariño, K.C. Nicolaou, M.O. Frederick, E. Cagide, I.R. Ares, M.C. Louzao, M.R. Vieytes, L.M. Botana: Cell growth inhibition and actin cytoskeleton disorganization induced by azaspiracid-1 structure–activity studies, Chem. Res. Toxicol. **19**(11), 1459–1466 (2006)

37.237 M. Bellocci, G.L. Sala, F. Callegari, G.P. Rossini: Azaspiracid-1 inhibits endocytosis of plasma membrane proteins in epithelial cells, Toxicol. Sci. **117**(1), 109–121 (2010)

37.238 P. Hess, P. McCarron, N. Rehmann, J. Kilcoyne, T. McMahon, G. Ryan, M.P. Ryan, G.J. Doucette: *Isolation and Purification of Azaspiracids from Naturally Contaminated Materials and Evaluation of Their Toxicological Effects. Final Project Report*, Marine Enviromental and Health Series, Vol. 129 (Marine Institute, Galway, Ireland 2007)

37.239 E. Ito, M. Satake, K. Ofuji, M. Higashi, K. Harigaya, T. McMahon, T. Yasumoto: Chronic effects in mice caused by oral administration of sublethal doses of azaspiracid, a new marine toxin isolated from mussels, Toxicon **40**(2), 193–203 (2002)

37.240 M. Satake, K. Ofuji, H. Naoki, K.J. James, A. Furey, T. McMahon, J. Silke, T. Yasumoto: Azaspiracid, a new marine toxin having unique spiro ring assemblies, isolated from Irish mussels, *Mytilus edulis*, J. Am. Chem. Soc. **120**(38), 9967–9968 (1998)

37.241 K. Ofuji, M. Satake, T. McMahon, J. Silke, K.J. James, H. Naoki, Y. Oshima, T. Yasumoto: Two analogs of azaspiracid isolated from mussels, *Mytilus edulis*, involved in human intoxication in Ireland, Nat. Toxins **7**(3), 99–102 (1999)

37.242 E. Ito, K. Terao, T. McMahon, J. Silke, T. Yasumoto: Acute pathological changes in mice caused by crude extracts of novel toxins isolated from Irish mussels. In: *Harmful Algae*, ed. by B. Reguera, J. Blanco, M.L. Fernández, T. Wyatt (Intergovernmental Oceanographic Commission of UNESCO, Santiago de Compostela 1998) pp. 588–589

37.243 E. Ito, M. Satake, K. Ofuji, T. McMahone, J. Silke, K. James, T. Yasumoto: Small intestinal injuries in mice caused by and new toxing, azaspiracid, isolated from Irish mussels. In: *Harmful and Toxic Algal Blooms*, ed. by T. Yasumoto,

Y. Oshima, Y. Fukuyo (Intergovernmental Oceanographic Commission of UNESCO, Santiago de Compostela 2000) p. 395

37.244 A.F. Flanagan, K.R. Callanan, J. Donlon, R. Palmer, A. Forde, M. Kane: A cytotoxicity assay for the detection and differentiation of two families of shellfish toxins, Toxicon **39**(7), 1021–1027 (2001)

37.245 Z. Cao, K.T. LePage, M.O. Frederick, K.C. Nicolaou, T.F. Murray: Involvement of caspase activation in azaspiracid-induced neurotoxicity in neocortical neurons, Toxicol. Sci. **114**(2), 323–334 (2010)

37.246 European Food Safety Authority: Marine biotoxins in shellfish-Saxitoxin group. Scientific opinion of the Panel on Contaminants in the Food Chain, EFSA Journal **1019**, 1–76 (2009)

37.247 R. Shinohara, T. Akimoto, O. Iwamoto, T. Hirokawa, M. Yotsu-Yamashita, K. Yamaoka, K. Nagasawa: Synthesis of skeletal analogues of saxitoxin derivatives and evaluation of their inhibitory activity on sodium ion channels $Na_v1.4$ and $Na_v1.5$, Chem. Eur. J. **17**(43), 12144–12152 (2011)

37.248 S.A. Murray, M. Wiese, B.A. Neilan, R.J.S. Orr, M. de Salas, S. Brett, G. Hallegraeff: A reinvestigation of saxitoxin production and *sxtA* in the 'non-toxic' *Alexandrium tamarense* group V clade, Harmful Algae **18**, 96–104 (2012)

37.249 M.C. Wylie, V.M. Johnson, E. Carpino, K. Mullen, K. Hauser, A. Nedder, J.N. Kheir, A.J. Rodriguez-Navarro, D. Zurakowski, C.B. Berde: Respiratory, neuromuscular, and cardiovascular effects of neosaxitoxin in isoflurane-anesthetized sheep, Reg. Anesth. Pain Med. **37**(2), 152–158 (2012)

37.250 A.M.S. Mayer, M.T. Hamann: Marine pharmacology in 2001–2002: Marine compounds with anthelmintic, antibacterial, anticoagulant, antidiabetic, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems and other miscellaneous mechanisms of action, Comp. Biochem. Physiol. C **140**(3–4), 265–286 (2005)

37.251 I.A. Ross, W. Johnson, P.P. Sapienza, C.S. Kim: Effects of the seafood toxin domoic acid on glutamate uptake by rat astrocytes, Food Chem. Toxicol. **38**(11), 1005–1011 (2000)

37.252 G. Giordano, T.J. Kavanagh, E.M. Faustman, C.C. White, L.G. Costa: Low-level domoic acid protects mouse cerebellar granule neurons from acute neurotoxicity: Role of glutathione, Toxicol. Sci. **132**(2), 399–408 (2013)

37.253 M.A. Friedman, L.E. Fleming, M. Fernandez, P. Bienfang, K. Schrank, R. Dickey, M.-Y. Bottein, L. Backer, R. Ayyar, R. Weisman, S. Watkins, R. Granade, A. Reich: Ciguatera fish poisoning: Treatment, prevention and management, Mar. Drugs **6**(3), 456–479 (2008)

37.254 R.W. Dickey, S.M. Plakas: Ciguatera: A public health perspective, Toxicon **56**(2), 123–136 (2010)

37.255 G.M. Nicholson, R.J. Lewis: Ciguatoxins: Cyclic polyether modulators of voltage-gated lion channel function, Mar. Drugs **4**(3), 82–118 (2006)

37.256 R.N. Murrell, J.E. Gibson: Brevetoxins 2, 3, 6, and 9 show variability in potency and cause significant induction of DNA damage and apoptosis in Jurkat E6-1 cells, Arch. Toxicol. **83**(11), 1009–1019 (2009)

37.257 N.V. Kulagina, T.J. O'Shaughnessy, W. Ma, J.S. Ramsdell, J.J. Pancrazio: Pharmacological effects of the marine toxins, brevetoxin and saxitoxin, on murine frontal cortex neuronal networks, Toxicon **44**(6), 669–676 (2004)

37.258 R.N. Murrell, J.E. Gibson: Brevetoxin 2 alters expression of apoptotic, DNA damage and cytokine genes in Jurkat cells, Hum. Exp. Toxicol. **30**(3), 182–191 (2010)

37.259 S.C. Hilderbrand, R.N. Murrell, J.E. Gibson, J.M. Brown: Marine brevetoxin induces IgE-independent mast cell activation, Arch. Toxicol. **85**(2), 135–141 (2011)

37.260 Z. Cao, J. George, W.H. Gerwick, D.G. Baden, J.D. Rainier, T.F. Murray: Influence of lipid-soluble gating modifier toxins on sodium influx in neocortical neurons, J. Pharmacol. Exp. Ther. **326**(2), 604–613 (2008)

37.261 P. Taupin: Brevetoxin derivative compounds for stimulating neuronal growth, Expert Opin. Ther. Pat. **19**(2), 269–274 (2009)

37.262 S. Bragadeeswaran, D. Therasa, K. Prabhu, K. Kathiresan: Biomedical and pharmacological potential of tetrodotoxin-producing bacteria isolated from marine pufferfish *Arothron hispidus* (Muller, 1841), J. Venom. Anim. Toxins Incl. Trop. Dis. **16**(3), 421–431 (2011)

37.263 B. Hegyi, I. Komaromi, K. Kistamas, F. Ruzsnavszky, K. Vaczi, B. Horvath, J. Magyar, T. Banyasz, P.P. Nanasi, N. Szentandrassy: Tetrodotoxin blockade on canine cardiac L-Type Ca^{2+} channels depends on pH and redox potential, Mar. Drugs **11**(6), 2140–2153 (2013)

37.264 F.R. Nieto, E.J. Cobos, M.A. Tejada, C. Sanchez-Fernandez, R. Gonzalez-Cano, C.M. Cendan: Tetrodotoxin (TTX) as a therapeutic agent for pain, Mar. Drugs **10**(2), 281–305 (2012)

37.265 A. Turabi, A.R. Plunkett: The application of genomic and molecular data in the treatment of chronic cancer pain, J. Surg. Oncol. **105**(5), 494–501 (2012)

37.266 F.R. Nieto, J.M. Entrena, C.M. Cendán, E. del Pozo, J.M. Vela, J.M. Baeyens: Tetrodotoxin inhibits the development and expression of neuropathic pain induced by paclitaxel in mice, Pain **137**, 520–531 (2008)

37.267 N.A. Hagen, P. du Souich, B. Lapointe, M. Ong-Lam, B. Dubuc, D. Walde, R. Love, A.H. Ngoc: Tetrodotoxin for moderate to severe cancer pain: A randomized, double blind, parallel design multicenter study, J. Pain Symptom Manag. **35**(4), 420–429 (2008)

37.268 N.A. Hagen, B. Lapointe, M. Ong-Lam, B. Dubuc, D. Walde, B. Gagnon, R. Love, R. Goel, P. Hawley, A.H. Ngoc, P. du Souich: A multicentre open-label safety and efficacy study of tetrodotoxin for cancer pain, Curr. Oncol. **18**(3), E109–E116 (2011)

37.269 F.R. Nieto, E.J. Cobos, M.Á. Tejada, C. Sánchez-Fernández, R. González-Cano, C. Miguel Cendán: Tetrodotoxin (TTX) as a therapeutic agent for pain, Mar. Drugs **10**, 281–305 (2012)

37.270 L. Wang, S.A. Shankarappa, R. Tong, J.B. Ciolino, J.H. Tsui, H.H. Chiang, D.S. Kohane: Topical drug formulations for prolonged corneal anesthesia, Cornea **32**(7), 1040–1045 (2013)

37.271 C. Vale: Palytoxins: Pharmacology and biological detection methods. In: *Seafood and Freshwater Toxins: Pharmacology, Physiology and Detection*, ed. by L.M. Botana (CRC/Taylor Francis, Boca Raton 2008) pp. 675–691

37.272 A. Tubaro, G. Del Favero, D. Beltramo, M. Ardizzone, M. Forino, M. De Bortoli, M. Pelin, M. Poli, G. Bignami, P. Ciminiello, S. Sosa: Acute oral toxicity in mice of a new palytoxin analog: 42-Hydroxy-palytoxin, Toxicon **57**(5), 755–763 (2011)

37.273 E.V. Wattenberg: Palytoxin: Exploiting a novel skin tumor promoter to explore signal transduction and carcinogenesis, Am. J. Physiol. Cell Physiol. **292**(1), C24–C32 (2007)

37.274 E.V. Wattenberg: Modulation of protein kinase signaling cascades by palytoxin, Toxicon **57**(3), 440–448 (2011)

37.275 R. Munday: Ocurrence and toxicology of palytoxins. In: *Seafood and Freshwater Toxins: Pharmacology, Physiology and Detection*, ed. by L.M. Botana (CRC/Taylor Francis, Boca Raton 2008) pp. 693–713

37.276 I. Valverde, J. Lago, A. Reboreda, J.M. Vieites, A.G. Cabado: Characteristics of palytoxin-induced cytotoxicity in neuroblastoma cells, Toxicol. In Vitro **22**(6), 1432–1439 (2008)

37.277 I. Valverde, J. Lago, J.M. Vieites, A.G. Cabado: In vitro approaches to evaluate palytoxin-induced toxicity and cell death in intestinal cells, J. Appl. Toxicol. **28**(3), 294–302 (2008)

37.278 M. Stewart, J.W. Blunt, M.H.G. Munro, W.T. Robinson, D.J. Hannah: The absolute stereochemistry of the New Zealand shellfish toxin gymnodimine, Tetrahedron Lett. **38**, 4889–4890 (1997)

37.279 R. Munday: Toxicology of cyclic imines: Gymnodimine, spirolides, pinnatoxins, pteriatoxins, prorocentrolide, spiro-prorocentrimine, and symbioimines. In: *Seafood and Freshwater Toxins: Pharmacology, Physiology, and Detection*, 2nd edn., ed. by L.M. Botana (CRC/Taylor Francis, Boca Raton 2008) pp. 581–594

37.280 S.M. Gueret, M.A. Brimble: Spiroimine shellfish poisoning (SSP) and the spirolide family of shellfish toxins: Isolation, structure, biological activity and synthesis, Nat. Prod. Rep. **27**(9), 1350–1366 (2010)

37.281 R. Kharrat, D. Servent, E. Girard, G. Ouanounou, M. Amar, R. Marrouchi, E. Benoit, J. Molgó: The marine phycotoxin gymnodimine targets muscular and neuronal nicotinic acetylcholine receptor subtypes with high affinity, J. Neurochem. **107**(4), 952–963 (2008)

37.282 Y. Bourne, Z. Radic, R. Aráoz, T.T. Talley, E. Benoit, D. Servent, P. Taylor, J. Molgó, P. Marchot: Structural determinants in phycotoxins and AChBP conferring high affinity binding and nicotinic AChR antagonism, Proc. Natl. Acad. Sci. USA **107**(13), 6076–6081 (2010)

37.283 C.B. Wandscheer, N. Vilariño, B. Espiña, M.C. Louzao, L.M. Botana: Human muscarinic acetylcholine receptors are a target of the marine toxin 13-desmethyl C spirolide, Chem. Res. Toxicol. **23**(11), 1753–1761 (2010)

37.284 C.L. Trevino, L. Escobar, L. Vaca, V. Morales-Tlalpan, A.Y. Ocampo, A. Darszon: Maitotoxin; a unique pharmacological tool for elucidating Ca^{2+}-dependent mechanisms. In: *Seafood and Freshwater Toxins: Pharmacology, Physiology, and Detection*, ed. by L. Botana (CRC/Taylor Francis, Boca Raton 2008) pp. 503–516

37.285 M.C. Louzao, E. Cagide, M.R. Vieytes, M. Sasaki, H. Fuwa, T. Yasumoto, L.M. Botana: The sodium channel of human excitable cells is a target for gambierol, Cell. Physiol. Biochem. **17**(5/6), 257–268 (2006)

37.286 E. Cagide, M.C. Louzao, B. Espina, I.R. Ares, M.R. Vieytes, M. Sasaki, H. Fuwa, C. Tsukano, Y. Konno, M. Yotsu-Yamashita, L.A. Paquette, T. Yasumoto, L.M. Botana: Comparative cytotoxicity of gambierol versus other marine neurotoxins, Chem. Res. Toxicol. **24**(6), 835–842 (2011)

37.287 E. Alonso, H. Fuwa, C. Vale, Y. Suga, T. Goto, Y. Konno, M. Sasaki, F.M. LaFerla, M.R. Vieytes, L. Gimenez-Llort, L.M. Botana: Design and synthesis of skeletal analogues of gambierol: Attenuation of amyloid-β and tau pathology with voltage-gated potassium channel and *n*-Methyl-*d*-aspartate receptor implications, J. Am. Chem. Soc. **134**(17), 7467–7479 (2012)