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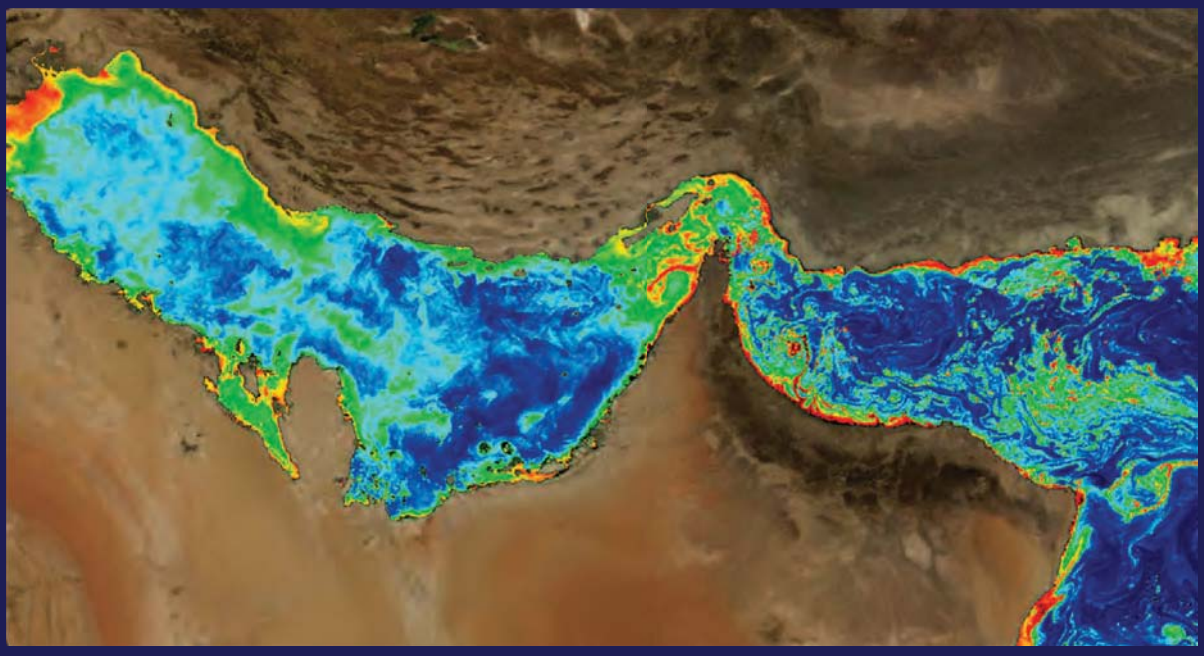
Harmful Algal Blooms (HABs) and Desalination: A Guide to Impacts, Monitoring, and Management



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Edited by:

Donald M. Anderson*
Biology Department, Woods Hole Oceanographic Institution
Woods Hole, MA 02543 USA

Siobhan F. E. Boerlage
Boerlage Consulting
Gold Coast, Queensland, Australia

Mike B. Dixon
MDD Consulting, Kensington
Calgary, Alberta, Canada

*Corresponding Author's email: danderson@whoi.edu

UNESCO 2017

2 ALGAL ISSUES IN SEAWATER DESALINATION

Philipp Hess[1], Loreen O. Villacorte[2,3], Mike B. Dixon[4], Siobhan F.E. Boerlage[5], Donald M. Anderson[6], Maria D. Kennedy[2], and Jan C. Schippers[2]

[1]IFREMER, Laboratoire Phycotoxines, 44311 Nantes, France
[2]IHE-Delft Institute for Water Education, Delft, The Netherlands
[3]GRUNDFOS Holding A/S, Bjerringbro, Denmark (current affiliation)
[4]MDD Consulting, Kensington, Calgary, Alberta, Canada
[5]Boerlage Consulting, Gold Coast, Queensland, Australia
[6]Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543 USA

2.1 INTRODUCTION

Once harmful algal blooms (HABs) reach a desalination plant, they can cause significant operational issues and potential health concerns for consumers. These issues stem from two factors – first, the algal cells produce organic matter that can cause filter clogging and membrane fouling, and secondly, some cells produce toxic substances or taste and odor compounds. This chapter first explains the mechanisms for cellular release of organic matter, the types of matter that are produced, and the relative contribution of each type of matter to fouling mechanisms. It then describes the wide range of toxins that are produced by HABs, their mode of toxicity, and analytical methods for detecting them. While taste and odor compounds are non-toxic, they are included in this chapter as they can create customer perception issues and distrust in the water supply system.

2.2 ALGAL ORGANIC MATTER (AOM) AND MEMBRANE FOULING

Natural organic matter in seawater is comprised of a diverse mixture of particulate, colloidal and dissolved organic substances which may originate from autochthonous (local) and allochthonous (external) sources. Algal blooms are believed to be responsible for about half of the autochthonous organic matter input to the earth's oceans (Field et al. 1998). These

algal-derived substances are collectively known as algal (or algogenic) organic matter (AOM). AOM can cause (directly or indirectly) operational problems in membrane-based desalination plants, affecting both the pretreatment processes and reverse osmosis membrane units. This section reviews the latest knowledge on the occurrence, composition, and characteristics of AOM from the perspective of seawater desalination.

2.2.1 AOM release and composition

Algal blooms are often responsible for the highest annual pulses of natural organic matter in seawater. A spike (>50% increase) in total organic carbon (TOC) concentration has been recorded in coastal seawater during algal blooms (e.g., Petry et al. 2007); however, AOM produced during algal blooms may vary substantially in terms of concentration, composition and characteristics, depending on the causative species and environmental conditions. AOM is generally released into seawater through metabolic excretion of active algal cells or through autolysis of damaged or dying cells. Active cells may excrete AOM in response to low-nutrient stress, unfavorable environmental conditions (e.g., light, pH and temperature) or invasion by bacteria or viruses (Leppard 1993). Several species of algae may also release AOM even under fairly favorable conditions (Fogg 1983).

Excessive production of AOM due to depletion of specific nutrients (e.g., phosphorus (P), nitrogen (N) and silicon (Si)) and pathogen invasion have been linked to the occurrence of marine mucilage (Mingazzini and Thake 1995). This phenomenon is characterized by the appearance of a sporadic but massive accumulation of gelatinous material at and below the water surface. Severe mucilage events occasionally occur in the North Sea, Adriatic Sea, and other parts of the Mediterranean Sea, and undoubtedly elsewhere in the world as well, though unreported. The proliferation of smaller mucilaginous aggregates known as “marine snow” has been reported in most oceanic and marine systems.

Specific conditions at the seawater intake and through the pretreatment processes may also induce further release of AOM. For instance, the addition of oxidizing or biocidal agents such as chlorine has been shown to cause damage to algal cell walls and membranes, resulting in the release of intracellular AOM (Daly et al. 2007). Moreover, exposure to hydrodynamic shear stress (e.g., valves and pumps) may cause breakage of soft-walled algal cells, releasing AOM which is normally stored inside the cells (Ladner et al. 2010; Voutchkov 2010).

The chemical composition of AOM usually includes proteins, polysaccharides, nucleic acids, lipids and other dissolved organic substances (Fogg 1983). For some algae, particularly diatoms such as *Chaetoceros affinis*, extracellular polysaccharides may comprise up to 90% of AOM released (Myklestad 1995). A major fraction of AOM is very sticky and is thought to cause operational issues in SWRO plants during blooms (Villacorte et al. 2015a). These substances are widely known as transparent exopolymer particles or TEPs (see Section 2.3.3 and Appendix 3).

2.2.2 AOM classification

Based on the mechanism of release by algae, AOM can be classified into two major groups, namely: (1) extracellular organic matter (EOM) - organic substances released through metabolic activity of algae, and (2) intracellular organic matter (IOM) - substances released through autolysis and/or during the process of cell decay. EOM substances can be either dis-

crete or attached (bound) to the algal cell as coatings. Discrete or free EOMs comprise mainly polysaccharides and tend to be more hydrophilic while bound EOM comprise more protein compounds and tend to be more hydrophobic (Qu et al. 2012; Henderson et al. 2008).

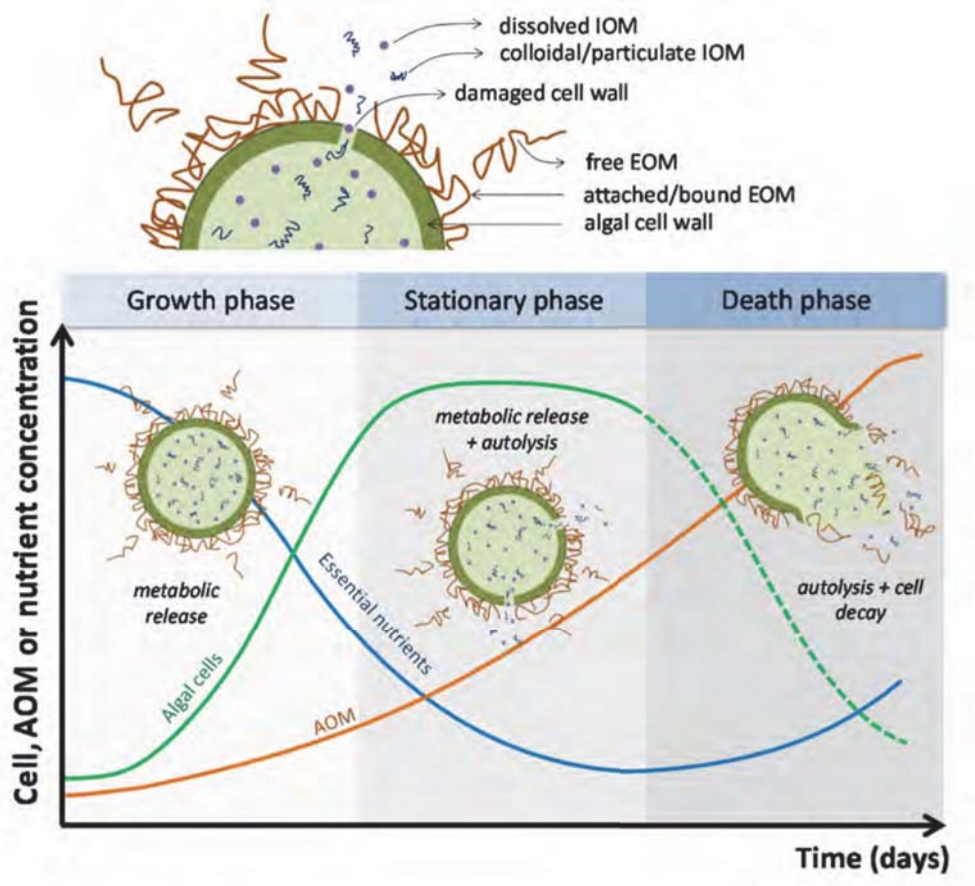

Figure 2.1. Graphical illustrations of how AOMs are released into seawater by algae at different phases of a bloom and in response to the availability of essential nutrients.

On the other hand, IOMs comprise mainly low molecular weight polymers released from the interior of damaged (e.g., broken cell wall), dying or decaying cells, which may include toxins as well as taste and odor compounds (see Section 2.3). Considering the conditions of how they are released, the contribution of IOM to the total AOM production is expected to increase during the stationary-death phase of the bloom (Figure 2.1).

AOM components can be also classified in terms of their molecular weight or size. The low molecular weight components of AOM include humic acid-like substances, nucleic acids, lipids, toxins, taste/odor compounds and other organic acids (Figure 2.2). In principle, these compounds also fall under the IOM classification because they are part of the intracellular components of an algal cell. High molecular weight AOM comprises protein and polysaccharide compounds, including TEP and their precursors. AOM typically cover a wide size spectrum, ranging from less than 1 nm to more than 1 mm. Based on their size cut-off, granular media filtration (GMF), microfiltration (MF), and ultrafiltration (UF) are expected to remove only part of high molecular weight AOM (Figure 2.2). Nanofiltration (NF) is expected to completely remove this fraction as well as part of the low molecular weight AOM while complete removal of AOM can be achieved by reverse osmosis.

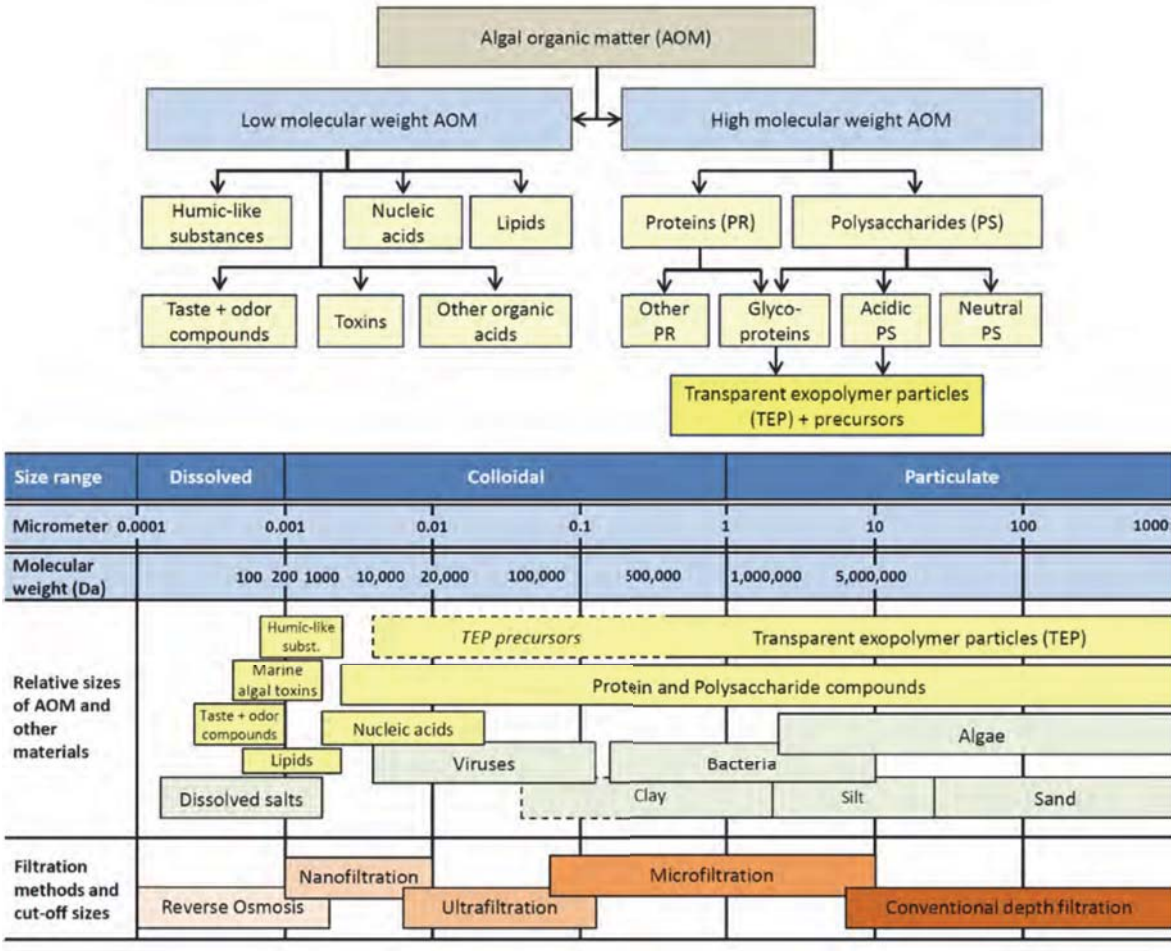

Figure 2.2. Classification of AOM components based on their molecular weight and size: (top chart) major components of AOM which belong to the low and high molecular fraction; (bottom chart) the size spectrum of major AOM components, in comparison with other suspended/dissolved matter in seawater and the size cut-off of relevant filtration methods (Villacorte 2014).

2.2.3 Transparent Exopolymer Particles (TEP)

TEPs are organic substances usually associated with algal blooms in both fresh and marine aquatic environments. These amorphous substances have been observed in various shapes (e.g., strings, disks, sheets or fibers) and sizes, ranging from a few nanometers in diameter up to hundreds of μm long as in the mucilage aggregates previously described (Passow 2000). In the ocean, TEPs are mainly produced by phytoplankton (micro-algae) and bacterioplankton but they may also originate from macro-algae, and shellfish (Passow 2002). Algae can directly release TEPs through shedding of cell mucus/coatings (Figure 2.3) or through disintegration of large algal colonies (Kiørboe and Hansen 1993).

By definition, the term 'TEP' refers to substances (including their associated components) stainable by Alcian Blue, a cationic dye, and are larger than 0.4 μm - they were originally discovered through retention on 0.4 μm pore size membrane filters (Alldredge et al. 1993). TEPs are not solid particles, but rather an agglomeration of particulate and colloidal hydrogels.

Colloidal AOM (1-10 kDa) may agglomerate to form TEP, first by spontaneous assembly of

free fibrils through alignment on hydrophobic surfaces, then through annealing and gelation to form micro-hydrogels, and eventually TEP (>0.4 μm) through aggregation (Verdugo et al. 2004). These sub-micron components (<0.4 μm) which have similar chemical properties as TEP are collectively known as TEP precursors (Passow 2000).

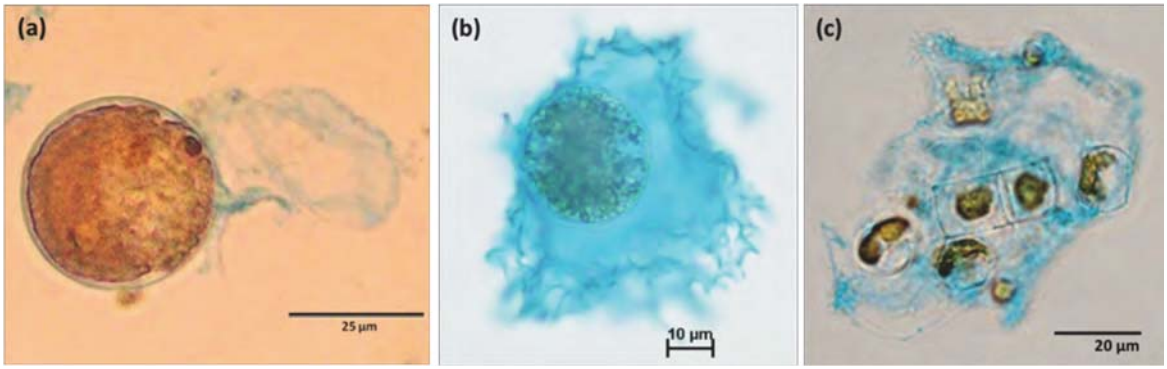

Figure 2.3. TEP release by marine algae through shedding of cell mucus or membrane coatings. Optical microscope images of Alcian Blue stained (a) *Alexandrium tamarense*, (b) *Lepidodinium chlorophorum* and (c) *Chaetoceros affinis*. Photos: (a) and (c) Villacorte et al. (2015a); (b) Claquin et al. (2008).

TEPs and their precursors are generally sticky, highly hydrophilic and comprise mainly negatively-charged polysaccharides and glycoprotein. Their stickiness and anionic charge are mainly attributed to the presence of sulfate half ester ($R\text{-}OSO_3^-$) functional groups (Mopper et al. 1995). In aquatic systems, they contain more than 99% water, which means they are neutrally buoyant and can bulk-up to more than 100 times their solid volume (Azetsu-Scott and Passow 2004). Moreover, they can be associated with or tend to absorb proteins, lipids, dissolved trace elements and metals in the water (Passow 2002). This makes them a nutritious platform and hotspot for bacterial activity (Berman and Holenberg 2005). Such characteristics have led some researchers to suspect that they may have an important role in the formation of aquatic biofilms. In 2005, Berman and Holenberg proposed the potential role of TEP and their precursors as a major initiator of biofilm leading to biofouling in reverse osmosis membranes (Berman and Holenberg 2005). Consequently, various studies were conducted to investigate the link between these substances and biofouling. Moreover, it was also demonstrated in lab- and pilot scale studies using seawater that they can cause severe organic fouling in micro-/ultra-filtration membranes (e.g., Villacorte et al. 2015b; Ladner et al. 2010; Kim and Yoon 2005).

Since the discovery of TEP in the early 90's, a number of methods have been developed and further modified to monitor TEP concentrations in the aquatic environment. The latest development of these methods and their potential application in monitoring the impact of HABs on SWRO plants and their removal are discussed in Chapter 5. Methods to measure TEP and TEP precursors are given in Appendix 3.

2.2.4 Fouling issues in SWRO plant during HAB events

The high AOM concentration in the raw water during algal blooms can cause fouling issues in both the pretreatment and RO systems of a SWRO desalination plant. RO membranes are primarily designed to remove dissolved constituents in the water, in particular, inorganic ions (dissolved salts). Membrane systems are vulnerable to fouling and clogging. Fouling occurs due to deposition of particulates and/or growth of bacteria to form a biofilm on the membrane surface, resulting in an increase in hydraulic resistance. Usually this resistance is compensated by increasing the feed pressure and ultimately membranes are chemically cleaned in place (see Appendix 5). Clogging of a membrane system is due to fouling of the

spacer of spiral wound SWRO elements or bundles of hollow fiber MF/UF membranes resulting from particulate matter deposition and/or biomass formation. To avoid frequent (chemical) cleaning, RO systems are generally preceded by a pretreatment process to minimize the particulate, organic and biofouling potential of the feed water. Conventional or advanced pretreatment techniques can be applied in seawater SWRO plants. Conventional techniques comprise various types of granular media filters. Frequently, 'in line coagulation' (addition of a coagulant in front of the filters) is applied to improve the hydraulic performance of the filter as well removal of smaller particles and organic matter. In some designs, these filters are preceded by coagulation/sedimentation or coagulation/flotation systems. Advanced pretreatment techniques include the application of micro- or ultra-filtration membranes. Dissolved air flotation (DAF) is often employed as an additional pretreatment step before GMF and/or MF/UF in seawater RO systems with the aim of making the pretreatment more reliable and robust.

Typically, algae and AOM are (partially) captured by the pretreatment process before the seawater is fed to the SWRO system. This means the pretreatment system will be the first to be exposed to AOM fouling. Clogging of granular media filters (GMF – the most widely used SWRO pretreatment technique) was one of the identified causes of SWRO plant shutdown during the severe HAB in the Gulf of Oman in 2008-2009 (Richlen et al. 2010; Berktay 2011) due to severely reduced operation times from 24 hours to 2 hours. GMF typically accumulate materials larger than 10 μm (Ripperger et al. 2012) which may include algal cells and large AOM (Figure 2.2). Considering the relatively high filtration rate (5-10 m/h) in GMF, a sudden spike in algae and AOM concentration will rapidly clog the interstices of the filter media and eventually form a slimy cake layer on the surface of the filter bed. In this scenario, the filtrate production of the GMF will rapidly decline, which would then require frequent backwashing and coagulant addition and hence, longer system downtime. In addition a significant part of the AOM can pass through these filters, resulting in pretreated water with high fouling potential (e.g., high silt density index) which is unacceptable for RO operation.

Several SWRO plants have MF/UF pretreatment systems that can remove algae and much smaller AOM than GMF (see Figure 2.2). During MF/UF pretreatment of algal bloom-impacted seawater, algal cells and AOM can block or foul the pores and eventually form a cake/gel layer on the surface of the membrane. These will cause rapid decline in the membrane permeability. More frequent hydraulic backwashing, chemical enhanced backwashing or chemical cleaning-in-place (CIP) is therefore required to recover the initial permeability, resulting in longer system downtime. Some MF/UF plants apply in-line coagulation, by dosing ferric salts, in front of the systems, to control the rate of non-backwashable fouling.

If AOM is not effectively removed by the pretreatment processes, it can accumulate to form a heterogeneous and compressible cake/gel layer on the surface of RO membranes. This can result in lower permeability and higher differential pressure along the RO feed channel (Her et al., 2004). The accumulated sticky substances may further initiate or promote particulate and biological fouling by enhancing deposition of bacteria and other particles from the feed water to the RO membrane and spacers (Berman and Holenberg 2005).

2.2.4.1 Biofouling in SWRO due to AOM deposition

Sticky and high charge-density TEPs produced during HABs can adhere and accumulate on the surface of the SWRO membranes and spacers. The accumulated TEPs may serve as a "conditioning layer" – a good platform for effective attachment and initial colonization by bacteria which may then accelerate biofilm formation (Berman and Holenberg 2005; Li et al.

2015; Villacorte 2014). TEPs are also (partially) biodegradable and may serve as a substrate for bacteria (Alldredge et al. 1993; BarZeev and Rahav 2015). Recently, Berman and co-workers proposed a "revised paradigm" of aquatic biofilm formation facilitated by TEPs (Berman and Holenberg 2005; Bar-Zeev et al. 2012). As illustrated in Figure 2.4, colloidal and particulate TEPs and protobiofilms in surface water can initiate, enhance, and possibly accelerate biofilm accumulation in RO modules.

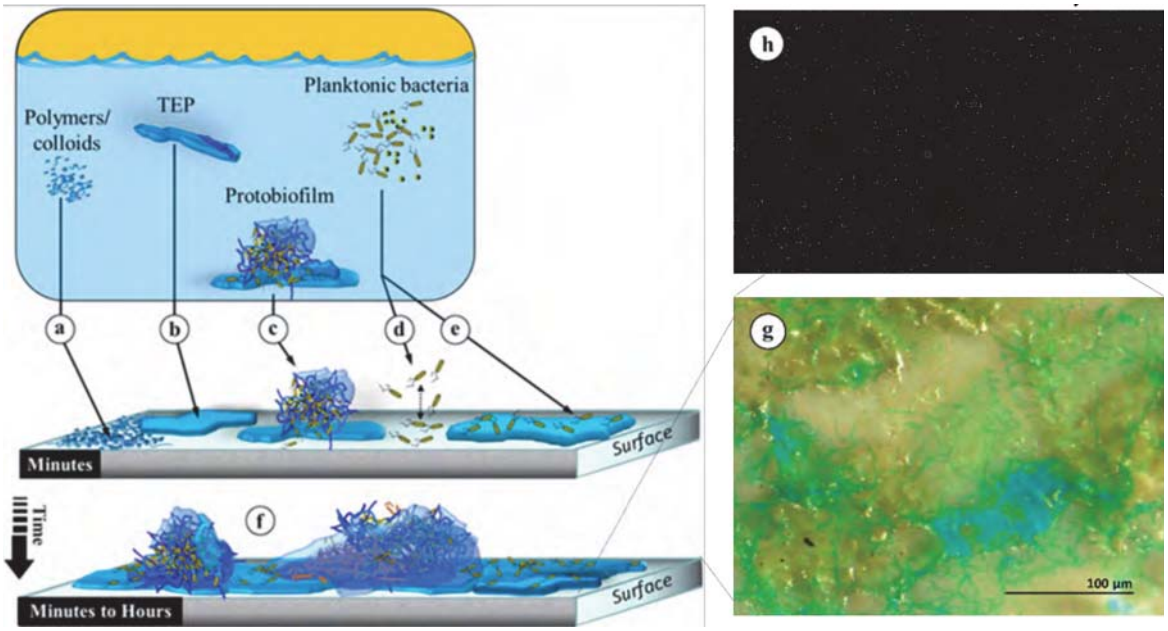

Figure 2.4. Schematic illustration of the possible contribution of (a) colloidal biopolymers, (b) TEP, and (c) protobiofilm (suspended TEP with extensive microbial outgrowth and colonization) in the initiation of aquatic biofilms. A number of planktonic bacteria (first colonizers) can attach (d) reversibly on clean surfaces or (e) irreversibly on TEP-conditioned surfaces. When nutrients are not limited in the water, (f) contiguous coverage of mature biofilm can develop within a short period of time (minutes to hours). Biofilm accumulation can cover a significant surface area of a (g) spiral wound membrane. Operational issues will occur when substantial accumulation (h) obstructs permeate and feed channel flow. Photos and descriptions adapted from (a-f) Bar-Zeev et al. (2012a), (g) Villacorte et al. (2009) and (h) Villacorte (2014).

Since bacteria require nutrients for energy generation and cellular synthesis, essential nutrients such as biodegradable or assimilable organic carbon (BDOC or AOC), phosphates and nitrates are likely the main factors dictating the formation and growth rate of biofilm in RO modules. During the peak of an algal bloom, some of these essential nutrients may be limited (e.g., phosphate, nitrates, silica) due to uptake by algae. However, when the bloom reaches the death phase, algal cells start to disintegrate while releasing some of these nutrients. Hence, biofouling initiated or enhanced by AOM will likely occur in SWRO some time (depending on available nutrients) after the termination of an algal bloom. So far, the role of AOM on membrane biofouling has only been illustrated in lab-scale experiments but autopsies of biofouled membrane elements from both brackish and seawater RO plants have shown the ubiquitous presence TEP among the biofilm accumulations (Figure 2.4; Villacorte et al. 2009; Villacorte 2014).

2.2.4.2 Particulate/Organic fouling of MF/UF during HABs

Various marine laboratory- and pilot-scale studies have demonstrated the effect of algal blooms on MF/UF operation. High molecular weight AOM (e.g., algal-derived biopolymers)

were identified to be the main causes of membrane fouling, more so than the algal cells themselves (Kim and Yoon 2005; Ladner et al. 2010; Schurer et al. 2013; Villacorte et al. 2015b); however, a synergistic effect between algal cells and AOM may intensify the rate of fouling in MF/UF membranes.

AOM such as TEPs are very sticky and can adhere strongly to the surface and pores of the membranes, rendering conventional hydraulic backwashing ineffective in recovering the initial membrane permeability (Figure 2.5). This scenario has been reported in recent studies (e.g., Qu et al. 2012; Schurer et al. 2013; Villacorte 2014), signifying that AOM accumulation not only causes rapid increase in operating pressures to maintain flux, but also can increase non-backwashable or hydraulically irreversible fouling in MF/UF.

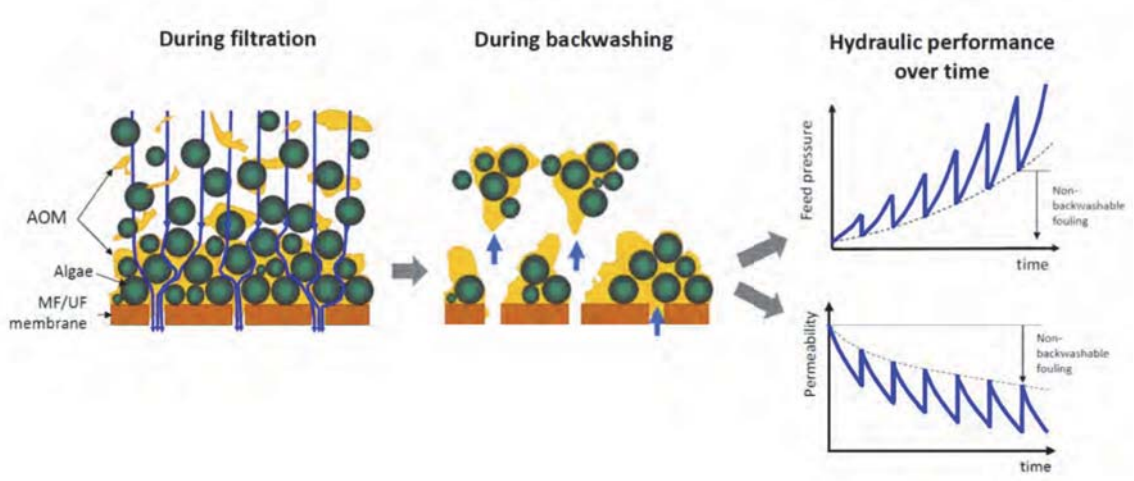

Figure 2.5: Fouling in MF/UF due to accumulation of algae and AOM and implications to membrane performance. During filtration, algal cells and AOM retained by the membrane form a (compressible) cake/gel layer which then causes rapid decline in permeability. This layer will only be partially removed during backwashing due to the gluey nature of some large AOMs (e.g., TEPs), resulting in progressively lower permeability (higher feed pressure if operated at constant flux) in the succeeding filtration cycles (non-backwashable fouling). Modified from Villacorte 2014.

When deposition of algae and AOM on the MF/UF membrane surface is relatively uniform, the impact on membrane permeability and backwashability can be explained with known fouling mechanisms, illustrated in Figure 2.6 and further discussed below.

a) **Membrane cake and pore constriction**. **(Figure 2.6a)**. Colloidal AOMs can enter into the narrow pores of MF/UF membranes, some of which will adsorb to the pore wall and eventually cause partial blockage of permeate flow (Herman and Bredee 1936). This can cause rapid increase in trans-membrane pressure (TMP) during the initial stage of filtration. Algal cells and large AOM can form a cake/gel layer on the surface of the membrane. Colloidal AOMs and other colloids will then fill up the large interstitial voids of the cake, narrowing the voids in the process. This may result in substantial increase in cake resistance due to the gradual reduction in cake porosity. During backwashing, the sticky AOM accumulated inside the membrane pores may not be completely removed, resulting in only partial recovery of the initial membrane permeability.

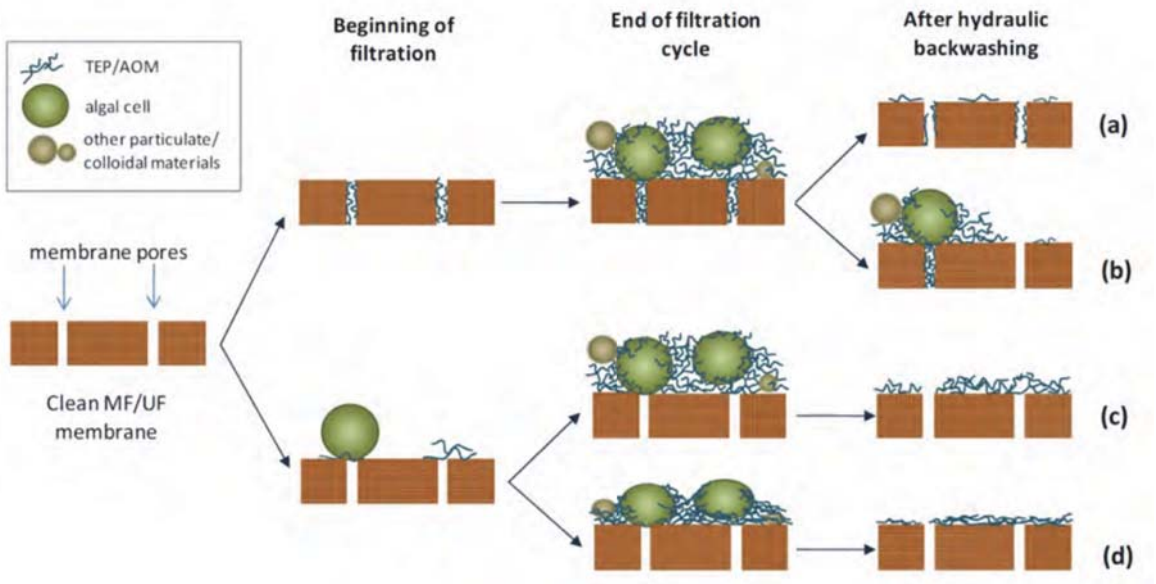

Figure 2.6. Possible fouling mechanisms involved due to uniform deposition of AOM and small algal cells in MF/UF. Each process is described in detail in the text. Modified from Villacorte (2014).

b) **Substantial loss in effective filtration area (Figure 2.6b)**. Colloidal AOM can accumulate inside membrane pores while algal cells and large AOM can accumulate at the entrance of the pores. In both cases, some pores may be completely blocked by the material and the active filtration area (membrane surface porosity) is substantially reduced, resulting in higher localized flux for the remaining active pores (Herman and Bredee 1936). An increase in flux can cause proportional increase in membrane resistance to filtration. Additionally, non-backwashable fouling can occur if the foulants blocking the pores are not effectively removed by hydraulic backwashing.

c) **Incomplete cake/gel removal during backwashing (Figure 2.6c)**. Since algal cells (typically range from 2 μm to 2 mm) and a substantial fraction of AOM are much larger than the pores of commercial MF/UF membranes (<1 μm), cake/gel formation can be mainly responsible for the increase in TMP. The accumulated AOM (like TEPs) are typically sticky and tend to adhere strongly to the membrane surface. During backwashing, a layer of the cake may remain on the surface of the membrane, which will then cause additional filtration resistance in the subsequent filtration cycle.

d) **Compression of accumulated cake/gel (Figure 2.6d).** Filter cake/gel comprising AOM and algal cells (soft-bodied) can be compressed due to localized increase of flux. Such localized increase in flux may be a consequence of pores narrowing and/or completely blocking as described above and hence occurs in combination with these fouling mechanisms.

Theoretically, small particles (e.g., small algal cells, colloidal TEPs) deposit uniformly along the capillary length whereas large particles (e.g., large algal cells and large particulate TEPs) tend to deposit non-uniformly (Panglisch 2003; Lerch et al. 2007). Modeling the transport of algal cells during dead-end filtration through a capillary membrane has shown that cells smaller than 5 μm tend to deposit evenly along the capillary length while cells larger than 25 μm tend to accumulate at the segment of the capillary with the lowest axial flow e.g.,

dead-end (Figure 2.7; Villacorte 2014). Considering that most bloom-forming algae in seawater are larger than 5 μm while TEPs and marine snow are in the range of hundreds of micrometers, it is expected that cake thickness is not uniform and filtration resistance can significantly vary along the length of a capillary membrane during severe HABs. Moreover, very large algal cells (e.g., *Noctiluca scintillans*) or algal aggregates/colonies (e.g., *Phaeocystis globosa*) may partially or fully plug the entrance of the capillary channels, which may then limit the productivity of the affected capillaries, resulting in substantial loss in overall permeability of the membrane module (Figure 2.7d; Heijman et al. 2007). Applying micro-screens with openings of 50 -150 μm upstream of UF membranes (current practice) can eliminate the possibility of capillary plugging. Increased capillary diameter, higher backwash flux/frequency or forward water/air flushing can potentially mitigate capillary plugging issues during HABs.

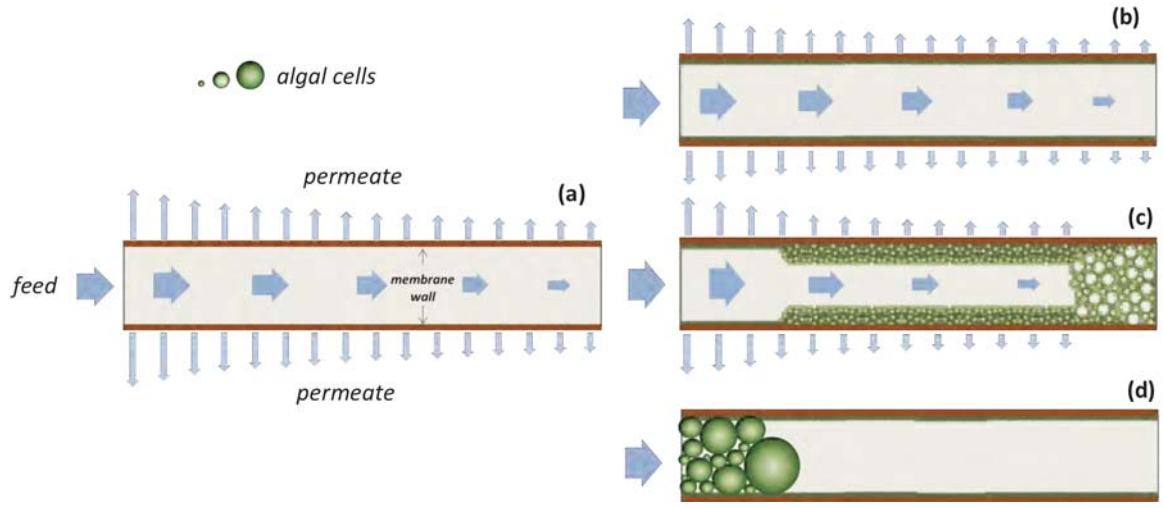

Figure 2.7. Graphical illustration of foulant accumulation in inside-out, dead-end capillary MF/UF membrane during filtration of algal bloom impaired seawater: axial and radial flow through (a) clean membrane and fouled capillary membrane with (b) uniform accumulation, (b) non-uniform accumulation and (d) entrance blockage. Figures modified from Panglisch (2003).

Pressure-driven capillary UF membranes have reportedly exhibited some degree of fouling during algal bloom periods due to the high concentrations of algal cells and associated organic matter. This is discussed in the case studies in Chapter 11 for the Jacobahaven demonstration plant on the North Sea and the Sohar plant on the Gulf of Oman. HABs at the Jacobahaven Plant impaired operation of UF membranes with chemically enhanced backwashing (CEBs) required as frequently as once in 6 hours (see Chapter 11-10). Similarly, at the Sohar plant (Chapter 11-3) with pressure driven, outside-in microfiltration membranes (PDO), shorter periods between CEB were required.

2.2.5 Summary and outlook

A spike in AOM concentration during algal blooms at a seawater desalination plant can adversely affect the operation of both pretreatment and RO systems. The high molecular weight fraction of AOM may enhance clogging of GMF and significantly increase non-backwashable fouling in MF/UF pretreatment. AOM, specifically TEP and their precursors, may initiate and accelerate biofouling in RO membranes. To ensure continuous operation in SWRO plants prone to algal blooms, the intake and/or the pretreatment system should be

reliable and robust to ensure continuous operation at design capacity and to minimize breakthrough of AOM to the downstream SWRO system. It is also advantageous to have monitoring instruments that can detect algal biomass (and algal species known to produce TEP and other organic material in high quantities) and alert operators that intervention or oversight is needed.

2.3 ALGAL ISSUES IN THERMAL DESALINATION PLANTS

Multi stage flash (MSF) and multi effect distillation (MED) are the most commonly employed methods to desalinate seawater for municipal use and for drinking water supply in the Middle East. MSF and MED thermal desalination account for 60% of the total seawater desalinated capacity in the region (20 Mm^3/d) (DesalData 2015). Thermal desalination plants have been known to be impacted by blinding of intake screens by seaweed (macroalgae) but generally are very forgiving of source water quality (Boerlage and Nada 2014). Unlike SWRO desalination plants, phytoplankton blooms seldom affect thermal desalination plants. During the prolonged 2008 algal bloom in the Gulf, the high concentration of the marine dinoflagellate *Cochlodinium polykrikoides* had limited impact on thermal plants. At Fujairah 1 in the UAE, the MSF plants continued to operate without issue while the SWRO desalination plant had to be shut down for more than one week. A minor shut down of thermal desalination plants did occur in Sharjah, UAE (less than 24 hours) due to odor issues associated with the product water. This was overcome through increased chlorination. Another thermal desalination plant (MED) at Kish Island in Iran reported a higher seawater pH during the bloom which required additional treatment measures to prevent alkaline scaling during this period.

2.4 MARINE AND FRESHWATER TOXINS

2.4.1 Background

Marine and cyanobacterial toxins have been identified globally in various coastal environments. Most of these toxins have been identified due to (i) poisoning events following the consumption of fish or shellfish or (ii) harmful effects through direct contact or exposure to aerosols. Therefore, the risks classically described for these compounds mostly relate to acute toxicity. However, acute poisoning from toxins following the consumption of desalinated drinking water has not yet been reported globally. The absence of such reports may relate to the absence of actual poisoning events or may reflect the typically experienced under-reporting of such events. In view of the risks these toxins pose, this section describes HAB toxins, their pharmacological activity, and methods of analysis. Risk assessment is presented in Chapter 8.

The following toxin groups have been identified for inclusion into this section: anatoxins (ATXs), azapiracids (AZAs), ß-methylamino alanine (BMAA), brevetoxins (BTXs), ciguatoxins (CTXs), cyclic imines (gymnodimines (GYM), spirolides (SPX), pinnatoxin (PnTX)), domoic acid (DA), microcystins (MCs), nodularins (NODs), okadaic acid and analogues (OA), palytoxins (PLTXs) incl. ovatoxins (OvTXs), saxitoxins (STXs), tetrodotoxins (TTXs) and trichotoxins (TRXs). Additional to this group of toxins are two taste and odor compounds, methyl isoborneol (MIB) and geosmin (GSM) that are non toxic, but can be a source of customer complaints. Geosmin is included in the information below in order of increasing molecular weight. Removal of these toxins is discussed in Chapter 10 with practical cases from the laboratory and full-scale plants. Note that not all of these toxins are direct threats to desalination plants, but all are presented here for completeness. For each toxin group there is a short description of the chemical nature of the compound and the

structure of a key compound from each group, as well as a listing of the algal or cyanobacterial species producing the toxins.

The toxins range dramatically in their polarity and interactions with water – described here in terms of lipophilicity (Table 2.1). Some are hydrophilic (soluble in water) and some lipophilic (soluble in fats, oils, etc.). Molecular weights range from just over 100 to close to 3500 Daltons (Table 2.1). Toxins from both pelagic (water column) and benthic (seafloor or epiphytic) micro-algae are considered since intake pipes of desalination plants can be close to surface and close to the seafloor. Likewise, some freshwater toxins are included because these can be found in brackish water, and because there is increasing evidence of them being washed into nearshore coastal waters via rivers.

Some HAB toxins are ubiquitous around the planet, e.g. DA, OA, STX and some cyclic imines (e.g. SPXs). Others are predominantly found in tropical and sub-tropical latitudes, e.g. CTXs, PLTXs and OvTXs. BTXs are typically only found in the Gulf of Mexico, and rarely in New Zealand, while the extent of the problems with some groups is not yet entirely clear, e.g. AZAs are distributed globally but most poisoning events have been reported from Irish shellfish. Most of the cyanobacterial toxins (e.g. MCs and NODs) have been reported to be of terrestrial or brackish water origin. Some of the toxins, however, such as homo-anatoxin a (homo-ATX-a) and trichotoxin, originate from benthic and pelagic marine organisms, respectively. Cyanobacterial toxins rarely occur in open seawater.

Assessment of potential public health problems requires the detection and quantitation of the HAB toxins in both intake and drinking water. To assess ecotoxicological problems it may also be necessary to analyze them in the concentrated waste streams from desalination plants. Classical detection methods for marine biotoxins have been based on whole animal assays, e.g. intraperitoneal (i.p.) mouse bioassays. Such assays typically do not possess detection limits (LODs) sufficiently low to detect the levels occurring in seawater or drinking water. Therefore, methods described here include physico-chemical methods of analysis (HPLC-UV/FLD/MS), antibody-based (e.g. ELISAs) or functional assays (PP2A, receptor-based assays). Recognizing that desalination plants are unlikely to have direct access to this type of sophisticated analytical equipment, Appendix 2 provides detailed instructions for some relatively simple, antibody-based screening methods for HAB toxins.

2.4.2 Chemistry and source organisms

Beta-methyl-amino alanine (BMAA) is a small amino acid (Figure 2.8) that has been implicated in a disease referred to as *amyotrophic lateral sclerosis* (ALS), following its discovery in Guam. As an amino acid, BMAA is a hydrophilic compound and has been shown to occur in association with proteins; it is still unclear whether this bonding is due to incorporation into proteins or due to nonspecific adsorption or inclusion.

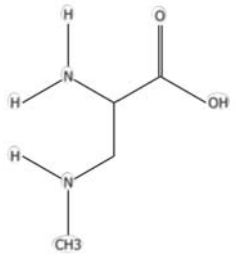

Figure 2.8. ß-methyl-amino alanine (BMAA).

Even though initially reported to be widely produced by marine and freshwater cyanobacteria, recent evidence points towards production in marine diatoms, some of which can be dominant species, e.g. *Chaetoceros* spp. (Jiang et al. 2014; Réveillon et al. 2015).

Anatoxin-A (ATX-a) is a potent neurotoxin that has been related to deaths of animals, (e.g. dogs), that have consumed contaminated surface waters from freshwater lakes or streams (Figure 2.9). The cyanobacterium *Anabaena circinalis* is a common producer. Chemically, ATX-a is a bicyclic secondary amine and belongs to the homotropane family (Wonnacott and

Gallagher 2006). While pinnamine had been isolated from a marine bivalve, *Pinna muricata* (Takada et al. 2000) and the actual biological origin has not yet been elucidated, homo-ATX-a has only been identified in a benthic, mat-forming marine cyanobacterium, *Hydrocoleum lyngbyaceum* (Méjean et al. 2010).

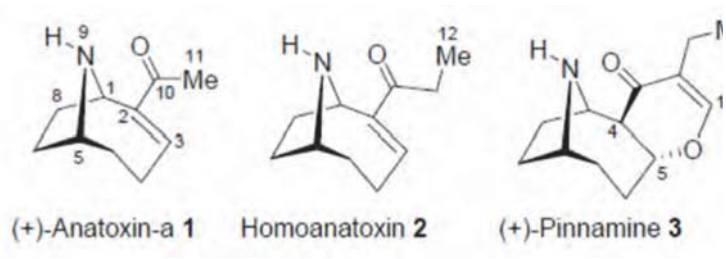

Figure 2.9. Homotropanes: anatoxin-a (ATX-a) and its methylated analogue homo-anatoxin-a (h-ATX-a) and pinnamine.

Table 2.1. Characteristics of marine and freshwater biotoxins: chemical formula, molecular weights, lipophilicity, toxicity and mode of action. (Note: geosmin is non-toxic) FW = predominantly freshwater origin; M = marine origin; FW + M = found in both freshwater and marine systems.

Toxin	Source	Chemical class	Formula	Molecular weight	Lipophilicity	Toxicity	Mode of action
BMAA	FW + M	amino acid	$C_4H_{10}N_2O_2$	118.1	hydrophylic	amyotrophic lateral sclerosis	unknown
anatoxin-a	FW	bicyclic amine alkaloid	$C_{10}H_{15}NO$	165.1	hydrophilic	fast acting neurotoxin	Na channel
geosmin	FW	bicyclic alcohol	$C_{12}H_{22}O$	182.3	lipophilic	odor disturbance	olfactive
saxitoxin	FW + M	alkaloid	$C_{10}H_{17}N_7O_4$	299.1	hydrophilic	fast acting neurotoxin	Na channel
domoic acid	M	cyclic amino acid	$C_{15}H_{21}NO_6$	311.1	hydrophilic	neurotoxin	glutamate agonist
trichotoxin	M	chlorinated phenyl-alkene	$C_{20}H_{27}CLO$	318.2	lipophilic	neurotoxin	unknown
tetrodotoxin	M	alkaloid	$C_{11}H_{17}N_3O_8$	319.1	hydrophilic	neurotoxin	Na channel
gymnodimine	M	cyclic imine, macrocycle	$C_{32}H_{45}NO_4$	507.3	lipophilic	fast acting neurotoxin	Na channel
13desmethyl-spirolide C	M	cyclic imine, macrocycle	$C_{41}H_{61}NO_7$	691.4	lipophilic	fast acting neurotoxin	Na channel
pinnatoxin G	M	cyclic imine, macrocycle	$C_{42}H_{63}NO_7$	693.5	lipophilic	fast acting neurotoxin	Na channel
okadaic acid	M	polyether	$C_{44}H_{68}O_{13}$	804.5	lipophilic	diarrhetic toxin	PP2a inhibitor
nodularin	FW	pentapeptide	$C_{41}H_{60}N_8O_{10}$	824.4	lipophilic	liver-damaging	PP2a inhibitor
azaspiracid	M	polyether	$C_{47}H_{71}NO_{12}$	841.5	lipophilic	diarrhetic toxin	unknown
brevetoxin-B	M	polyether	$C_{50}H_{70}O_{14}$	894.5	lipophilic	diarrhetic neurotoxin	Na channel
microcystin-LR	FW + M	heptapeptide	$C_{49}H_{74}N_{10}O_{12}$	994.5	lipophilic	liver-damaging	PP2a inhibitor
ciguatoxin	M	polyether	$C_{60}H_{85}O_{16}$	1061.6	lipophilic	diarrhetic neurotoxi	Na channel
palytoxin	M	polyether	$C_{129}H_{223}N_3O_{54}$	2677	amphiphilic	neurotoxin	Na K-ATPase
maitotoxin	M	polyether	$C_{164}H_{258}O_{68}S_2$	3380	amphiphilic	neurotoxin	Ca-channel

Saxitoxin (STX) and ***tetrodotoxin*** (TTX). Saxitoxin (Figure 2.10) and its analogues are very potent neurotoxins that induce symptoms in humans within minutes after consumption of contaminated shellfish; severe poisoning may lead to rapid death in patients (Rossini and Hess 2010). Tetrodotoxins have a different chemical structure from STX (Figure 2.10), but act in a very similar fashion by blocking sodium ion channels. Effects in humans are also very similar in that rapid death can occur as a result of paralysis. Tetrodotoxin has long been known as the causative agent in puffer fish poisoning (Fuchi et al. 1988; Kodama et al. 1983). Saxitoxins are a family of toxins based on a tetrahydropurine skeleton. The tetrahydropurine group renders the molecule highly water-soluble. To date, 57 analogs have been reported in cyanobacteria, marine dinoflagellates, and in molluscs (Wiese et al. 2010). STX analogues do not exhibit a strong ultraviolet (UV) absorbance or fluorescence. They are typically stable to heat treatment up to 100°C. Different acid and base treatments will lead to various transformations. In particular, all C11-epimeric pairs (e.g. GTX2 and 3 or GTX1 and 4) will interconvert and equilibrate to a constant ratio at high pH. Similarly, carbamoyl and sulfocarbamoyl analogues will convert to decarbamoyl analogues through cleavage of the carbamoyl-ester group at pH > 9 (e.g. C1 to dc-GTX2 and C2 to dc-GTX3). Under acidic conditions, the carbamoylester is relatively stable but the sulphate ester will be cleaved to convert sulfocarbamoyl-groups into the more toxic carbamoyl groups (e.g. C1 to GTX2 and C2 to GTX3). These transformations are important because the STX analogues can differ by well over an order of magnitude in potency.

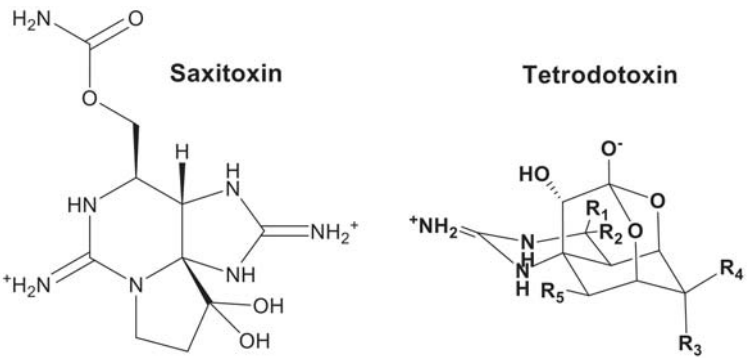

Figure 2.10: Saxitoxin and tetrodotoxin.

TTX and analogs have recently been found as contaminants in bivalves in temperate waters, i.e. the English Channel (Turner et al. 2015). These authors have also been able to demonstrate that bacteria associated with the same shellfish are capable of biosynthesizing TTXs. The association of tetrodotoxins with the marine dinoflagellate *Prorocentrum* and accumulation in bivalve mollusks, recently discovered in the Mediterranean (Vlamis et al. 2015), suggest that microalgal blooms may well act as carriers for TTX-producing bacteria.

Domoic acid (DA) Due to the common occurrence of one of its source organisms (the diatom *Pseudo-nitzschia* spp.), DA occurs throughout the world. As DA has weak diarrheic properties, the seaweed *Chondria armata* (which also produces DA) has been used in Japan as an anti-worming agent; however, the severe poisoning of over 100 people following consumption of DA-containing mussels 1987 in Canada, including 3 fatalities, stopped this practice. DA is a small cyclic amino acid, with three carboxylic acid groups (Figure 2.11). These groups are responsible for its solubility in water and its relatively high polarity. The acid constants (pK_as) of the three carboxylic acids and the cyclic amino group have been determined using NMR techniques by Walter et al. (Walter et al. 1992). Although numerous isomers and several analogues have been reported, so far only DA and its C5-diastereomer have been shown to be of

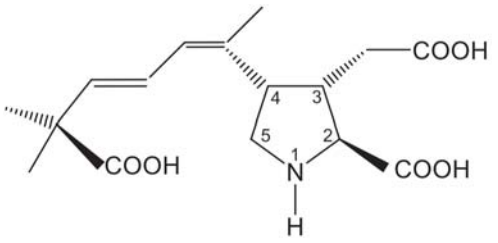

Figure 2.11. Domoic acid.

toxicological relevance (Rossini and Hess 2010). DA transforms into its diastereomer through heat or long-term storage (Quilliam et al. 1995) and analysis has focused on determination of the sum of these two isomers as best estimate of the total toxicity. A conjugated double bond in the aliphatic side chain allows for detection of DA by UV absorbance and both UV and MS detection are commonly used for the physico-chemical determination of DA (Hess et al. 2005). The conjugated double bond also leads to light-sensitivity and is the cause of radical-mediated oxidative metabolism.

Domoic acid has been reported in a wide variety of seafood organisms, including mussels, scallops and anchovies. As a contaminant in shellfish tissues, DA is heat stable and cooking does not destroy the toxin. Its stability under various conditions has been studied, and storage of raw or autoclaved tissues only resulted in approximately 50% degradation of the toxin after 5 months (McCarron et al. 2007).

Trichotoxin (TRI) Cytotoxicity of trichotoxin is ca. 1000-fold less than STX, but negative effects of *Trichodesmium* sp. have been reported on marine fauna and humans, so the toxin has to be considered (Schock et al. (2011)). TRI is a small, lipophilic, organic molecule (Figure 2.12) that has been isolated as an oil from *Trichodesmium thiebauthii*, a ubiquitous nitrogen-fixing marine cyanobacterium (Schock et al. 2011). This species is pelagic and known to form extensive and dense blooms in tropical and subtropical areas. Some claim the Red Sea derived its name from extensive blooms of this organism.

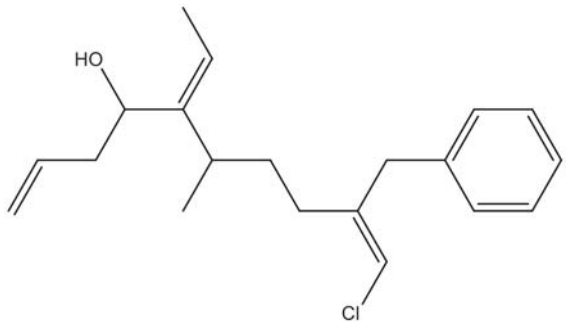

Figure 2.12. Trichotoxin isolated from a field sample of *Trichodesmium thiebauthii.*

Cyclic imines: pinnatoxins (PnTXs), ***gymnodimines*** (GYM) and ***spirolides*** (SPX). These compounds are all classified as fast acting toxins (FTAs) due to the rapid death of mice following intraperitoneal injection. Acute poisoning in humans has not been proven, however, despite an initial suspicion following consumption of the mussel *Pinna muricata* (Zheng et al. 1990). Even though acute toxicity has not been demonstrated to date, the risk of long-term exposure to sub-lethal doses is of concern given these toxins capacity to cross the intestinal and blood–brain barriers, and their high affinities for human neuronal nicotinic acetylcholine receptors (Aráoz et al. 2015).

The group of cyclic imine toxins was discovered due to their rapid response in the lipophilic mouse bioassay (Hu et al. 1995; Seki et al. 1995; Uemura et al. 1995). They all have the chemical functional groups of a cyclic imine and a macrocycle (Figure 2.13); breakage of either ring will result in loss of toxicity. Spirolides are produced by *Alexandrium ostenfeldii*

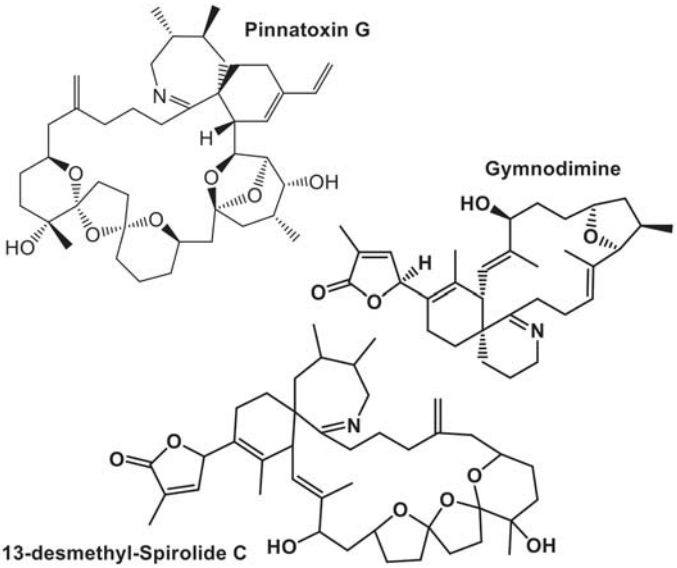

Figure 2.13. Cyclic imine toxins: pinnatoxin G, gymnodimine, 13-desmethyl spirolide C.

and *Alexandrium peruvianum* (Hu et al. 1995; Van Wagoner et al. 2011); gymnodimines are produced by *Karenia selliformis* and *A. peruvianum* (Miles et al. 2003; Seki et al. 1995; Van Wagoner et al. 2011). All of these organisms are marine, pelagic dinoflagellates. In contrast, pinnatoxins are produced by *Vulcanodinium rugosum*, a semi-benthic dinoflagellate (Hess et al. 2013; Rhodes et al. 2011; Rhodes et al. 2010; Selwood et al. 2010). Chromatographic behavior suggests intermediate lipophilicity and studies using passive samplers or seawater pre-concentration with lipophilic resins demonstrate that detectable concentrations are dissolved in seawater following HAB occurrences (Fan et al. 2014; Fux et al. 2009; Garcia-Altares et al. 2014; Rundberget et al. 2009; Zendong et al. 2014).

Microcystins and ***nodularins*** are of intermediate lipophilicity. They are produced by fresh- and brackish water cyanobacteria (Figure 2.14). Like okadaic acid, these compounds inhibit phosphoprotein phosphatases and have been linked to liver damage in humans. Microcystins are found in coastal and fresh water environments, either accumulated in shellfish or directly in the water (Morais et al. 2008; Amorim and Vasconcelos 1999; Kohoutek et al. 2010, Kudela 2011; Vasconcelos 1995, 1999). Transfer from freshwater to coastal marine waters and subsequent uptake by coastal mammals has recently been shown by Californian researchers (Gibble and Kudela 2014; Miller et al. 2010). Analogs of these groups are numerous and a Norwegian group has recently reported a large number congeners of microcystins (Ballot et al. 2014; Miles et al. 2013a; Miles et al. 2012; 2013b).

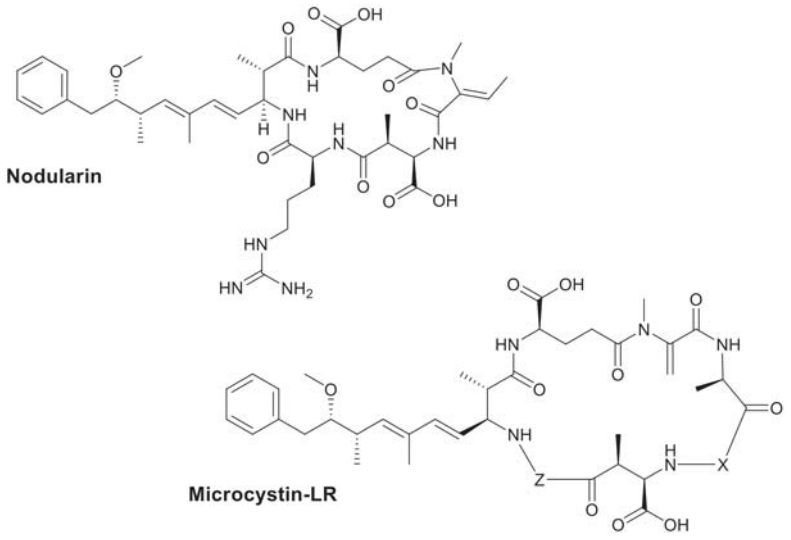

Figure 2.14. Microcystin-LR and nodularin.

Okadaic acid **(OA)** and ***dinophysistoxins*** **(DTXs)**. OA and DTXs are phosphoprotein phosphatase inhibitors and potentially tumor promoters and have been responsible in many areas for diarrhetic shellfish poisoning (EFSA, 2008b). Chemically, OA and DTXs belong to the polyether family and possess a carboxylic acid group rendering them somewhat water soluble (Figure 2.15). Numerous analogs have been reported but all are based on these three main skeletons. OA had initially been discovered as a bioactive metabolite in a marine sponge of the genus *Halichondria*, but was rapidly also attributed to the benthic dinoflagellate *Prorocentrum lima* (Murakami et al. 1982; Tachibana et al. 1981). Around the same time a closely related compound, dinophysistoxin-1 (DTX1), was described as a metabolite of the pelagic dinoflagellate *Dinophysis* following a major series of human shellfish poisoning (Murata et al. 1982; Yasumoto et al. 1978). Since then, many species of

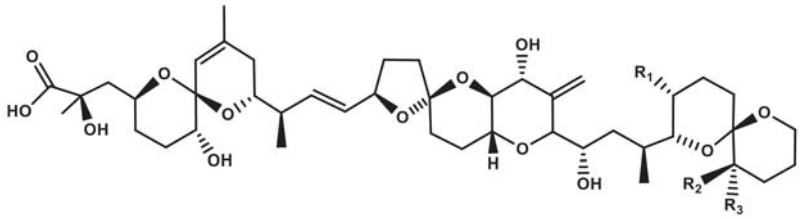

Figure 2.15. Okadaic acid and dinophysistoxins-1 and -2: OA (R1 = CH3, R2 and R3 = H), DTX1 (R1 and R2 = CH3, R3 = H), DTX2 (R1 and R2 = H, R3 = CH3).

the genera *Dinophysis* and *Prorocentrum* have been described in all seas and oceans and most of these are considered ubiquitous and toxic in all areas (Henrichs et al. 2013; Hoppenrath et al. 2013; Reguera et al. 2012). Solubility and persistence of OA and DTXs in seawater have been shown for several weeks to months after blooms via field studies using passive samplers (Fux et al. 2009; MacKenzie et al. 2004; Zendong et al. 2015a).

Azaspiracids (AZAs) are another diarrhetic shellfish poisoning group that were discovered following human poisoning from consumption of mussels (*Mytilus edulis*) produced in Ireland (McMahon and Silke 1996; Satake et al. 1998a). Toxicity in humans clearly targets the digestive tract, but the mechanism of action has not yet been elucidated despite many efforts (EFSA 2008a; Hess et al. 2015; Twiner et al. 2014). Almost 40 analogs of this polyether have been described, and the distribution of the toxins and causative organisms has been shown to be ubiquitous (Hess et al. 2014; Tillmann et al. 2014; Twiner et al. 2014).

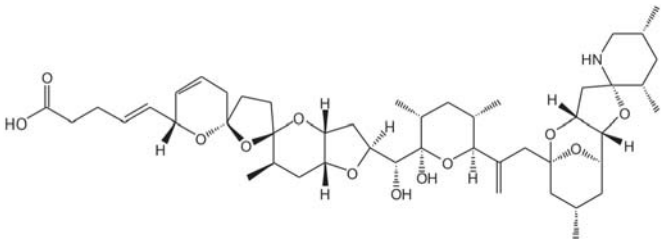

Figure 2.16. Azaspiracid.

The AZA producing organisms are all small, pelagic dinoflagellates belonging to the closely related genera of *Amphidoma* and *Azadinium*. Consistent with its polar functional groups (carboxylic acid and secondary amine), AZA is soluble in seawater (Fux et al. 2009).

Brevetoxin (BTX) toxicology is complex because this family of compounds causes toxicity from consumption of contaminated seafood as well as from direct contact with seawater or inhalation of spray from seawater. Despite their documented relation to harmful microalgae in US since the 1960s (Spikes et al. 1968), controversy existed until recently as to which were the toxicologically most relevant analogs (Bottein et al. 2007; Bottein et al. 2010; Henri et al. 2014). Thus, a compound-specific maximum permissible limit has not yet been agreed upon, and furthermore, risk assessment and management at international level will continue to remain very difficult for this toxin group (EFSA 2010; Lawrence et al. 2011).

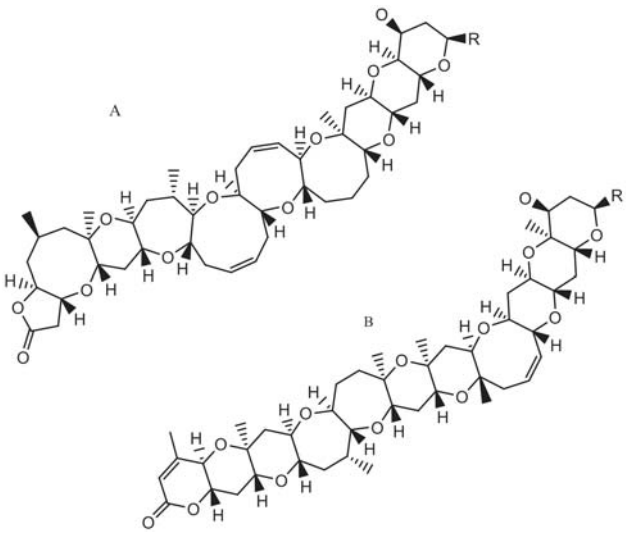

Figure 2.17. Brevetoxins: lipophilic polyethers with contiguously fused rings. Two main structural skeletons can be distinguished with analogues mainly changing at the position indicated with R.

BTXs are polyethers with contiguously fused rings which make the molecule somewhat more rigid than other polyethers, e.g. OA and AZAs (Figure 2.17). BTXs are much more lipophilic than most previously described toxins and little is known about their absolute environmental dissolved concentration, even when passive samplers or very sensitive methods have been developed to detect them in seawater (Kulagina et al. 2006a; Shea et al. 2006). The causative organism is *Karenia brevis*, a major, pelagic bloom-forming dinoflagellate, which has undergone a number of taxonomic revisions; synonyms include *Gymnodinium breve* and *Ptychodiscus brevis*. Distribution has

mostly been reported from the Gulf of Mexico region, but one major event also occurred in New Zealand, suggesting a wider distribution than initially believed (Ishida et al. 2004).

Ciguatoxin (CTX) and maitotoxin (MTX). CTXs are among the most toxic compounds known and current US-FDA guideline values in fish are not to exceed 0.01 µg P-CTX1 eq. kg^{-1} fish flesh. Like BTXs, they belong to the family of polyethers with contiguously fused rings (Figure 2.18). Both are produced by dinoflagellates belonging to the tropical marine genera *Gambierdiscus* and *Fukuyoa* (Litaker et al. 2010). These are epiphytic or benthic dinoflagellates, meaning that they live attached to surfaces on the sea floor. They are responsible for the syndrome called ciguatera fish poisoning (CFP) which is a major source of illness in tropical countries dependent upon reef fish for protein. *Gambierdiscus* species also swim, and thus can be drawn into a desalination plant intake under certain situations, though it seems unlikely that large numbers of cells would be encountered in this way. Furthermore, although some species or strains produce ciguatoxins directly, the major metabolites of *Gambierdiscus* species are metabolized to the much more potent ciguatoxins following consumption by fish. Ciguatoxins and maitotoxins are not likely to be a concern to desalination plants.

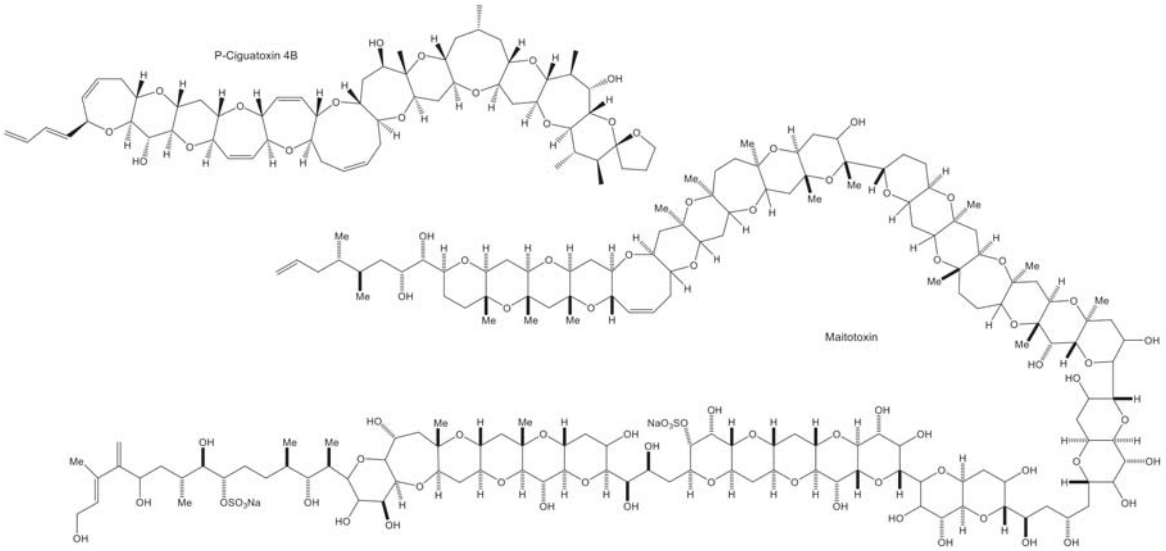

Figure 2.18. Ciguatoxin (CTX) and maitotoxin (MTX) are lipophilic polyethers with contiguously fused rings (similar to BTXs). CTX4B is shown here. CTXs are among the most lipophilic compounds while MTX is an amphiphilic polyhydroxy- polyether with two sulphate-groups.

Palytoxin (PLTX) and ovatoxin (OVTX). PLTX is atypical of most known marine toxins in that it poses risks to humans through multiple routes of exposure (oral, inhalational, and dermal). Palytoxins have been associated with human deaths following consumption of fish (Onuma et al. 1999) and with respiratory and dermatological syndromes from exposure through household aquarium supplies (Cortini et al. 2015; Davey et al. 2015) or environmental exposure to bathers and beachgoers (Funari et al. 2015; Tartaglione et al. 2015). These compounds are amongst the largest non-proteinaceous natural molecules (Figure 2.19) and have very high intrinsic toxicity. Palytoxin and its analogs ostreocins and ovatoxins are produced by zooanthids, e.g. *Palythoa* spp. (Kimura et al. 1972), dinoflagellates, e.g. *Ostreopsis* spp. (Usami et al. 1995) and potentially cyanobacteria (Kerbrat et al. 2011). PLTXs are found in dinoflagellates distributed throughout tropical and sub-tropical habitats, as well as in temperate waters of the Mediterranean and Adriatic Seas. Their chemistry and pharmacology have been recently reviewed (Carmen Louzao et al. 2015; Ciminiello et al. 2015).

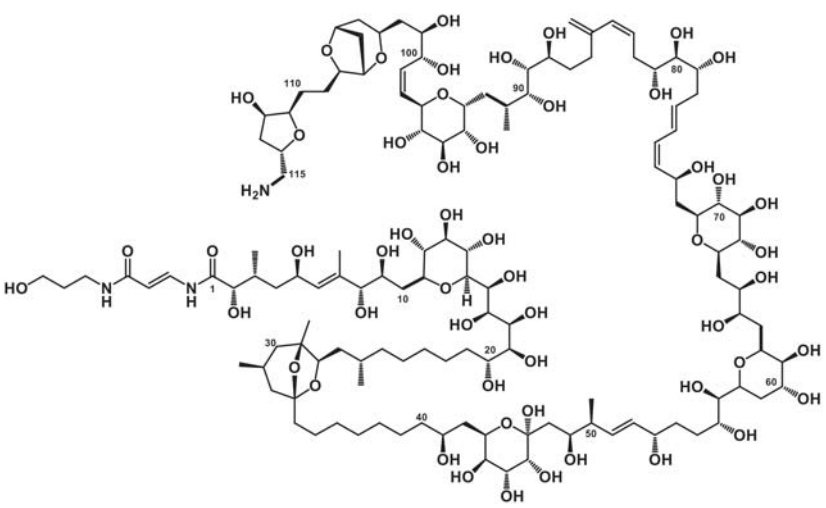

Figure 2.19: Palytoxins are amongst the largest non-proteinaceous natural molecules and have very high intrinsic toxicity.

Although the algal source for palytoxins (*Ostreopsis* species) are benthic organisms, they also do occur in dense blooms in shallow coastal waters, with accumulations of cells embedded in mucilage (Funari et al. 2015, Tartaglione et al. 2015) that could be of concern to desalination plants. Of particular interest are the observations of acute toxicity following aerosol exposures to these blooms. One noteworthy example occurred in 2005 when ~ 200 beach-goers experienced symptoms of rhinorrhea, cough, mild dyspnea, bronchoconstriction, and fever that coincided with a bloom of *Ostreopsis ovata* along the Mediterranean coast near Genoa, Italy (Ciminello et al. 2006). Altogether, over 650 cases have now been reported throughout the northern Mediterranean and Adriatic seas in association with exposure to waters containing *Ostreopsis ovata*. The concentrations of PLTX and/or PLTX-like compounds required to cause these effects through inhalational, dermal, and ocular exposures are still unknown.

2.5 TASTE AND ODOR COMPOUNDS

Geosmin (GSM) and ***methylisoborneol*** (MIB). Geosmin and MIB are both non toxic volatiles produced by cyanobacteria and marine species. Geosmin is a bicyclic alcohol (Figure 2.20) with a distinct earthy flavor and aroma produced by a type of actinobacteria, and is responsible for the earthy taste of beets and a contributor to the strong scent that occurs in the air when rain falls after a dry spell of weather or when soil is disturbed. In chemical terms, it is a lipophilic compound and an analogue of decalin. Its name is derived from the Greek geo- "earth" and osmin- "smell". Cyanobacteria are also major producers of geosmin and MIB, another compound potentially adding to poor smelling drinking water (Polak and Provasi 1992; Suurnäkki et al. 2015).

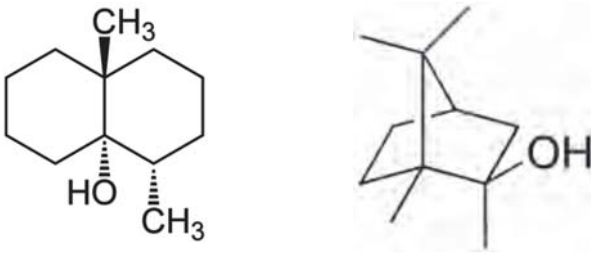

Figure 2.20 Structures of geosmin (left) and methylisoborneol (right).

2.6 DETECTION TECHNIQUES

There is an increasing range of analytical methods available for the detection and quantification of marine and cyanotoxins, and they vary greatly in the manner of detection, the information they provide and level of sophistication (Botana 2014; Harada et al. 1999; Lawrence et al. 2011; Meriluoto and Codd 2005; Nicholson and Burch 2001). For

convenience, geosmin and MIB will be included in this section, even though they are not toxins.

As mentioned above, assays based on whole, live animal exposure are excluded from this discussion due to their lack of sensitivity for desalination processes. In some cases, assays based on immortalized cell lines are also available for screening. A comprehensive discussion of the range of cell-based screening assays used to detect cyanotoxins is given in the Water Quality and Treatment Research Report 60 (Froscio et al. 2008). Such cellular approaches have also been developed for many marine biotoxins (Canete et al. 2010; Canete and Diogene 2010; Ledreux et al. 2012; Ledreux et al. 2010); however, the techniques have been only rarely validated for seawater matrix (Kulagina et al. 2006a). Still, they may be used for the estimation of toxin concentrations present in concentrated phytoplankton from seawater.

Similarly, some lateral flow immunoassays have been developed for DA, OA, STXs, and other HAB toxins (Laycock et al. 2010; McLeod et al. 2015; Vale et al. 2009); some of these may also be used for analysis of concentrated phytoplankton from seawater. Appendix 2 provides protocols for the use of some of these as screening assays.

Quantitative techniques available include immunological or biochemical screening techniques based on enzyme-linked immunosorbent assays (ELISA) or enzyme activity (protein phosphatase inhibition, PPIA) assays respectively. Some techniques, here referred to as assays, will give a sum response for all compounds, either related to the sum of concentrations present (immunological assays) or relating to the sum of toxic equivalents present (functional assays). Other methods, mainly those based on separation by gas- or liquid chromatography with various detectors will give results on individual compounds for which the sum toxicity present needs to be calculated via multiplication with toxic equivalence factors, specific to each compound.

One technique, liquid chromatography coupled to tandem mass spectrometry has been extensively used for all biotoxin compound groups except the very volatile geosmin and MIB. Even though it is an expensive and sophisticated technique, it has also been adapted for detection and quantitation of multiple groups of toxins in a single analysis (Brana-Magdalena et al. 2014; Fux et al. 2007; Quilliam et al. 2001; These et al. 2011; van den Top et al. 2011; Zendong et al. 2015b); however, sensitivity of the technique by itself is not good enough for direct analysis of seawater and thus, pre-concentration or other sample pretreatment must typically be used to achieve required detection limits for analysis in sea- or drinking water; this has recently been effectively demonstrated for okadaic acid group toxins (Zendong et al. 2015a).

A summary of analytical techniques that are available for different classes of toxins and their detection limits are given in Table 2.2. For the techniques described in the table, the detection limits may vary depending upon the standards that are available and instrumentation used. A range of other methods used within various research laboratories for screening and analysis includes ELISA methods for microcystins (Appendix 2), neuroblastoma cytotoxicity assay, saxiphilin and single-run HPLC methods for saxitoxins. The following section gives a brief overview of major methods available for individual compound groups.

2.6.1 Geosmin and methylisoborneol

The chemical procedures used to analyze organic taste and odor compounds in water must be very sensitive, because many of these substances can be detected by sensory analysis (i.e. the human nose) at ng/L levels. The most common method currently used for quantitative analysis is gas chromatography combined with mass spectrometry (GC/MS). As these compounds need to be detected at very low concentrations, a pre-concentration method often

is required. The most important methods used for the pre-concentration step are summarized below.

Closed-loop stripping analysis (CLSA) has been widely used for the analysis of non-polar volatile organic compounds of intermediate molecular weight, at the ng/L to μg/L level. The compounds are stripped from the water by a recirculating stream of air and then adsorbed from the gas phase onto a few milligrams of activated carbon. They are then extracted from the carbon with a few μL of carbon disulphide for direct analysis. This method can be applied to both raw and treated waters. The main advantage of the method is that it does not require further concentration of the solvent. Prior to the widespread adoption of solid phase micro-extraction (see below) this method was considered the standard for isolation of MIB and geosmin (Krasner et al. 1983). The limit of detection (LOD) for this method is usually reported as 1-2 ng/L.

Solid phase microextraction (SPME) is simpler and more cost-effective than CLSA, and has thus gained popularity in recent years (Huang et al.; 2004). The LOD for this method is usually reported as 1-2 ng/L for geosmin and slightly higher for MIB at 4ng/L.

2.6.2 Cylindrospermopsin

The method recommended for cylindrospermopsin is an HPLC method with SPE pre concentration (Nicholson and Burch 2001; Metcalf et al. 2002). A protein synthesis inhibition assay has been developed for cylindrospermopsin (Froscio et al. 2001).

2.6.3 Saxitoxins and tetrodotoxins

The analytical methods available for saxitoxins are continuously evolving and are based upon either high performance liquid chromatography and fluorescence detection or mass spectral detection (LC/MS/MS). Internationally, the only technique recognized by the Association of Official Analytical Chemists (AOAC) for analyzing saxitoxins from shellfish (where they are commonly found) other than mouse bioassay is a technique based upon liquid chromatography with pre-column derivatization (Nicholson and Burch 2001; Lawrence et al. 2005). This technique is not yet widely used for analysis of cyanobacterial material. Similarly, TTXs may be detected using LC-MS/MS (Boundy et al. 2015, Turner et al. 2015).

2.6.4 Domoic acid

This toxin is one of the rare compounds where detection of a single entity is sufficient to characterize the risk. Thus, several methods have been developed and validated, or cross-validated (Hess et al. 2001; Kleivdal et al. 2007; Quilliam et al. 1995). More recent developments have also allowed for a significant lowering of detection limits that permit detection of relevant levels (Table 2.3), with LODs sufficiently low to ascertain relevant levels in purified drinking water.

2.6.5 Microcystins and nodularin

Congener-independent immunoassay techniques have recently been developed for microcystin and nodularin (Fischer et al. 2001; Samdal et al. 2014). These techniques have the most appropriate detection and quantitation limits. It is important to select the appropriate analytical method for each situation, which may change regionally. For example, the technique considered most suitable to monitor microcystins in relation to the Australian Drinking Water Guidelines is high performance liquid chromatography with photo diode array detection or mass spectral detection (HPLC-PDA or HPLC-MS) (Nicholson and Burch 2001.)

Table 2.2. Toxin or taste and odor compounds, limits of detection (LOD) or quantitation (LOQ) in seawater. When an assay was not developed for seawater analysis, the LOD and LOQ values were estimated from the working range.

Toxins	Quantitative detection techniques	LOD/LOQ	Reference
Geosmin, methyl-isoborneol	GC-MS(/MS)	2 ng/L / 6 ng/L	Huang et al. 2004
Domoic acid	Biosense ELISA	3 µg/kg / 11 µg/kg §	McLeod et al. 2015; Trainer et al. 2009
	LC-FLD (direct injection)	15 ng/L / 45 ng/L in seawater	Devez and Delmas 2013
	LC-MS/MS	15 ng/L / 45 ng/L in seawater	Mafra Jr et al. 2009
	LC-MS/MS	30 ng/L / 100 ng/L	Wang et al. 2007a
	LC-MS/MS (SPE-disks)	20 ng/L (LOD)	de la Iglesia et al. 2008
	LC-UV	43 ng/L / 130 ng/L	Mafra Jr et al. 2009
Saxitoxin	^{3}H-STX-RBA	45 µg/kg / 126 µg/kg §	van Dolah et al. 2012
	Abraxis ELISA	200 µg/kg shellfish (LOQ) §	McLeod et al. 2015
	Neuronal network	76 pM in seawater (LOD)	Kulagina et al. 2006a
	LC-FLD	> 2000 nM (LOD) *§	Dell'Aversano et al. 2005
	LC-MS/MS	> 4000-6000 nM (LOD) *§	Dell'Aversano et al. 2005
Cylindrospermopsin	ELISA	0.5 µg/L	Froscio et al. 2001
	HPLC	1 µg/L	Metcalf et al. 2002
Microcystins	ELISA	40 ng/L (LOQ) in drinking water	Samdal et al. 2014
	PPIA	100 ng/L	Carmichael et al. 1999
	Radioactive PP2a binding assay	50 pM (LOD)	Serres et al. 2000
	HPLC-PDA	0.1 µg/L	Ho et al. 2006
	LC-MS/MS (MYC-LR)	2.5 ng/L (LOQ)	Wang et al. 2007b
Nodularins	ELISA	50 ng/L	Samdal et al. 2014
	PPIA	100 ng/L	Nicholson and Burch 2001
	Radioactive PP2a binding assay	40 ng/L / 120 ng/L	Serres et al. 2000
	HPLC	0.5 µg/L	Nicholson and Burch 2001

Table 2.2 *(Continued)*

Toxins	Quantitative detection techniques	LOD/LOQ	Reference
Okadaic acids	Abraxis ELISA	100 µg/kg shellfish §	McLeod et al. 2015
	PPIA	63 pg/mL (LOD) in aqueous solution	Tubaro et al. 1996
	Radioactive PP2a binding assay	200 pM (LOD)	Serres et al. 2000
	LC-MS/MS (without preconcentration)	0.16 ng/mL (LOD) §	Brana-Magdalena et al. 2014
	LC-MS/MS (with HP-20 pre-concentration)	0.2 ng/L seawater (LOQ)	Zendong et al. 2015a
Azaspiracids	ELISA	57 µg/kg LOQ) §	Samdal et al. 2015
	LC-MS/MS (without preconcentration)	0.4 µg/kg (LOD) §	Zendong et al. 2015b
	Neuronal network	0.5 nM LOD solution (IC_{50}=2nM)	Kulagina et al. 2006b
13-desmethyl Spirolide C	LC-MS/MS (without preconcentration)	0.15 µg/kg (LOD) §	Zendong et al. 2015b
PnTX-G	LC-MS/MS (without preconcentration)	0.1 µg/kg (LOD) §	Zendong et al. 2015b

*Per analog (sum of toxic equivalents may be significantly higher)
§ Not validated for seawater matrix but for shellfish matrix

2.6.6 Azaspiracids, brevetoxins, ciguatoxins, cyclic imines, okadaic acid and dinophysistoxins

These toxins are all lipophilic toxins and may be detected by LC-MS/MS (Plakas et al. 2008; Yogi et al. 2014, Zendong et al. 2015b), preferentially following pre-concentration with passive sampling resins (Zendong et al. 2015a). Alternative techniques such as ELISAs exist for some groups (e.g. OA and AZA groups) but are not necessarily more sensitive (Table 2.3).

2.7 GAPS AND PERSPECTIVES ON ANALYTICAL TECHNIQUES

Improvements are direly required for a methodology allowing for the detection and quantitation of large numbers of toxins in seawater and drinking water. Most of the currently available techniques have been developed for detection of algal toxins in shellfish and the concentration levels are typically 100 – 1000 fold higher in this matrix compared to seawater or drinking water. Pre-concentration techniques using resins, either *in situ* or in the laboratory, have recently been shown to be an effective approach; however, none of these methods have been brought to validation at the interlaboratory level.

A further need to implement regular testing of seawater and drinking water in desalination plants would be proficiency testing for this matrix. Currently, proficiency testing for algal toxins in shellfish matrices is available internationally via a registered provider: Quality Assurance in Marine Environmental Matrices (QUASIMEME 2015). A scheme for seawater and drinking water matrices could be added through this provider, subject to an expert laboratory providing test materials and analytical services to characterize such test materials.

A promising area that is developing rapidly is the application of molecular techniques (quantitative PCR) for determination of genes for toxin production. Among the algal toxins from diatoms and dinoflagellates, this approach has only been applied to STX thus far, as the toxin-producing genes are not known for most of the other toxins. Still, this approach will only apply to detection of toxin-producing algae, not the toxins themselves.

2.8 REFERENCES

Alldredge, A. L., Passow, U., and Logan, B. E. 1993. The abundance and significance of a class of large, transparent organic particles in the ocean. Deep-Sea Research I: 40, 1131–1140.

Amorim, Á. And Vasconcelos, V. 1999. Dynamics of microcystins in the mussel *Mytilus galloprovincialis*. *Toxicon* 37, 1041-1052.

Aráoz, R., Hess, P., Pelissier, F., Benoit, E., Servent, D., Zakarian, A., and Molgó, J. 2015. Cyclic imine toxins: From shellfish poisoning to neuroscience: The case of acyl derivatives. *Biochemical Pharmacology* 97, 622.

Azetsu-Scott, K. and Passow, U. 2004. Ascending marine particles: Significance of transparent exopolymer particles (TEP) in the upper ocean. *Limnology and Oceanography* 49(3), 741-748.

Ballot, A., Sandvik, M., Rundberget, T., Botha, C. J., and Miles, C. O. 2014. Diversity of cyanobacteria and cyanotoxins in Hartbeespoort Dam, *South Africa*. *Marine and Freshwater Research* 65, 175-189.

Bar-Zeev, E., Berman-Frank, I., Girshevitz, O., and Berman, T. 2012. Revised paradigm of aquatic biofilm formation facilitated by microgel transparent exopolymer particles. *Proceedings of the National Academy of Sciences* 109(23), 9119-9124.

Bar-Zeev, E. and Rahav E. 2015. Microbial metabolism of transparent exopolymer particles during the summer months along a eutrophic estuary system. *Frontiers in Microbiology* 6:403. doi: 10.3389/fmicb.2015.00403.

Berktay, A. 2011. Environmental approach and influence of red tide to desalination process in the middle-east region. *International Journal of Chemical and Environmental Engineering* 2(3), 183-188.

Berman, T. and Holenberg, M. 2005. Don't fall foul of biofilm through high TEP levels. *Filtration & Separation* 42(4), 30-32.

Botana, L.M. 2014. Seafood and Freshwater toxins: pharmacology, physiology, and detection. Third Edition. CRC Press, Boca Raton, FL, USA.

Boerlage, S. F. E. and Nada, N. 2014. Algal toxin removal in seawater desalination processes, *In Proceedings of European Desalination Society*, Cyprus.

Bottein, M.-Y. D., Fuquay, J. M., Munday, R., Selwood, A. I., van Ginkel, R., Miles, C. O., Loader, J. I., Wilkins, A. L., and Ramsdell, J. S. 2010. Bioassay methods for detection of N-palmitoylbrevetoxin-B2 (BTX-B4). *Toxicon* 55, 497-506.

Bottein, M.-Y. D, Wang, Z., and Ramsdell, J. S. 2007. Intrinsic potency of synthetically prepared brevetoxin cysteine metabolites BTX-B2 and desoxyBTX-B2. *Toxicon* 50, 825-834.

Boundy, M. J., Selwood, A. I., Harwood, D. T., McNabb, P. S., and Turner, A. D. 2015. Development of a sensitive and selective liquid chromatography–mass spectrometry method for high throughput analysis of paralytic shellfish toxins using graphitised carbon solid phase extraction. *Journal of Chromatography A* 1387, 1-12.

Brana-Magdalena, A., Leao-Martins, J. M., Glauner, T., Gago-Martinez, A. 2014. Intralaboratory validation of a fast and sensitive UHPLC/MS/MS method with fast polarity switching for the analysis of lipophilic shellfish toxins. *Journal of AOAC International* 97, 285-292.

Canete, E., Campas, M., de la Iglesia, P., and Diogene, J. 2010. NG108-15 cell-based and protein phosphatase inhibition assays as alternative semiquantitative tools for the screening of lipophilic toxins in mussels. Okadaic acid detection. *Toxicology in Vitro* 24, 611-619.

Canete, E., Diogene, J. 2010. Improvements in the use of neuroblastoma x glioma hybrid cells (NG108-15) for the toxic effect quantification of marine toxins. *Toxicon* 55, 381-389.

Carmen Louzao, M., Fraga, M., and Vilariño, N. 2015. Pharmacology of palytoxins and ostreocins, Phycotoxins. John Wiley & Sons, Ltd, pp. 113-135.

Carmichael, W. W. and An, J. 1999. Using an enzyme linked immunosorbent assay (ELISA) and a protein phosphatase inhibition assay (PPIA) for the detection of microcystins and nodularins. *Journal of Natural Toxins* 7: 377-385 .

Ciminiello, P., Dell'Aversano, C., Fattorusso, E., Forino, M., Magno, G. S., Tartaglione, L., Grillo, C., and Melchiorre, N. 2006. The Genoa 2005 outbreak. Determination of putative palytoxin in Mediterranean *Ostreopsis ovata* by a new liquid chromatography tandem mass spectrometry method. *Analytical Chemistry*, 78(17), 6153-6159.

Ciminiello, P., Dell'Aversano, C., and Forino, M. 2015. Chemistry of palytoxin and its analogues, Phycotoxins. John Wiley & Sons, Ltd, pp. 85-111.

Claquin, P., Probert, I., Lefebvre, S., AND Veron, B. 2008. Effects of temperature on photosynthetic parameters and TEP production in eight species of marine microalgae. *Aquatic Microbial Ecology* 51, 1-11.

Cortini, E., Lonati, D., Petrolini, V. M., Giampreti, A., Tubaro, A., and Locatelli, C. A. 2015. Dangerous palytoxin exposure after boiling coral. *Clinical Toxicology* 53, 339-339.

Daly, R., Ho, L., and Brookes, J. 2007. Effect of chlorination on *Microcystis aeruginosa* cell integrity and subsequent Microcystin release and degradation. *Environmental Science and Technology* 41, 4447-4453.

Davey, M. P., McKeown, N., and Hendrickson, R. 2015. Palytoxin yoxicity in a coral enthusiast and his family from unmanipulated coral. *Clinical Toxicology* 53, 776-777.

de la Iglesia, P., Giménez, G., and Diogène, J. 2008. Determination of dissolved domoic acid in seawater with reversed-phase extraction disks and rapid resolution liquid chromatography tandem mass spectrometry with head-column trapping. *Journal of Chromatography A* 1215, 116-124.

Dell'Aversano, C., Hess, P., and Quilliam, M. A. 2005. Hydrophilic interaction liquid chromatography–mass spectrometry for the analysis of paralytic shellfish poisoning (PSP) toxins. *Journal of Chromatography A* 1081, 190-201.

DesalData, 2015. https://www.desaldata.com/, accessed 3 November 2015.

Devez, A. and Delmas, D. 2013. Selective liquid chromatographic determination of trace domoic acid in seawater and phytoplankton: improvement using the o-phthaldialdehyde/9-fluorenylmethylchloroformate derivatization. *Limnology and Oceanography: Methods* 11, 327-336.

EFSA, 2008a. Marine biotoxins in shellfish - Azaspiracid group, Scientific Opinion of the Panel on Contaminants in the Food chain, adopted on 9 June 2008. *EFSA Journal* 723, 1-52.

EFSA, 2008b. Marine biotoxins in shellfish - Okadaic Acid and analogues, Scientific Opinion of the Panel on Contaminants in the Food chain, adopted on 27 November 2007. *EFSA Journal* 589, 1-62.

EFSA, 2010. Marine biotoxins in shellfish - Emerging Toxins: Brevetoxin-group, Scientific Opinion of the Panel on Contaminants in the Food chain; adopted on 5 July 2010. *EFSA Journal* 8, 1-29.

Fan, L., Sun, G., Qiu, J., Ma, Q., Hess, P., and Li, A. 2014. Effect of seawater salinity on pore-size distribution on a poly(styrene)-based HP20 resin and its adsorption of diarrhetic shellfish toxins. *Journal of Chromatography A* 1373, 1-8.

Field, C. B., Behrenfeld, M. J., Randerson, J. T., and Falkowski, P. 1998. Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science* 281, 237-240.

Fischer, W. J., Garthwaite, I., Miles, C. O., Ross, K. M., Aggen, J. B., Chamberlin, A. R., Towers, N. R., Dietrich, and D. R. 2001. Congener-independent immunoassay for microcystins and nodularins. *Environmental Science and Technology* 35, 4849-4856.

Fogg, G. E. 1983. The ecological significance of extracellular products of phytoplankton photosynthesis. *Botanica Marina* 26, 3–14.

Froscio, S., Fanok, S., King, B., and Humpage, A. R. 2008. Screening assays for water-borne toxicants research report 60. CRC for Water Quality and Treatment.

Froscio, S. M., Humpage, A. R., Burcham, P. C. and Falconer, I. R. 2001. Cell-free protein synthesis inhibition assay for the cyanobacterial toxin cylindrospermopsin. *Environmental Toxicology* 16: 408–412.

Fuchi, Y., Morisaki, S., Nagata, T., Shimazaki, K., Noguchi, T., Ohtomo, N., and Hashimoto, K. 1988. Determination of tetrodotoxin in puffer fish and shellfish by high performance liquid chromatography. *Shokuhin Eiseigaku Zasshi* 29, 306-312.

Funari, E., Manganelli, M., and Testai, E. 2015. *Ostreospis* cf. *ovata* blooms in coastal water: Italian guidelines to assess and manage the risk associated to bathing waters and recreational activities. *Harmful Algae* 50, 45-56.

Fux, E., Biré, R., and Hess, P. 2009. Comparative accumulation and composition of lipophilic marine biotoxins in passive samplers and in mussels (*M. edulis*) on the West Coast of Ireland. *Harmful Algae* 8, 523-537.

Fux, E., McMillan, D., Biré, R., and Hess, P. 2007. Development of an ultra-performance liquid chromatography-mass spectrometry method for the detection of lipophilic marine toxins. *Journal of Chromatography A* 1157, 273-280.

Garcia-Altares, M., Casanova, A., Bane, V., Diogene, J., Furey, A., and de la Iglesia, P. 2014. Confirmation of pinnatoxins and spirolides in shellfish and passive samplers from catalonia (Spain) by liquid chromatography coupled with triple quadrupole and high-resolution hybrid tandem mass spectrometry. *Marine Drugs* 12, 3706-3732.

Gibble, C. M. and Kudela, R. M. 2014. Detection of persistent microcystin toxins at the land–sea interface in Monterey Bay, California. *Harmful Algae* 39, 146-153.

Harada, K. I., Kondo, F., and Lawton, L. 1999. Toxic cyanobacteria in water. A guide to their public health consequences, monitoring and management, in: Chorus, I., Bartram, J. (Eds.). World Health Organisation, pp. 369-405.

Heijman, S. G. J., Vantieghem, M., Raktoe, S., Verberk, J. Q. J. C., and van Dijk, J. C. 2007. Blocking of capillaries as fouling mechanism for dead-end ultrafiltration. *Journal of Membrane Science*, 287(1), 119-125.

Henderson, R. K., Baker, A., Parsons, S. A., and Jefferson, B. 2008. Characterisation of algogenic organic matter extracted from cyanobacteria, green algae and diatoms. *Water Research* 42:3435-3445.

Henri, J., Leighfield, T. A., Lanceleur, R., Huguet, A., Ramsdell, J. S., and Fessard, V. 2014. Permeability of dihydro- and cysteine-brevetoxin metabolites across a Caco-2 cell monolayer. *Harmful Algae* 32, 22-26.

Henrichs, D. W., Scott, P. S., Steidinger, K. A., Errera, R. M., Abraham, A., and Campbell, L. 2013. Morphology and Phylogeny of *Prorocentrum texanum* sp nov (Dinophyceae): A new toxic dinoflagellate from the Gulf of Mexico coastal waters exhibiting two distinct morphologies. *Journal of Phycology* 49, 143-155.

Her, N., Amy, G., Park, H.-R., and Song, M. 2004. Characterizing algogenic organic matter (AOM) and evaluating associated NF membrane fouling. *Water Research* 38, 1427–1438.

Herman, P.H., Bredee, H.L. 1936. Principles of the mathematical treatment of constant pressure filtration. *Journal of the Society of Chemical Industry* 1-4.

Hess, P., Abadie, E., Herve, F., Berteaux, T., Sechet, V., Araoz, R., Molgo, J., Zakarian, A., Sibat, M., Rundberget, T., Miles, C. O., and Amzil, Z. 2013. Pinnatoxin G is responsible for atypical toxicity in mussels (*Mytilus galloprovincialis*) and clams (*Venerupis decussata*) from Ingril, a French Mediterranean lagoon. *Toxicon* 75, 16-26.

Hess, P., Gallacher, S., Bates, L. A., Brown, N., and Quilliam, M. A. 2001. Determination and confirmation of the amnesic shellfish poisoning toxin, domoic acid, in shellfish from Scotland by liquid chromatography and mass spectrometry. *Journal of the AOAC International* 84, 1657-1667.

Hess, P., McCarron, P., Krock, B., Kilcoyne, J., and Miles, C. O. 2014. Azaspiracids: Chemistry, Biosynthesis, Metabolism, and Detection, Seafood and Freshwater Toxins. CRC Press, Boca Raton, Florida, USA, pp. 799-822.

Hess, P., Stobo, L. A., Brown, N, A., McEvoy, J. D. G., Kennedy, G., Young, P. B., Slattery, D., McGovern, E., McMahon, T., and Gallacher. S. 2005. LC-UV and LC-MS methods for the determination of domoic acid. *Trends in Analytical Chemistry* 24, 358-367.

Hess, P., Twiner, M., Kilcoyne, J., and Sosa, S. 2015. Azaspiracid Toxins: Toxicological Profile, in: Gopalakrishnakone, P., Haddad Jr, V., Kem, W.R., Tubaro, A., Kim, E. (Eds.), Marine and Freshwater Toxins. Springer Netherlands, pp. 1-19.

Ho, L., Onstad, G., von Gunten, U., Rinck-Pfeiffer, S., Craig, K., and Newcombe, G. 2006. Differences in the chlorine reactivity of four microcystin analogues, *Water Research* 40(6), 1200-1209.

Hoppenrath, M., Chomérat, N., Horiguchi, T., Schweikert, M., Nagahama, Y., and Murray, S. 2013. Taxonomy and phylogeny of the benthic *Prorocentrum* species (Dinophyceae)—A proposal and review. *Harmful Algae* 27, 1-28.

Hu, T., Curtis, J. M., Oshima, Y., Quilliam, M. A., Walter, J. A., Watson-Whright, W. M., and Wright, J. L. C. 1995. Spirolides B and D, two novel macrocycles isolated from the digestive glands of shellfish. *Journal of the Chemical Society, Chemical Communications*, 2159-2161.

Huang, Y., Ortiz, L., Garcia, J., Aguirre, P., Mujeriego, R., and Bayona, J. M. 2004. Use of headspace solid-phase microextraction to characterize odour compounds in subsurface flow constructed wetland for wastewater treatment. *Water Science and Technology* 49, 89-98.

Ishida, H., Nozawa, A., Nukaya, H., Rhodes, L., McNabb, P., Holland, P. T., and Tsuji, K. 2004. Confirmation of brevetoxin metabolism in cockle, *Austrovenus stutchburyi*, and greenshell mussel, *Perna canaliculus*, associated with New Zealand neurotoxic shellfish poisoning, by controlled exposure to *Karenia brevis* culture. *Toxicon* 43, 701-712.

Jiang, L. Y., Eriksson, J., Lage, S., Jonasson, S., Shams, S., Mehine, M., Ilag, L. L., and Rasmussen, U. 2014. Diatoms: A novel source for the neurotoxin BMAA in aquatic environments. *PLoS One* 9.

Kerbrat, A. S., Amzil, Z., Pawlowiez, R., Golubic, S., Sibat, M., Darius, H. T., Chinain, M., and Laurent, D. 2011. First evidence of palytoxin and 42-hydroxy-palytoxin in the marine cyanobacterium *Trichodesmium*. *Marine Drugs* 9, 543-560.

Kim, S. H. and Yoon, J. S. 2005. Optimization of microfiltration for seawater suffering from red-tide contamination. *Desalination* 182(1–3), 315–321.

Kimura, S., Hashimoto, Y., and Yamazato, K. 1972. Toxicity of the zoanthid *Palythoa tuberculosa*. *Toxicon* 10, 611.

Kiørboe, T. and Hansen, J. L. 1993. Phytoplankton aggregate formation: Observations of patterns and mechanisms of cell sticking and the significance of exopolymeric material. *Journal of Plankton Research*, 15(9), 993-1018.

Kleivdal, H., Kristiansen, S. I., Nilsen, M. V., Goksoyr, A., Briggs, L., Holland, P., and McNabb, P. 2007. Determination of domoic acid toxins in shellfish by Biosense ASP ELISA - A direct competitive enzyme-linked immunosorbent assay: Collaborative study. *Journal of the AOAC International* 90, 1011-1027.

Kodama, M., Ogata, T., Noguchi, T., Maruyama, J., and Hashimoto, K. 1983. Occurrence of saxitoxin and other toxins in the liver of the pufferfish *Takifugu pardalis*. *Toxicon* 21, 897-900.

Kohoutek, J., Maršálek, B., and Bláha, L. 2010. Evaluation of the novel passive sampler for cyanobacterial toxins microcystins under various conditions including field sampling. *Analytical and Bioanalytical Chemistry* 397, 823-828.

Krasner, S. W., Hwang, C. J., and McGuire, M. J. 1983. A standard method for quantification of earthy-musty odorants in water, sediments, and algal cultures. *Water Science and Technology* 15, 127-138.

Kudela, R. M. 2011. Characterization and deployment of Solid Phase Adsorption Toxin Tracking (SPATT) resin for monitoring of microcystins in fresh and saltwater. *Harmful Algae* 11, 117-125.

Kulagina, N. V., Mikulski, C. M., Gray, S., Ma, W., Doucette, G. J., Ramsdell, J. S., and Pancrazio, J. J. 2006a. Detection of marine toxins, brevetoxin-3 and saxitoxin, in seawater using neuronal networks. *Environmental Sciemce amd Technology* 40, 578-583.

Kulagina, N. V., Twiner M. J., Hess, P., McMahon, T., Satake, M., Yasumoto, T., Ramsdell, J. S., Doucette, G. J., Ma, W., and O'Shaughnessy, T. J. 2006b. Azaspiracid-1 inhibits bioelectrical activity of spinal cord neuronal networks. *Toxicon* 47, 766-773.

Ladner, D. A., Vardon, D. R., and Clark, M. M. 2010. Effects of shear on microfiltration and ultrafiltration fouling by marine bloom-forming algae. *Journal of Membrane Science* 356, 33-43.

Lawrence, J., Loreal, H., Toyofuku, H., Hess, P., Iddya, K., and Ababouch, L. 2011. Assessment and management of biotoxin risks in bivalve molluscs. FAO Fisheries and Aquaculture Technical Paper No. 551, 337 pages.

Lawrence, J. F., Niedzwiadek, B., and Ménard, C. 2005. Quantitative determination of paralytic shellfish poisoning toxins in shellfish using prechromatographic oxidation and liquid chromatography with fluorescence detection: Collaborative study. *Journal of the AOAC International* 88, 1714-1732.

Laycock, M. V., Anderson, D. M., Naar, J., Goodman, A., Easy, D. J., Donovan, M. A., Li, A. F., Quilliam, M. A., Al Jamali, E., and Alshihi, R. 2012. Laboratory desalination experiments with some algal toxins. *Desalination* 293, 1-6.

Laycock, M. V., Donovan, M. A., and Easy, D. J. 2010. Sensitivity of lateral flow tests to mixtures of saxitoxins and applications to shellfish and phytoplankton monitoring. *Toxicon* 55, 597-605.

Ledreux, A., Serandour, A. L., Morin, B., Derick, S., Lanceleur, R., Hamlaoui, S., Furger, C., Bire, R., Krys, S., Fessard, V., Troussellier, M., and Bernard, C. 2012. Collaborative study for the detection of toxic compounds in shellfish extracts using cell-based assays. Part II: application to shellfish extracts spiked with lipophilic marine toxins. *Analytical and Bioanalytical Chemistry* 403, 1995-2007.

Ledreux, A., Thomazeau, S., Catherine, A., Duval, C., Yepremian, C., Marie, A., and Bernard, C. 2010. Evidence for saxitoxins production by the cyanobacterium *Aphanizomenon gracile* in a French recreational water body. *Harmful Algae* 10, 88-97.

Leppard, G. G. 1993. Organic flocs in surface waters: their native state and aggregation behavior in relation to contaminant dispersion. In: Rao, S.S. Lewis (Ed.), Particulate Matter and Aquatic Contaminants, Boca Raton, FL, pp. 169– 195.

Lerch, A., Uhl, W., and Gimbel, R. 2007. CFD modelling of floc transport and coating layer build-up in single UF/MF membrane capillaries driven in inside-out mode. *Water Science and Technology: Water Supply* 7(4), 37-47.

Li, S., Winters, H., Villacorte, L. O., Ekowati, Y., Abdul-Hamid, E., Kennedy, M. D., and Amy, G. L. 2015. Compositional similarities and differences between Transparent Exopolymer Particles (TEP) from two Marine Bacteria and two Marine Algae: Significance to Surface Biofouling. *Marine Chemistry* 174, 131–140.

Litaker, R. W., Vandersea, M. W., Faust, M. A., Kibler, S. R., Nau, A. W., Holland, W. C., Chinain, M., Holmes, M. J., and Tester, P. A. 2010. Global distribution of ciguatera causing dinoflagellates in the genus *Gambierdiscus*. *Toxicon*, 56(5), pp.711-730.

MacKenzie, L., Beuzenberg, V., Holland, P., McNabb, P., and Selwood, A. 2004. Solid phase adsorption toxin tracking (SPATT): a new monitoring tool that simulates the biotoxin contamination of filter feeding bivalves. *Toxicon* 44, 901-918.

Mafra, Jr, L. L., Léger, C., Bates, S. S., and Quilliam, M. A. 2009. Analysis of trace levels of domoic acid in seawater and plankton by liquid chromatography without derivatization, using UV or mass spectrometry detection. *Journal of Chromatography A* 1216, 6003-6011.

McCarron, P., Burrell, S., and Hess, P. 2007. Effect of addition of antibiotics and an antioxidant on the stability of tissue reference materials for domoic acid, the amnesic shellfish poison. *Analytical and Bioanalytical Chemistry* 387, 2495-2502.

McLeod, C., Burrell, S., and Holland, P. 2015. Review of the currently available field methods for detection of marine biotoxins in shellfish flesh. Report FS102086 UK-FSA Crown Copyright, website access.

McMahon, T. and Silke, J. 1996. West coast of Ireland; winter toxicity of unknown aetiology in mussels. *Harmful Algae News* 14, 2.

Méjean, A., Peyraud-Thomas, C., Kerbrat, A. S., Golubic, S., Pauillac, S., Chinain, M., and Laurent, D. 2010. First identification of the neurotoxin homoanatoxin-a from mats of *Hydrocoleum lyngbyaceum* (marine cyanobacterium) possibly linked to giant clam poisoning in New Caledonia. *Toxicon* 56, 829-835.

Meriluoto, J., and Codd, G. A. 2005. Toxic cyanobacterial monitoring and cyanotoxin analysis. Åbo Akademi University Press, Turku, Finland.

Metcalf, J. S., Beattie, K. A., Saker, M. L., and Codd, G. A. 2002 Effects of organic solvents on the high performance liquid chromatographic analysis of the cyanobacterial toxin cylindrospermopsin and its recovery from environmental eutrophic waters by solid phase extraction. *FEMS Microbiology Letters* 216(2), 159.

Miles, C. O., Sandvik, M., Haande, S., Nonga, H., and Ballot, A. 2013a. LC-MS analysis with thiol derivatization to differentiate [Dhb(7)]- from [Mdha(7)]-Microcystins: Analysis of cyanobacterial blooms, planktothrix cultures and european crayfish from Lake Steinsfjorden, Norway. *Environmental Science and Technology* 47, 4080-4087.

Miles, C. O., Sandvik, M., Nonga, H. E., Rundberget, T., Wilkins, A. L., Rise, F., Ballot, and A. 2012. Thiol derivatization for LC-MS identification of Microcystins in complex matrices. *Environmental Science and Technology* 46, 8937-8944.

Miles, C. O., Sandvik, M., Nonga, H. E., Rundberget, T., Wilkins, A. L., Rise, F., and Ballot, A. 2013b. Identification of microcystins in a Lake Victoria cyanobacterial bloom using LC–MS with thiol derivatization. *Toxicon* 70, 21-31.

Miles, C. O., Wilkins, A. L., Stirling, D. J., and MacKenzie, A. L. 2003. Gymnodimine C, an isomer of gymnodimine B, from *Karenia selliformis*. *Jouranl of Agricultural and Food Chemistry* 51, 4838-4840.

Miller, M. A., Kudela, R. M., Mekebri, A., Crane, D., Oates, S. C., Tinker, M. T., Staedler, M., Miller, W. A., Toy-Choutka, S., Dominik, C., Hardin, D., Langlois, G. W., Murray, M., Ward, K., and Jessup, D. A. 2010. Evidence for a novel marine harmful algal bloom: cyanotoxin (Microcystin) transfer from land to sea otters. *PLoS One 5*, e12576.

Mingazzini, M. and Thake, B. 1995. Summary and conclusions of the workshop on marine mucilages in the Adriatic Sea and elsewhere. *Science of the Total Environment*, 165:9-14.

Mopper, K., Zhou, J., Sri Ramana, K., Passow, U., Dam, H. G., and Drapeau, D. T. 1995. The role of surface-active carbohydrates in the flocculation of a diatom bloom in a mesocosm. *Deep-Sea Research Part II*, 42(1), 47-73.

Morais, J., Augusto, M., Carvalho, A. P., Vale, M., and Vasconcelos, V. M. 2008. Cyanobacteria hepatotoxins, microcystins: bioavailability in contaminated mussels exposed to different environmental conditions. *European Food Research and Technology* 227, 949-952.

Murakami, Y., Oshima, Y., and Yasumoto, T. 1982. Identification of okadaic acid as a toxic component of a marine dinoflagellate *Prorocentrum lima*. *Nippon Suisan Gakkaishi* 48, 69-72.

Murata, M., Shimatani, M., Sugitani, H., and Oshima, Y. T. Y. 1982. Isolation and structural elucidation of the causative toxin of the diarrhetic shellfish poisoning. *Bulletin of the Japanese Soc*iety *for the Science of Fish* 48, 549-552.

Myklestad, S. M. 1995. Release of extracellular products by phytoplankton with special emphasis on polysaccharides. *Science of the Total Environment* 165, 155-164.

Nicholson, B. and Burch, M. 2001. Evaluation of analytical methods for the detection and quantification of cyanotoxins in relation to Australian drinking water guidelines. NHMRC, Water Services Association of Australia and the Cooperative Research Centre for Water Quality and Treatment report, National Health and Medical Research Council of Australia, Canberra, Australia.

Onuma, Y., Satake, M., Ukena, T., Roux, J., Chanteau, S., Rasolofonirina, N., Ratsimaloto, M., Naoki, H., and Yasumoto, T. 1999. Identification of putative palytoxin as the cause of clupeotoxism. *Toxicon* 37, 55-65.

Panglisch, S. 2003. Formation and prevention of hardly removable particle layers in inside-out capillary membranes operating in dead-end mode. *Water Science and Technology: Water Supply* 3 (5-6), 117-124.

Passow, U. 2000. Formation of transparent exopolymer particles (TEP) from dissolved precursor material. *Marine Ecology Progress Series* 192, 1-11.

Passow, U. 2002. Transparent exopolymer particles (TEP) in aquatic environments. *Progress in Oceanography* 55(3), 287-333.

Petry, M., Sanz, M. A., Langlais, C., Bonnelye, V., Durand, J.-P., Guevara, D., Nardes, W. M., and Saemi, C. H. 2007. The El Coloso (Chile) reverse osmosis plant. *Desalination* 203(1-3), 141-152.

Plakas, S. M., Jester, E. L. E., El Said, K. R., Granade, H. R., Abraham, A., Dickey, R. W., Scott, P. S., Flewelling, L. J., Henry, M. S., Blum, P., and Pierce, R. H. 2008. Monitoring of brevetoxins in the *Karenia brevis* bloom-exposed Eastern oyster (*Crassostrea virginica*). *Toxicon* 52, 32-38.

Polak, E. H. and Provasi, J. 1992. Odor sensitivity to geosmin enantiomers. *Chemical Senses* 17, 23-26.

Qu, F., Liang, H., He, J., Ma, J., Wang, Z., Yu, H., and Li, G. 2012. Characterization of dissolved extracellular organic matter (dEOM) and bound extracellular organic matter (bEOM) of *Microcystis aeruginosa* and their impacts on UF membrane fouling. *Water Research* 46, 2881-2890.

QUASIMEME, 2015. http://www.quasimeme.org/,. website accessed 31 December 2015.

Quilliam, M. A., Hess, P., and Dell'Aversano, C. 2001. Recent developments in the analysis of phycotoxins by liquid chromatography-mass spectrometry, Mycotoxins and Phycotoxins in Perspective at the Turn of the Century, pp. 383-391.

Quilliam, M. A., Xie, M., and Hardstaff, W. R. 1995. A rapid extraction and cleanup procedure for the liquid chromatographic determination of domoic acid in unsalted seafood. *Journal of AOAC International* 78, 543-554.

Reguera, B., Velo-Suárez, L., Raine, R., and Park, M. G. 2012. Harmful Dinophysis species: A review. *Harmful Algae* 14, 87-106.

Réveillon, D., Abadie, E., Séchet, V., Masseret, E., Hess, P., and Amzil, Z. 2015. β-N-methylamino-l-alanine (BMAA) and isomers: Distribution in different food web compartments of Thau lagoon, *French Mediterranean Sea. Marine Environmental Research* 110, 8-18.

Rhodes, L., Smith, K., Selwood, A. I., McNabb, P., Molenaar, S., Munday, R., Wilkinson, C., and Hallegraeff, G. M. 2011. Production of pinnatoxins E, F and G by scrippsielloid dinoflagellates isolated from Franklin Harbour, South Australia. *New Zealand Journal of Marine and Freshwater Research* 45, 703-709.

Rhodes, L., Smith, K., Selwood, A. I., McNabb, P., van Ginkel, R., Holland, P. T., and Munday, R. 2010. Production of pinnatoxins by a peridinoid dinoflagellate isolated from Northland, New Zealand. *Harmful Algae* 9, 384-389.

Richlen, M. L., Morton, S. L., Jamali, E. A., Rajan, A., and Anderson, D. M. 2010. The catastrophic 2008–2009 red tide in the Arabian Gulf region, with observations on the identification and phylogeny of the fish-killing dinoflagellate *Cochlodinium polykrikoides*. *Harmful Algae* 9(2), pp.163-172.

Ripperger, S., Gösele, W., and Alt, C. 2012. Filtration, 1. Fundamentals. Ullmann's Encyclopedia of Industrial Chemistry. pp. 677–709.

Rossini, G. P. and Hess, P. 2010. Phycotoxins: chemistry, mechanisms of action and shellfish poisoning. *Exs* 100, 65-122.

Rundberget, T., Gustad, E., Samdal, I. A., Sandvik, M., and Miles, C. O. 2009. A convenient and cost-effective method for monitoring marine algal toxins with passive samplers. *Toxicon* 53, 543-550.

Samdal, I. A., Ballot, A., Lovberg, K. E., Miles, C. O. 2014. Multihapten approach leading to a sensitive ELISA with broad cross-reactivity to microcystins and nodularin. *Environmental Science and Technology* 48, 8035-8043.

Samdal, I. A., Lovberg, K. E., Briggs, L. R., Kilcoyne, J., Xu, J., Forsyth, C. J., and Miles, C. O. 2015. Development of an ELISA for the Detection of Azaspiracids. *Journal of Agriculture and Food Chemistry* 63, 7855-7861.

Satake, M., Ofuji, K., James, K., Furey, A., and Yasumoto, T. 1998a. New toxic event caused by Irish mussels, in: Reguera, B., Blanco, J., Fernandez, M.L., Wyatt, T. (Eds.), *Harmful Algae.* Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO pp. 468-469.

Schock, T. B., Huncik, K., Beauchesne, K. R., Villareal, T. A., and Moeller, P. D. R. 2011. Identification of Trichotoxin, a novel chlorinated compound associated with the bloom forming cyanobacterium, *Trichodesmium thiebautii*. *Environmental Science and Technol*ogy 45, 7503-7509.

Schurer, R., Tabatabai, A., Villacorte, L., Schippers, J. C., and Kennedy, M. D. 2013. Three years operational experience with ultrafiltration as SWRO pretreatment during algal bloom. *Desalination and Water Treatment* 51 (4-6), 1034-1042.

Seki, T., Satake, M., MacKenzie, A. L., Kaspar, H. F., and Yasumoto, T. 1995. Gymnodimine, a new marine toxin of unprecedented structure isolated from New Zealand oysters and the dinoflagellate, *Gymnodinium* sp. *Tetrahedron Letters* 36, 7093-7096.

Selwood, A. I., Miles, C. O., Wilkins, A. L., van Ginkel, R., Munday, R., Rise, F., and McNabb, P. 2010. Isolation, structural determination and acute toxicity of pinnatoxins E, F and G. *Journal of Agriculture and Food Chemistry* 58, 6532-6542.

Serres, M. H., Fladmark, K. E., and Doskeland, S.O. 2000. An ultrasensitive competitive binding assay for the detection of toxins affecting protein phosphatases. *Toxicon* 38, 347-360.

Shea, D., Tester, P., Cohen, J., Kibler, S., and Varnam, S. 2006. Accumulation of brevetoxins by passive sampling devices. *African Journal of Marine Science* 28, 379-381.

Spikes, J. J., Ray, S. M., Aldrich, D. V., Nash, J. B. 1968. Toxicity variations of *Gymnodinium breve* cultures. *Toxicon* 5, 171-174.

Suurnäkki, S., Gomez-Saez, G. V., Rantala-Ylinen, A., Jokela, J., Fewer, D. P., and Sivonen, K. 2015. Identification of geosmin and 2-methylisoborneol in cyanobacteria and molecular detection methods for the producers of these compounds. *Water Research* 68, 56-66.

Tachibana, K., Scheuer, P. J., Tsukitani, Y., Kikuchi, H., Van Engen, D., Clardy, J., Gopichand, Y., and Schmitz, F. J. 1981. Okadaic acid, a cytotoxic polyether from two marine sponges of the genus *Halichondria*. *Journal of the American Chemical Society* 103, 2469-2471.

Takada, N., Iwatsuki, M., Suenaga, K., and Uemura, D. 2000. Pinnamine, an alkaloidal marine toxin, isolated from *Pinna muricata*. *Tetrahedron Letters* 41, 6425-6428.

Tartaglione, L., Dell'Aversano, C., Mazzeo, A., Forino, M., Wieringa, A., and Ciminiello, P. 2015. Determination of palytoxins in soft coral and seawater from a home aquarium. comparison between *Palythoa*- and *Ostreopsis*-related inhalatory poisonings. *Environmental Science and Technology* 50(2):1023-30.

These, A., Klemm, C., Nausch, I., and Uhlig, S. 2011. Results of a European interlaboratory method validation study for the quantitative determination of lipophilic marine biotoxins in raw and cooked shellfish based on high-performance liquid chromatography–tandem mass spectrometry. Part I: collaborative study. *Analytical and Bioanalytical Chemistry* 399, 1245-1256.

Tillmann, U., Salas, R., Jauffrais, T., Hess, P., and Silke, J. 2014. AZA: The producing organisms - Biology and trophic transfer, in: Botana., L.M. (Ed.), Seafood and Freshwater Toxins. CRC Press, Boca Raton, Florida, USA, pp. 773-798.

Trainer, V. L., Wells, M. L., Cochlan, W. P., Trick, C. G., Bill, B. D., Baugh, K. A., Beall, B. F., Herndon, J., and Lundholm, N. 2009. An ecological study of a massive bloom of toxigenic *Pseudo-nitzschia cuspidata* off the Washington state coast. *Limnology and Oceanography* 54, 1461-1474.

Tubaro, A., Florio, C., Luxich, E., Sosa, S., DellaLoggia, R., and Yasumoto, T. 1996. A protein phosphatase 2A inhibition assay for a fast and sensitive assessment of okadaic acid contamination in mussels. *Toxicon* 34, 743-752.

Turner, A. D., Powell, A., Schofield, A., Lees, D. N., and Baker-Austin, C. 2015. Detection of the pufferfish toxin tetrodotoxin in European bivalves, England, 2013 to 2014. *Eurosurveillance* 20, 2-8.

Twiner, M.J., Hess, P., and Doucette, G. J. 2014. Azaspiracids: Toxicology, Pharmacologiy, and Risk Assessment, Seafood and Freshwater Toxins. CRC Press, Boca Raton, Florida, USA, pp. 823-856.

Uemura, D., Chou, T., Haino, T., Nagatsu, A., Fukuzawa, S., Zheng, S., and Chen, H. 1995. Pinnatoxin A: a toxic amphoteric macrocycle from the Okinawan bivalve *Pinna muricata*. *Journal of the American Chemical Society* 117, 1155-1156.

Usami, M., Satake, M., Ishida, S., Inoue, A., Kan, Y., and Yasumoto, T. 1995. Palytoxin analogs from the dinoflagellate *Ostreopsis siamensis*. *Journal of the American Chemical Society* 117, 5389-5390.

Vale, P., Gomes, S. S., Lameiras, J., Rodrigues, S. M., Botelho, M. J., and Laycock, M. V. 2009. Assessment of a new lateral flow immunochromatographic (LFIC) assay for the okadaic acid group of toxins using naturally contaminated bivalve shellfish from the Portuguese coast. *Food Additives and Contaminants Part A-Chem.* 26, 214-220.

van den Top, H. J., Gerssen, A., McCarron, P., and van Egmond, H. P. 2011. Quantitative determination of marine lipophilic toxins in mussels, oysters and cockles using liquid chromatography-mass spectrometry: inter-laboratory validation study. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 28, 1745-1757.

van Dolah F. M., Fire S. E., Leighfield T. A., Mikulski C. M., and Doucette G. J. 2012. Determination of paralytic shellfish toxins in shellfish by receptor binding assay: collaborative study. *Journal of AOAC Internationa*. 95, 795-812.

Van Wagoner, R. M., Misner, I., Tomas, C., and Wright, J. L. C. 2011. Occurrence of 12-methylgymnodimine in a spirolide-producing dinoflagellate *Alexandrium peruvianum* and the biogenetic implications. *Tetrahedron Letters* 52, 4243-4246.

Vasconcelos, V. M., 1995. Uptake and depuration of the heptapeptide toxin microcystin-LR in *Mytilus galloprovincialis*. *Aquatic Toxicology* 32, 227-237.

Vasconcelos, V. M. 1999. Cyanobacterial toxins in Portugal: effects on aquatic animals and risk for human health. *Brazilian Journal of Medical and Biological Research* 32, 249-254.

Verdugo, P., Alldredge, A. L., Azam, F., Kirchman, D. L., Passow, U., and Santschi, P. H. 2004. The oceanic gel phase: a bridge in the DOM–POM continuum. *Marine Chemistry* 92, 67-85.

Villacorte, L. O. 2014. Algal blooms and membrane-based desalination technology. ISBN 978-1-138-02626-1, CRC Press/Balkema, Leiden.

Villacorte, L. O., Ekowati, Y., Neu, T. R., Kleijn, J. M., Winters, H., Amy, G., Schippers J. C., and Kennedy M. D. 2015a. Characterisation of algal organic matter produced by bloom forming marine and freshwater algae. *Water Research* 73, 216–230.

Villacorte, L. O., Ekowati, Y., Winters, H., Amy, G., Schippers, J. C., and Kennedy, M. D. 2015b. MF/UF rejection and fouling potential of algal organic matter from bloom-forming marine and freshwater algae. *Desalination* 367, 1-10.

Villacorte, L. O., Kennedy, M. D., Amy, G. L., and Schippers, J. C. 2009. The fate of Transparent Exopolymer Particles (TEP) in integrated membrane systems: Removal through pretreatment processes and deposition on reverse osmosis membranes. *Water Research* 43 (20), 5039-5052.

Voutchkov, N. 2010. Considerations for selection of seawater filtration pretreatment system. *Desalination* 261 (3), 354-364.

Vlamis, A., Katikou, P., Rodriguez, I., Rey, V., Alfonso, A., Papazachariou, A., Zacharaki, T., Botana, A. M., and Botana, L. M. 2015. First detection of Tetrodotoxin in greek shellfish by UPLC-MS/MS potentially linked to the presence of the dinoflagellate *Prorocentrum minimum*. *Toxins* 7, 1779-1807.

Walter, J. A., Leek, D. M., and Falk, M. 1992. NMR study of the protonation of domoic acid. *Canadian Journal of Chemistry* 70, 1156-1161.

Wang, Z., King, K. L., Ramsdell, J. S., and Doucette, G. J. 2007a. Determination of domoic acid in seawater and phytoplankton by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A* 1163, 169-176.

Wang, J., Pang, X., Ge, F., and Ma, Z. 2007b. An ultra-performance liquid chromatography–tandem mass spectrometry method for determination of microcystins occurrence in surface water in Zhejiang Province, China. *Toxicon* 49, 1120-1128.

Wiese, M., D'Agostino, P. M., Mihali, T. K., Moffitt, M. C., and Neilan, B. A. 2010. Neurotoxic alkaloids: Saxitoxin and its analogs. *Marine Drugs* 8, 2185-2211.

Wonnacott, S. and Gallagher, T. 2006. The chemistry and pharmacology of anatoxin-a and related homotropanes with respect to nicotinic acetylcholine receptors. *Marine Drugs* 4, 228-254.

Yasumoto, T., Oshima, Y., and Yamaguchi, M. 1978. Occurrence of a new type of shellfish poisoning in the Tohoku district. *Bulletin of the Japanese Society for the Science of Fish*, 1249-1255.

Yogi, K., Sakugawa, S., Oshiro, N., Ikehara, T., Sugiyama, K., and Yasumoto, T. 2014. Determination of toxins involved in ciguatera fish poisoning in the Pacific by LC/MS. *Journal of AOAC International* 97, 398-403.

Zendong, Z., Abadie, E., Mazzeo, A., Hervé, F., Herrenknecht, C., Amzil, Z., Dell'Aversano, C., and Hess, P. 2015a. Determination of the concentration of dissolved lipophilic algal toxins in seawater using pre-concentration with HP-20 resin and LC-MS/MS detection, in: MacKenzie, L. (Ed.), 16th International Conference on Harmful Algae 27th-31st October 2014. Cawthron Institute, Nelson, New Zealand and International Society for the Study of Harmful Algae, Wellington, New Zealand.

Zendong, Z., Herrenknecht, C., Abadie, E., Brissard, C., Tixier, C., Mondeguer, F., Séchet, V., Amzil, Z., and Hess, P. 2014. Extended evaluation of polymeric and lipophilic sorbents for passive sampling of marine toxins. *Toxicon* 91, 57-68.

Zendong, Z., McCarron, P., Herrenknecht, C., Sibat, M., Amzil, Z., Cole, R. B., and Hess, P. 2015b. High resolution mass spectrometry for quantitative analysis and untargeted screening of algal toxins in mussels and passive samplers. *Journal of Chromatography A* 1416, 10-21.

Zheng, S. Z., Huang, F. L., Chen, S. C., Tan, X. F., Zuo, J. B., Peng, J., Xie, R. W. 1990. The isolation and bioactivities of pinnatoxin. *Chinese Journal of Marine Drugs* 33, 33-35.